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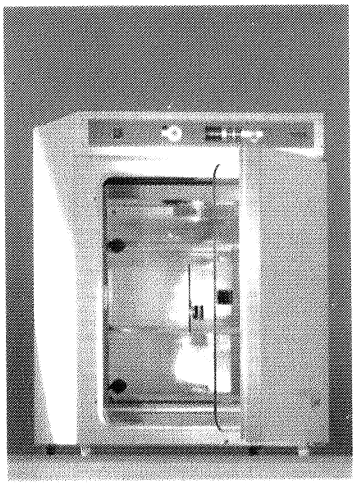
PUBLICACION DE LA SOCIEDAD
ESPAÑOLA DE MICROBIOLOGIA

Microbiología

Heraeus
INSTRUMENTS

Incubador de CO2 BB-16
Hemos incubado el huevo de Colón .....



En esta nueva generación de aparatos nos hemos concentrado especialmente en las caracteísticas de funcionamiento esenciales. Naturalmente no hemos hecho ninguna concesión en lo referente a la calidad de nuestros aparatos.

Sencillo y funcional
Las características técnicas de la BB 16 han sido estudiadas para facilitar el trabajo al usuario para las aplicaciones de rutina. Este aparato no representa complicaciones en su manejo, y está equipado con los dispositivos de seguridad necesarios.

Pequeño y compacto
La BB16 necesita poco sitio. Especialmente diseñado para espacios reducidos, el montaje especial de su puerta permite su colocación prácticamente a ras de los muebles de laboratorio.

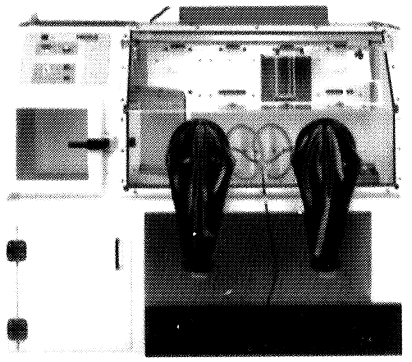
Next to in vivo
Un sistema de medición de CO2 de nueva creación, basado en la medición de la termoconductividad, asegura un valor pH estable de larga duración, incluso en el rango inferior de CO2.

Equipamiento orientado a la aplicación
Para condiciones especialmente estables se puede instalar, sobre demanda, una puerta de vidrio subdividida dotada de 3 pequeñas puertas, que permite un acceso segmentado.

Técnicas para el futuro
La BB-16 Function Line posee la acreditada calidad de Heraeus: Es un incubador con el que Ud. podrá contar también en el próximo milenio.

Camara para el cultivo de Anaerobios
Mod. WA 6200

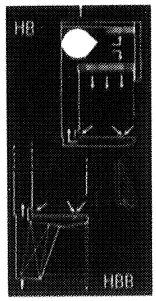
- ***Trabajo sin guantes***
- ***Manejo simple***
- ***Visión óptima***
- ***Control completamente automático***
- ***Incubador integrado hasta 70º C***
- ***Exento de condesación***
- ***Conexión para dos tipos de gas***
- ***Sistema completo***



Para trabajos en atmósfera exenta de oxigeno.
Cabina de flujo laminar para aplicaciones en el campo de la microbiología, genética, farmacia microbiología médica en una atmósfera exenta de oxígeno. Apropiada para sobreinocuación de microorganismos sensibles al contacto con el oxigeno. Perfectamente adecuada para su empleo en laboratorios para la comprobación de la calidad de medicamentos y productos químicos que exigen atmósferas controlables, así como para la producción de componentes electrónicos en atmósfera de gas protector.

Construcción
Construcción en acero inoxidable barnizada con cristal frontal de plexiglás y dos aperturas con cierre para trabajar sin guantes. Incubador de aerobios hasta 70º C integrado y controlado por microprocesador, así como disyuntor de sobre-temperatura ajustable. Esclusa de anaerobios integrada, con carro móvil para la introducción de las muestras. Control de vacío completamente automático para el gar[illegible]miento rápido de la esclusa. Sis[illegible] automático de regulación de la humedad del aire. Dispositivo de seguridad manométrico de la camara.

Probablemente la mejor cabina de flujo laminar del mundo

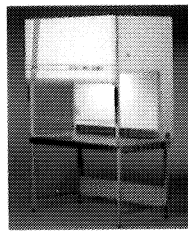


HB/HBB Cabinas de seguridad clase I

Seguridad clase II con 2 juegos de filtros HEPA para máxima protección producto, operario y medio ambiente. Verificador y construida segun todas normas internacionales.

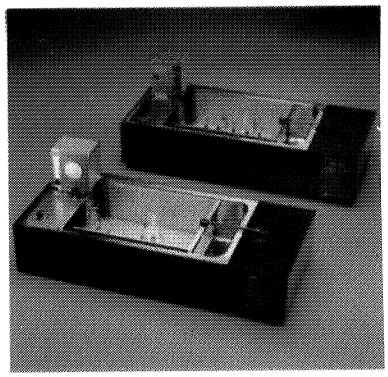
- Diseño insuperable y confortable para el usuario
- Bajo nivel de ruido
- Fácil mantenimiento, filtros facilmente intercambiables
- Alarma de seguridad
- Mesa de acero inoxidable
- Laterales en cristal para mejor iluminación
- Para usarse en laboratorios P-3

El modelo HBB tiene un sistema de filtros HEPA debajo de la mesa, proporcionando un filtrado extra de aire.
Corta distancia entre la parte superior de la mesa y el 1er filtro HEPA lo que proporciona una mínima area contaminada.



Heto es mucho más!

Adicionalmente al ámplio rango de baños presentados en este catalogo HETO ofrece más equipos para control de muestras. Aquí introducimos baños de agitación, baños termostatizados y mezcladores de muestras.



Hetomix

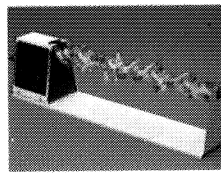
Heto ofrece un amplio rango de baños de agitación y completo rango de accesorios para el equipamiento completo del baño.
El baño de agitación Heto combinado con termostato Heto proporciona un sistema de control de temperatura preciso entre - 25°C y + 110° C.

Preguntar por catalogo adicional.

Baños de agua termostatizados

Para altas temperaturas o tareas básicas de regulación Heto ofrece un rango de baños de agua termostatizados con rasgos desde ambiente hasta + 100° C. Con compensación de agua evaporada. Existe un amplio rango de tapas y accesorios.

Preguntar por catalogo adicional.



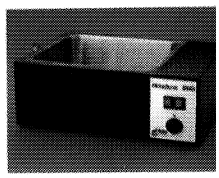
Mezcladores de muestra

Un amplio rango de mezcladores
rotación y tareas de agitación. Capacida
hasta 150 tubos simultaneamente
adaptable a casi todo tipo de muestras.

- ***Tubos Eppendorf***
- ***Tubos conicos***
- ***Cajas Petri***
- ***Tubos de recogida de sangre***
- ***Soporte de protamuestras***

Con lectura digital de velocidad y tien
velocidades extra 1-45 RPM.Amplio rang
anillos para muestras opcionales.

(Preguntar por catalogo adicional).



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Para mayor información, recortar este cupón y remitir a:

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❑ Incubador de CO2 BB-16 ❑ Cabina Flujo Laminar ❑ Camara de Anaerobios ❑ Termostatos
❑ Baños con Agitación ❑ Agitadores de Tubos ❑ Baños de Agua

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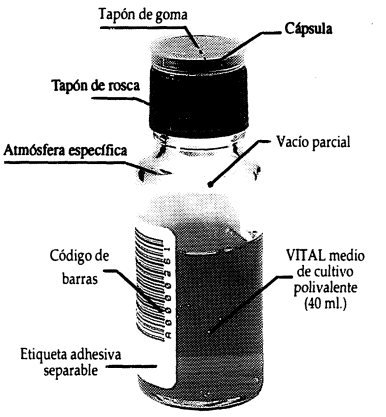
VITAL

Sistema automático para hemocultivos



ecnología H.F.T.

ectura no invasiva:
- en continuo las 24 horas del día cada 15 minutos.

ntroducción inmediata de frascos uevos en el ciclo de análisis.

- Detección del crecimiento bacteriano mediante:
  - producción CO_2.
  - variación del PH.
  - modificación del potencial de oxido-reducción.
- Facilidad de Manejo.

- Interfase windows.
- Sistema modular.
- Conexión bidireccional.

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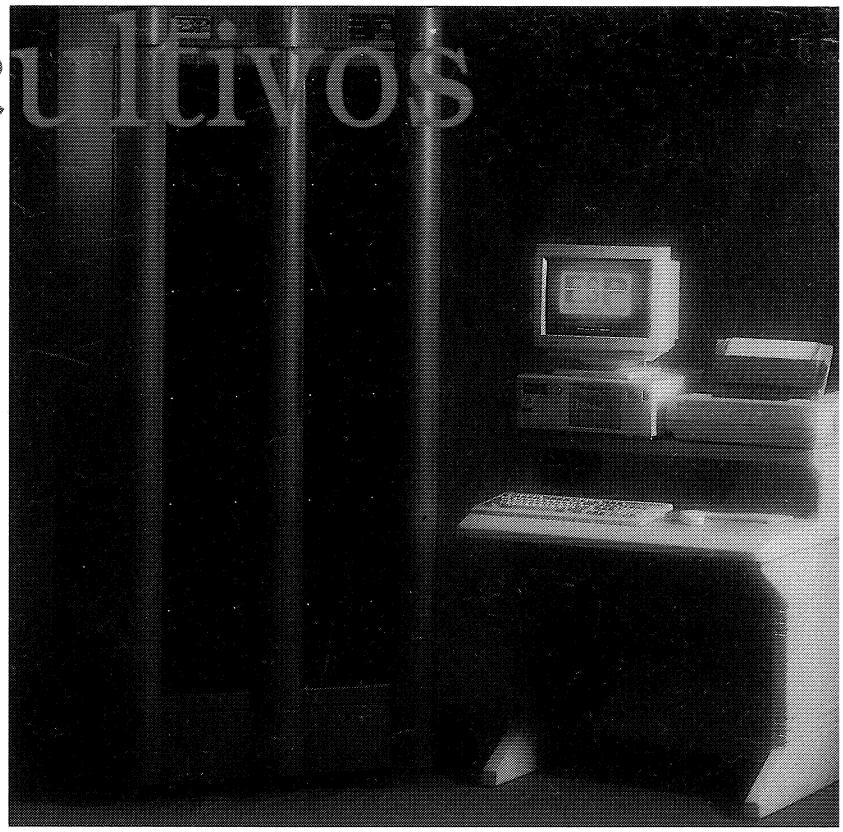
Hemocultivos ESP

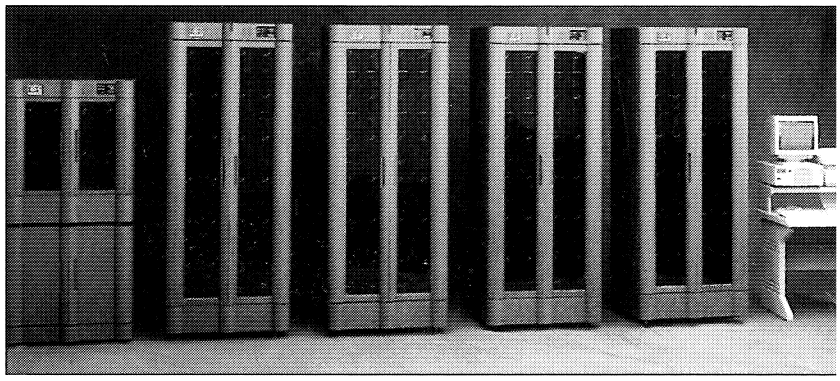
LA NUEVA TECNOLOGIA CONDUCE A UNA MAYOR RAPIDEZ

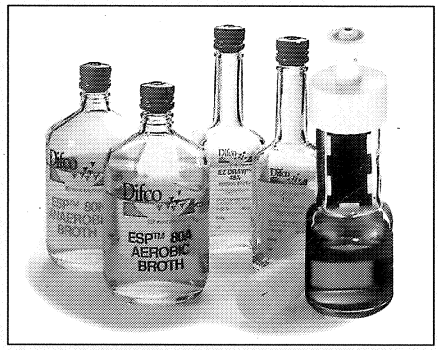
ESP ofrece lectura contínua, no invasiva, de los hemocultivos con automatización total y monitorización tanto de consumo como de producción de cualquier gas.



Configuraciones

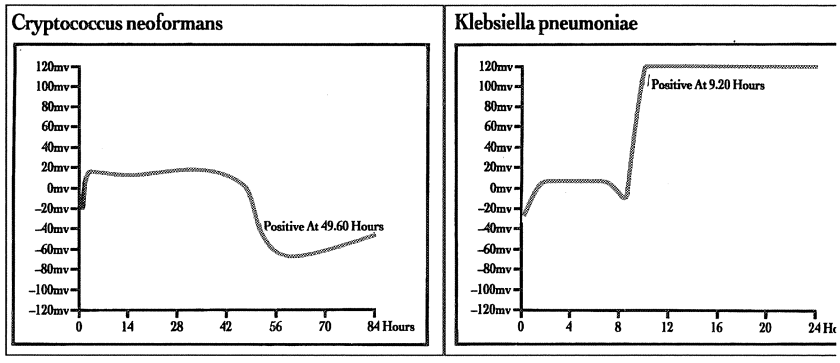
El sistema de Hemocultivos ESP, se ajusta con precisión a su volúmen de trabajo. Los instrumentos están disponibles en dos tamaños, 128 y 384 botellas. El ordenador controla hasta 5 instrumentos de cualquier capacidad.





Formatos de botellas

Los medios de nuevo desarrollo se ofrecen en una selección de tamaños. La botella de 30 ml. acepta muestras entre 0,1 y 10 ml. La botella pediátrica acepta hasta 5 ml. en extracción directa.
La botella bifásica contiene además una lengueta con Agar Chocolate y Sabouraud Dextrose.



Mayor recuperación. Mayor rapidez

Los sensores están contínuamente monitorizando tanto el consumo como la producció cualquier gas de forma que los positivos son detectados antes que con sistemas que monitorizan la producción de CO_2.

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***Instructions to Authors*:**
Information about the Journal, including instructions on the preparation and submission of manuscripts, is published on pp. 415–416 of this issue, and may also be obtained from the Editorial Office.

ÍNDICE

Página

Editorial. *Ruiz-Berraquero, F.* ... 293

New statements on the Gaia theory. *Lovelock, J. E.* ... 295

Understanding the causes of aging and cancer. *Ames, B. N.* ... 305

Formation and microbial utilization of amorphous aggregates in the sea: ecological significance. *Iriberri, J., Herndl, G. J.* ... 309

Analysis of bacterial genomes by pulsed field gel electrophoresis. *Méndez-Álvarez, S., Pavón, V., Esteve, I., Guerrero, R., Gaju, N.* ... 323

Effect of cultivation conditions on glycerophosphate oxidase production by a mutant strain of *Aerococcus viridans*. *Macková-Suchová, M., Kostál, J., Demnerová, K.* ... 337

Detection of *Bacillus larvae* spores in Argentinian honeys by using a semi-selective medium. *Alippi, A. M.* 343

Photosynthetic oxidation of MnS and FeS by *Chlorobium* spp. *Borrego, C., García-Gil, J.* ... 351

Breve historia de la Sociedad Española de Microbiología, I. De 1946 a 1971. *García Mendoza, C.* ... 359

Algunas contribuciones a los Simposios y Mesas Redondas del XV Congreso Nacional de Microbiología, Madrid, 25–28 de septiembre, 1995

- Plasmid transfer during growth and survival of *Escherichia coli*. *Muela, A.* ... 369
- Regulation mechanisms of the gene expression involved in ciliate cryptobiosis. *Gutiérrez, J. C., Palacios, G., Benítez, L., Martín González, A.* ... 373
- Protein kinases and phosphatases during the developmental cycle of myxobacteria. *Muñoz Dorado, J., Arias, J. M.* ... 376
- Intraspecific diversity in Bacteria. The case of *Escherichia coli*. *Rodríguez-Valera, F., García Martínez, J., Martínez-Murcia, A. J.* ... 379
- Genetic bases of the chromosomal polymorphism of industrial yeasts. *Benítez, T., Codón, A. C.* ... 383
- The yeast *Phaffia rhodozyma* as an industrial source of astaxanthin. *Calo, P., González, T.* ... 386
- Survival strategies of enteric bacteria in aquatic systems. *Barcina, I.* ... 389
- The survival of enteric viruses in the water environment. *Bosch, A.* ... 393
- Bacterial survival mechanisms in microbial mats. *Esteve, I., Gaju, N., Mas-Castellà, J., Guerrero, R., Margulis, L.* ... 397
- Ciliated populations in activated sludge plants: structure, dynamics and indicator value. *Fernández-Galiano, D., Guinea, A., Serrano, S., Martín-Cereceda, M., Arregui, L., Rodríguez, B. Calvo, P., Campos, I., Suárez, J.* ... 400
- Molecular identification of pathogenic actinomycetes: a new approach. *Fernández-Garayzábal, J. F.* 403

Restoring good manners in research. *Maddox, J.* ... 405

Science, Culture & Communication for the 21st century. *Fasella, P.* ... 407

Maria Therezinha Martins (1936-1995) and the Seventh International Symposium on Microbial Ecology. Campos, V., Toranzo, G. A. ... 409

Revisión de libros ... 411

Normas para los autores ... 415

Direcciones de los miembros del Consejo Editorial ... 417

ISSN 02 13-4101

CONTENTS

Page

Editorial. *Ruiz-Berraquero, F.* ... 293

New statements on the Gaia theory. *Lovelock, J. E.* ... 295

Understanding the causes of aging and cancer. *Ames, B. N.* ... 305

Formation and microbial utilization of amorphous aggregates in the sea: ecological significance. *Iriberri, J., Herndl, G. J.* ... 309

Analysis of bacterial genomes by pulsed field gel electrophoresis. *Méndez-Álvarez, S., Pavón, V., Esteve, I., Guerrero, R., Gaju, N.* ... 323

Effect of cultivation conditions on glycerophosphate oxidase production by a mutant strain of *Aerococcus viridans*. *Macková-Suchová, M., Kostál, J., Demnerová, K.* ... 337

Detection of *Bacillus larvae* spores in Argentinian honeys by using a semi-selective medium. *Alippi, A. M.* 343

Photosynthetic oxidation of MnS and FeS by *Chlorobium* spp. *Borrego, C., García-Gil, J.* ... 351

Short history of the Spanish Society for Microbiology, I. 1946–1971. *García Mendoza, C.* [In Spanish] ... 359

Some contributions to the Simposia and Round Tables of the XV National Congress of Microbiology, Madrid, September 25–28, 1995

- Plasmid transfer during growth and survival of *Escherichia coli*. *Muela, A.* ... 369
- Regulation mechanisms of the gene expression involved in ciliate cryptobiosis. *Gutiérrez, J. C., Palacios, G., Benítez, L., Martín González, A.* ... 373
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- The survival of enteric viruses in the water environment. *Bosch, A.* ... 393
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Restoring good manners in research. *Maddox, J.* ... 405

Science, Culture & Communication for the 21st. century. *Fasella, P.* ... 407

Maria Therezinha Martins (1936-1995) and the Seventh International Symposium on Microbial Ecology. Campos, V., Toranzos, G. A. ... 409

Book reviews [In Spanish] ... 411

Instructions to authors ... 416

Editorial Board addresses ... 417

ISSN 02 13-4101

MICROBIOLOGÍA SEM **11** (1995), 293–294

Editorial

Cincuenta años de vida, tanto para una persona como para una institución, son ya un número suficiente para considerar con madurez el puesto que se ocupa, la labor realizada y encarar el futuro con realismo, aunque no por ello carente de ilusión. La Sociedad Española de Microbiología (SEM) va a cumplir su cincuenta aniversario.

Hace ya algunos meses que, hablando de ese acontecimiento y de las posibles actuaciones para conmemorarlo, decidimos revolver archivos, papeles y memorias (personales) para desandar el camino hasta sus inicios y trazar desde allí lo que ha sido su historia, hasta el momento. Eso es lo que, en este mismo número de *Microbiología SEM*, nos cuenta nuestra compañera Concha García Mendoza (referente a los primeros veinticinco años de la SEM) y promete seguir contando en números sucesivos. Nadie se crea que ha sido tarea fácil. De todo esto, del pasado y del futuro, tendremos ocasión de hablar durante el XV Congreso Nacional de Microbiología, ya en puertas, que, con la participación mayoritaria de los socios, constituirá una digna celebración del Cincuentenario.

Llegados a este punto, es preciso reconocer la labor que realizaron los que acometieron la tarea de crearla (aislarla), ponerla en marcha (enriquecerla) y desarrollarla (cultivarla, aunque a veces haya habido que agitarla) durante cincuenta años. Unos nos acompañan y otros ya nos dejaron. Lo cierto es que a partir de la iniciativa, el esfuerzo y la actividad de nuestros colegas hemos llegado a lo que somos y tenemos hoy, a ese lugar que ocupamos y del cual, creo poder decir en nombre de los socios, nos sentimos orgullosos. Y, ya que por voluntad de esos mismos socios me encuentro ejerciendo este cargo, que no oficio (hay quien se lo toma como oficio y quiere ejercerlo durante toda su vida), se me va a permitir que a modo de contable, muestre la columna del haber de nuestra Sociedad. Se me ocurre que, al contrario de la cuenta bancaria, donde esa columna dice la cantidad que hay, pero no el trabajo que nos ha costado ganarla, la somera descripción de lo conseguido en la SEM nos tendría que evocar el esfuerzo de quienes la pusieron en marcha.

En la SEM somos hoy más de dos mil socios. La Sociedad pertenece a la Federation of European Microbial Societies (FEMS), de la que actualmente César Nombela es presidente. Publica el *Boletín,* de aparición semestral, una publicación ágil que anima a los socios a que expresen sus opiniones y ofrece información de las actividades de interés. Tiene una revista propia, *Microbiología SEM*, de publicación trimestral, con artículos científicos en inglés o en español, sometidos al sistema de "peer review". Organiza cada dos años un Congreso Nacional, la actividad más destacada de la Sociedad, porque permite realizar un balance de la actividad y de la producción científica en microbiología de nuestro país, y es motivo de intercambio de ideas y experiencias en todos los ámbitos de nuestra

especialidad. Organiza, desde hace algunos años, el Ciclo de Conferencias de Iniciación a la Investigación en Microbiología, destinado a estudiantes excelentes de los últimos cursos de carrera, que es una de las mejores cosas a las que puede dedicarse tanto la SEM como la universidad: estimular a los jóvenes, estudiantes y profesores, dedicados y con aptitudes. Existen los siguientes Grupos Especializados: Biodeterioro, Micología, Microbiología de los alimentos, Microbiología clínica, Microbiología industrial, Microbiología del medio acuático, Microbiología molecular, Protozoología y Taxonomía bacteriana. Además de estas actividades, de organización propia, participa en otras muchas, como las que llevan a cabo los miembros de los grupos especializados, por ejemplo el Sexto Simposio Internacional de Ecología Microbiana (celebrado en Barcelona en septiembre de 1992), el congreso de Microbiología de los Alimentos (Lérida, octubre de 1994), el de Biodeterioro (Madrid, octubre de 1994), la Reunión de Taxonomía bacteriana (Valencia, septiembre de 1994). Etcétera. Todo ello es una muestra de la vitalidad y el dinamismo de la SEM.

Por otro lado, proyectos no faltan y entre ellos tenemos el largamente acariciado de conseguir que nuestra revista aparezca en los índices internacionales, algo en lo que todos podemos colaborar. También se va a abordar la conveniencia de una licenciatura en Microbiología, para cuya discusión se ha organizado una Mesa Redonda en el XV Congreso. Recordemos que este tema ya se trató en 1971 en Salamanca, durante la celebración del XIII Congreso. Sobre estos y otros temas en marcha, es posible que haya discrepancias, pero no es la uniformidad de criterios lo que hace avanzar ni la ciencia ni la sociedad. Se necesita voluntad para analizar los problemas y para buscar la mejor solución, aunque no coincida con la propia. Igual que en un experimento científico, si los procesos seguidos son correctos, los resultados serán válidos.

Para acabar, voy a imbuirme de la vena paternalista de un representante de algo que tiene cincuenta años y me voy a dirigir a los jóvenes, microbiólogos recientes o en ciernes, para decirles que yo no conozco personalmente a los más de dos mil microbiólogos de la SEM, aunque sí a bastantes. Y la mayoría, independientemente de la compensación económica por su trabajo (más bien escasa), son unos enamorados de su profesión. Con el permiso de las diversas entidades que suministran dinero para investigar, y a pesar de las penurias, les aplicaría aquello que decía uno de los primeros científicos modernos, Francis Bacon (1561–1626): "Todo hombre está en deuda con su profesión, porque de ella recibe sustento y la satisfacción de hacer lo que le gusta."

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New statements on the Gaia theory

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Summary

Gaia is the name the ancient Greeks gave to their goddess of the Earth and is the root of words like geography and geology. Gaia is also a straightforward scientific theory about the Earth and the organisms that inhabit it. Gaia theory is testable and has a proper mathematical basis in a set of closely coupled differential equations. We do not yet know if it is a good explanation of the way our planet works; the evidence is only partially gathered. Its main value at this stage is to provide a different way to look at the Earth. In science, Gaia theory has already led to significant discoveries but just as important it forces us to question whether the good of humankind is the only thing that matters. The true value of the journeys into space was to reveal the Earth as a live planet. They made us realise for the first time that humanism is not enough. The view from space teaches that we are part of a greater entity, the Earth, and that our survival and its good health are inextricably entwined. Perhaps in time we can expand our view to encompass the larger systems of the galaxy and the Universe. Now the Earth needs our full attention.

Key words: Gaia theory, Earth's regulation, biogeochemical cycles, life's evolution, atmospheric gases

Resumen

Gaia es el nombre que dieron los antiguos griegos a la diosa de la Tierra, y es parte de términos como geografía o geología. Gaia es también una sólida teoría científica que trata de nuestro planeta y de los organismos que lo habitan. La teoría de Gaia puede ser sometida a pruebas y tiene una base matemática adecuada, con un conjunto de ecuaciones diferenciales acopladas. Todavía no sabemos si es una buena

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explicación de cómo funciona nuestro planeta; sólo hemos podido reunir algunas pruebas. En el presente estado del conocimiento, su principal valor es aportar una visión totalmente nueva de la Tierra. La teoría de Gaia ha conducido a importantes descubrimientos científicos, pero también nos fuerza a cuestionarnos si lo único que debe importarnos es el bienestar de la humanidad. El verdadero valor de los viajes espaciales ha sido revelarnos que la Tierra es un planeta vivo; nos hicieron aprender, por primera vez, que preocuparnos sólo de nuestra especie no es suficiente. La visión desde el espacio nos enseña que somos parte de una entidad mayor, la Tierra, y que nuestra supervivencia y su buena salud están entrelazadas inextricablemente. Tal vez en el futuro podamos expandir nuestra visión para abarcar sistemas mayores, como nuestra galaxia o el universo. Pero, por el momento, la Tierra requiere toda nuestra atención.

This article is based on the lecture given by the author on January 25, 1995, at the Congress Hall of Barcelona, under the auspices of the Department of Culture of the Autonomous Government of Catalonia.

The author Lewis Thomas once said: "If we look at the Earth and people from far away, from a planetary perspective we are like ants." He went on to say "They are so much like people as to be an embarrassment. They farm fungi, raise aphids as livestock, launch armies for wars, use chemical sprays to alarm and confuse their enemies, capture slaves. The families of weaver ants engage in child labour, holding their larvae like shuttles to spin out the thread that sews the leaves together for their fungus gardens. They exchange information ceaselessly. They do everything that we do except watch television."

To see the Earth as if it were an ant's nest is one of the gifts brought back from our journeys into space. Scientists call the nests of social insects like wasps and ants superorganisms because they can regulate their inner environment almost as well as we can regulate our bodies. These insects keep their nest always comfortable without any sense of purpose and without a plan. They do it is automatically. Now the view from space also led me to see our planet as if it were a superorganism, something able to regulate the climate and atmosphere so as always to be comfortable for life. And this view of the Earth called Gaia theory is the subject of my talk today.

So let us assume that the Earth self regulates; that on our planet organisms, rocks, air and ocean all act in unison to keep the climate and chemistry comfortable. I am not asking you to suspend science and believe in some mysterious Earth mother with teleological powers. All I ask is that you imagine a planet sized ecosystem, Gaia, something that emerged when organisms and their material environment evolved together. Why do it anyway? Simply because our place as one of many species on this planet is more clearly seen in a view of the whole Earth than in the subdivided parts of it. More than this I do believe that those voyages outside the Earth thirty years ago were one of our greatest achievements. They made us aware for the first time that our world was really finite and let us see how beautiful and different it was from those barren dead sister planets Mars and Venus. We then began to understand that unless we recognised the needs of the Earth, humans had no future.

The birth of Gaia theory

But first I must return to the beginning and tell you how the idea of Gaia began thirty four years ago. In 1961 the American space agency NASA invited me to join with them and explore the Moon and the planets. Before that invitation I thought space travel was science fiction, but I soon discovered that their intention was serious. An important goal of the Lunar and Planetary Division of NASA was the search for the presence of life on Mars. The work involved brief visits to that famous institute, the Jet Propulsion Laboratories (JPL), in Pasadena, California. They wanted me for my ability to design and make sensitive analytical instruments, but soon after joining them I became interested in their methods for detecting life on Mars. I expected that the biological experiments would have the same excellence that I found in the exquisite engineering and physics of the JPL. Instead, I found them unimaginative and unlikely to work even if Mars had life on it. Perhaps I was overcritical. It is not easy to design an experiment to find life on a distant planet when there is no knowledge of the life from being sought. Most of them were an automated version of the biologist's laboratory here on Earth, wonderfully engineered but based on the doubtful assumption that life on Mars would be the same as it is here. The bacteriologists, for example, proposed a robot to scoop a sample of Martian soil and apply in to a culture plate. It would then look for the growth of bacteria from the soil. There were many reasons why such an experiments could fail to detect life. Martian life might not include bacteria; even if it did, their biochemistry might be different. The experiment might land at a barren site. Even on the Earth if the experiment landed on a polar ice cap it would not have found life.

Reacting to my criticisms, the biologists challenged me to offer an alternative life detection experiment that would work. After much hard thinking I suggested that they tried a top down view of the whole planet. The simplest and most general life detection experiment would be the chemical analysis of the Martian atmosphere. The reasoning behind such a test for life goes like this. A lifeless planet would have an atmosphere determined by physics and chemistry alone, and the chemical composition would be close to the chemical equilibrium state. But on a planet that bore life, the organisms at the surface would be obliged to use the atmosphere as a source of raw materials and as a depository for wastes. Such a use of the atmosphere would change its chemical composition. It would depart from equilibrium in a way that would show the presence of life. Dian Hitchcock joined me then, and together we examined atmospheric evidence from the infrared astronomy of Mars.

We compared this evidence with evidence about the sources and skins of atmospheric gases on the one planet we knew bore life, Earth. We found an astonishing difference between the two planetary atmospheres. Mars was close to chemical equilibrium, and its atmosphere dominated by carbon dioxide. The Earth's atmosphere, in great contrast, is in a state of deep chemical disequilibrium. In our atmosphere, carbon dioxide is a mere trace gas, and the coexistence of abundant oxygen with methane and other reactive gases shows a near infinite degree of chemical disequilibrium, something impossible on a lifeless planet. Even the abundant nitrogen and water of the Earth are difficult to explain by geochemistry. No such anomalies are present in the atmospheres of Mars or Venus, and their existence in the Earth's atmosphere signals the presence of living organisms at the surface. There was no escaping the probable conclusion of chemistry: Mars was lifeless.

This was not the news our sponsors in NASA wanted to hear. They were preparing at great expense the Viking space craft to go to Mars to find life, and here were we saying there was none there. Worse

than this, we used NASA funds to view the Earth from space and conclude that there was life on it, something that could have led to the criticism of the whole space program. They asked me, what could possibly be the value of such a discovery? I was unrepentant and answered that I saw great value in it. They, NASA, had unintentionally set up an environment in which it became, for the first time, natural to ask questions about the nature of the Earth's atmosphere in the context that it was a planet with life upon it. No one had looked at the atmosphere this way before and had had the opportunity to see what a strange and beautiful anomaly is the Earth. We who live on Earth take for granted the steady constant chemical composition of our atmosphere. Changes do occur but only slowly compared with the residence times of the gases. No one had wondered how our atmosphere could remain constant and stable in composition by blind chemistry when it is a mixture of reactive gases. One afternoon in 1965 at the JPL in California, when thinking about the contradiction of our constant but highly unstable atmosphere, the thought came to me in a flash that such constancy required the existence of a regulator.

Then, I lacked any idea of the nature of what could be regulating the composition of the Earth's atmosphere, except that the organisms on the Earth's surface were part of it. I learnt from astrophysicists that the stars increase their heat output as they age, and that our Sun has grown in luminosity by 25% since life began. I realised that in the long term there might be climate regulation also. The notion of a control system involving the whole planet and the life upon it was now firmly established in my mind. Sometime, near the end of the 1960's I discussed this idea with my near neighbour the novelist William Golding. He suggested the name Gaia as the only one appropriate for so powerful an entity. Not long after this I began a collaboration on Gaia with the eminent American biologist, Lynn Margulis, that has continued until now.

Testing Gaia

What is Gaia? Gaia is the name the ancient Greeks gave to their goddess of the Earth, and is the root of words like geography and geology. The goddess Gaia was at once gentle, feminine and nurturing, but also ruthlessly cruel to those who transgressed. Gaia is also a straightforward scientific theory about the Earth and the organisms that inhabit it. A theory that views the Earth as if it were alive. As if it were able to regulate the climate and chemistry to keep it comfortable for life. Gaia theory is testable and has a proper mathematical basis in a set of closely coupled differential equations. We do not yet know if it is a good explanation of the way our planet works; the evidence is only partially gathered. I see its main value at this stage is to provide a different way to look at the Earth. In science, Gaia theory has already led to significant discoveries (Table 1), but, just as important, it forces us to question whether the good of humankind is the only thing that matters. If Gaia does exist, then it must come before us for we cannot live without it.

The principles of Gaia are not new, they were first proposed over two hundred years ago by the father of geology, James Hutton. He said in 1795 "I consider the Earth to be a superorganism and its proper study should be by physiology." His wise words were forgotten in the next century when science flourished abundantly, but also grew like a tree and separated into many separate branches. Hutton's view and Gaia are broad general science and almost incomprehensible to modern scientists, most of

TABLE 1. Some predictions from Gaia theory that have led to significant planetary discoveries

Year	Prediction	Test, result, and year
1968	That Mars was lifeless (from atmospheric evidence).	Viking Mission, 1977. Strong confirmation.
1971	That organisms would make compounds that can transfer essential elements from the oceans to the land surfaces.	Dimethylsulphide and methyliodide both found, 1973.
1973	That oxygen has stayed at $21 \pm 5\%$ of total atmospheric gases for the last 200 million years.	Still under test.
1981	That climate may be regulated by the control of carbon dioxide concentration through biologically enhanced rock weathering.	Microorganisms greatly increase rock weathering, 1989.
1987	That climate regulation via cloud density control is linked to algal sulphur gas emissions.	Still under test. Evidence that oceanic cloud cover geographically matches algal distribution, 1990.
1988	That Archean atmospheric chemistry was dominated by methane.	Still under test.

whom are specialists. Gaia is an evolutionary theory that includes the material Earth and the organisms in a single tightly coupled process. It is entirely consistent with Darwinian natural selection. The self regulation of the climate and the chemistry of the Earth are emergent properties that arises automatically. Regulation goes on entirely without foresight or planning and there is no teleology involved. Let me say that, as a scientist, I wholly reject dogmatic certainties. I do not know if Gaia theory is right; only time and evidence will bring an answer.

It is right to ask what is the use of Gaia theory? What has it done for science? Working scientists usually judge a new theory from the usefulness of its predictions. By this measure, Gaia research is useful for it has advanced both Earth and Life sciences. Let me tell you about three of these advances. The first was the discovery that the elements iodine and sulphur are transported through the environment in the form of the gases methyl iodide and dimethyl sulphide, and that both of these substances are the natural products of marine algae living at the ocean surface. I made this discovery during the voyage on a small ship from England to the Southern hemisphere and back. Everywhere the ship sailed these gases were found to be part of the ocean environment. Before the voyage scientists wrongly assumed sulphur and iodine to pass through the atmosphere as fine particles of sea salt floating in the air. Life they said plays no part in regulating the composition of the Earth, organisms merely turn over the chemicals that blind inorganic chemistry leaves for them to find.

The second discovery was that the long term climate of the Earth is regulated by the pump down of carbon dioxide in a controlled way by organisms living in the soil.

There is only one source of the gas carbon dioxide: volcanos and volcanic processes, which bring it from the Earth's interior. There is only one long term sink for carbon dioxide: its removal from the air during the weathering of calcium silicate rocks. Geochemists had assumed that weathering was a purely inorganic process in which organisms played no part. With my colleagues Michael Whitfield and Andrew Watson, we proposed that weathering takes place at least thirty times faster when organisms are present. Our proposition was experimentally confirmed by the American scientists Schwartzman and Volk. It means that organisms control the abundance of atmospheric carbon dioxide and therefore the climate also. When it is cold, soil organisms grow poorly and the pump down of carbon dioxide is slow, as a consequence carbon dioxide builds up in the air, and the world warms. When it is hot, organisms grow fast and pump down carbon dioxide rapidly so as to cool the Earth. This is one process by which the Earth could have kept cool and comfortable in spite of a 25% increase in solar heat since life began. But there is a puzzle concerning the working of this pump in the present world. It is hotter now than it was in the ice age, but the carbon dioxide has increased, not decreased, or remained constant as would be expected if regulation were taking place. How could this be?

Before I answer let me tell you of the third discovery to come from Gaia, because it is part of the explanation. The gas dimethyl sulphide has been found to do much more than merely carry sulphur from the ocean, where sulphur is abundant, to the land, where it is scarce and needed. Dimethyl sulphide oxidises in the air to produce two strong acids, methane sulphonic acid (MSA) and sulphuric acid. These acids are known to be the major source of the nucleating particles on which cloud droplets form. Without the production of dimethyl sulphide by the organisms living in the oceans there would be fewer and less dense clouds and the Earth would be a hotter place. This work was done in collaboration with my colleagues Robert Charlson, Andi Andreae and Stephen Warren and reported in 1987. Again it was in Gaian view that motivated the search for a connection between the organisms living in the oceans and climate. We wondered if the algal productions of dimethyl sulphide could be part of a Gaian feedback mechanism for keeping the Earth cool. As evidence accumulated, it pointed not to regulation but the opposite, a tendency to destabilise climate. The strongest evidence came from the chemical analysis of the ice cores taken in Antarctica. These showed clearly that as the Earth grew warmer following the last glaciation so the quantity of MSA laid down in the ice grew less. This means the warmer it becomes, the fewer the clouds and the hotter the climate. Just like the carbon dioxide puzzle, there is a positive feedback on warming, the opposite of climate self regulation in the Gaian way.

I learnt early in my life as a scientist that evidence that appears to contradict a theory under test is more likely in the end to confirm it. The bad news for theory testers is neutral or uncertain evidence. The apparent contradiction can come simply from viewing the problem in the wrong way. In the present instance, if we consider the Earth system to be in a temporary state of failure then positive feedback is not unexpected. Consider the last time you had a fever. When a fever starts, the processes that normally cool, like sweating and the dilatation of the blood vessels of the skin, cease to operate; we also produce more heat by shivering. These are all positive feedbacks characteristic of disease. Yet, who would doubt normally we regulate our body temperature very well?

If we look at the present Earth as a fevered system, then the positive feedback between climate and carbon dioxide and between climate and clouds makes sense. But, what is the justification for looking

at the Earth this way? The first thing to note is that the Earth's climate for most of the present geological period, the pleistocene, has been cold. Only about one tenth of the time is spent in interglacials like now. Moreover, the Antarctic ice records the deposition during the glaciation of seven times as much sulphur acid as now; in addition, the carbon dioxide was less than 200 parts per million in the glacial state. Both facts imply a more abundant, or more vigorous, biosphere than we have now. Perhaps the system really does prefer it cool, and the present interglacial, although comfortable for us, is a fever as far as the planet is concerned.

To test these ideas further, my colleague Lee Kump and I have made numerical models based on Gaia theory. These models included plant life on the land surfaces to pump down carbon dioxide and algal growth in the ocean to generate cloud cover. We assumed that the optimum temperature for plant growth was 18°C and the optimum sea temperature for algal growth 10°C. This was not because algae have a different temperature preferences to land organisms but because, for geophysical reasons, ocean temperatures above 10°C are associated with the formation of stable layers of warm water floating at the surface. These layers soon become depleted of nutrients and the algae cease to flourish. Our models predicted the self regulation of climate during the glacial cool state. The production of clouds by marine organisms and the pumping down of carbon dioxide both exerted a negative feedback on warming and kept a steady climate. The models also predicted the system to be close to the limit of stable operation and that even a small increase of solar heat could precipitate a change to a warmer and less stable climate. Before and after this change the system was in positive feedback.

Gaia from space

A glance at a satellite view of the Earth taken to show plant growth on the land and algal growth in the oceans confirms that dense algal growth is limited to the ocean near the poles, and to upwelling water near the edge of continents, in both of these regions the temperature is below 10°C.

The land surfaces between latitudes 30°N and 30°S, where the temperature is above 18°C for most of the year, are either desert or tropical rain forest. We know that such forests are unstable and do not recover if removed; they are also ecosystems that are managing their own regional climate and would be likely to decline with increasing temperature. The models and the evidence are consistent with Gaian self regulation in the long term, but with a temporary failure in the present feverish world. The immediate cause of the change from glacial to interglacial was almost certainly one of the small regular changes in the Earth's inclination and orbital position with respect to the Sun, the Milankovich effect. By itself the increase in heat received is insufficient to cause the large change in climate from the ice age to the interglacial. The Milankovich effect was the trigger not the prime cause of the change.

If this planetary view of the present climate is correct, it suggests that we have chosen a bad moment to add greenhouse gases to the air and to use so much of the natural ecosystems of the land surface for agriculture. More seriously, the consequences of these acts could be amplified by the positive feedback of the system, and the climate of the coming century made hotter than is usually forecast.

I started this lecture by praising journeys into space, and explained how they led to the discovery of Gaia. It is important to distinguish space exploration, which allows us to see and understand the Earth, from space exploitation, which sees space as the new frontier. Space exploration would not have

happened when it did, had it not been for the cold war between the super-powers; the enormous cost could only come from a military budget. This is forgotten when scientists and politicians talk of space exploitation for the good of mankind. It is naive and full of hubris to think of having dominion over the other planets. It is foolish and vain to plan to make Mars a second home for people, when we are so far from knowing how to live with ourselves and with the Earth. Even more absurd is the idea of colonies floating in space. Those who look to space as the new frontier ignore the mess they leave behind. Their bravado is in stark contrast to the words of the true space explorers, those brave astronauts who made their journeys to the Moon. They saw the awesome immensity of space and how small is the Earth and realised poignantly that it really is home. I recall so well Jim Lovell, one of the three that nearly did not return, telling me that even his thumb nail, held at arm's length, masked the Earth when seen from the distance of the Moon.*

The exploration of space changed the balance of power between religion and science, and strengthened the authority of science in its claim to explain the mysteries of the Earth and the Universe. The superstitious and dogmatic side of religion lost much of its power over simple people when it became known that men had walked on the Moon. In 1969, when the first men landed on the moon, I was with my family in the far west of Ireland; in those days one of the most beautiful, least touched, and devoutly religious places in Europe. During the week that followed the landing, people from the region called at our home and asked was it true that men had landed on the moon. I said yes, did you not hear it on the radio? They replied yes but we needed to hear it from your lips to know that it was true. When I asked why, they said: "We need to know the truth. We want to know that there are no angels and heaven up there in the sky."

The true value of those journeys into space, whether real or in the mind, was to reveal the Earth as a live planet. They made us realise for the first time that humanism is not enough. The view from space teaches that we are part of a greater entity, the Earth, and that our survival and its good health are inextricably entwined. Perhaps in time we can expand our view to encompass the larger systems of the galaxy and the Universe. Now the Earth needs our full attention.

What dangers lie ahead

Even if we reform immediately we shall still see the Earth change and we, its first social intelligent species, are privileged to be both the cause and the spectators. The imminent change in climate is as large as between the last ice age and now.

To comprehend the magnitude of the charge ahead glance back to the depth of the last ice age, some tens of thousands of years ago. Then the glaciers reached as far south as latitude 35° in North America

* Jim Lovell statement, talking on the Apollo 8 mission (December 1968): "What I can recall vividly is how quickly the Earth began to shrink, it began to become quite small right away; really was a kind of unusual feeling. You could actually put your thumb up to the window and hide the Earth behind your thumb, which gave us the feeling of how humble we are, because everything we knew, all our loved ones, all of our experiences, all the problems the Earth had in 1968, was behind our thumb. And it made you realize just how insignificant we are with regards to the vastness of the Universe."

and to the Alps in Europe. The sea was more than 100 m lower than now, and therefore an area of land as large as Africa was above water and where plants grew. The tropics were like the warm temperature regions are now. In all it was a pleasant world to live on and there was more land. What could take place, as a result of our presence so far, is a change as great as that from the last ice age until about 100 years ago.

To understand what has already begun and could happen in the next century, imagine the start of a heat age. An age when temperatures and sea levels climbed, by fits and starts, until eventually the world was torrid, ice free, and all but unrecognisable. Eventually is a long time ahead, it might never happen to that extent; what we have to prepare for now are the incidents of a changing climate, just about to begin. These are likely to be surprises, things that even the most detailed of big science models do not predict. Think of the ozone hole, this was a real surprise. The most expensive computer modelling and monitoring of the Earth's ozone layer failed to see or predict it. It was seen by observers looking at the sky with simple instruments. Surprise may comes as climatic extremes, like ferocious storms, or as unexpected atmospheric events. Nature is nonlinear and unpredictable and never more so than in a period of change.

This is an occasion when we cannot look to Gaia for help. If the present warm period is a planetary fever, we should expect than the Earth left to itself would be relaxing into its normal comfortable ice age. Such comfort may be unattainable because we have been busy removing its skin for farm land, taking away the trees that are the means for recovery. We also are adding vast blanket of greenhouse gases to the already feverish patient. Gaia is more likely to shudder, then move over to a new stable state, fit for a different and more amenable biota. It could be much hotter, but whatever it is, no longer the comfortable world we know. These are not fictional doom predictions, they have real possibility. We have already changed the atmosphere to an extent unprecedented in recent geological history. We seem to be driving ourselves heedlessly down a slope into a sea that is rising to drown us.

Among the things we must not do is cling to the illusion that we can manage the Earth. Management implies that contemporary science can fully explain the Earth, and that people are willing and able to work together to keep the Earth a fit and comfortable place for life.

These assumptions are naive. They are like assuming the passengers of a plane, whose pilot had died, could land it safely with no more help than pilot's manual. Does anyone believe that we, intelligent carnivores prone to tribal genocide, could, by some act of common will, change our natures and become wise and gentle gardeners, stewards, taking care of all of the natural life of our planet? I would sooner expect a goat to succeed as a gardener as expect humans to become managers of the Earth.

Do we want to be the bureaucrats in charge of the Earth? Do we want to be made accountable for its health? There can be no worse fate for people than to conscript them in such a hopeless task; to make them responsible for the smooth running of the climate. To make them responsible for the chemistry of the oceans, the air, and the soil. Something, that until we began to dismantle it, Gaia gave free.

So, what should we do? The President of the Czech Republic, the playwright Havel, has a way of looking at the Earth and ourselves that at least offers guidance. On the occasion of his receipt of the Freedom Medal of the United States of America (Philadelphia, July 4, 1994), Vaclav Havel gave an address to the people of America in which he made as the theme "We are not here for ourselves alone". He went on to remind his audience that science had been successful in displacing religion as the source of knowledge about the Universe, life and the Earth. This he said was a triumph but at the same time modern science, while destroying the older faith, has failed to offer any alternative code of moral

conduct. Because of this, the post modern world has no code of behaviour other than a belief in human rights. There is now no code of obligation to guide behaviour among ourselves and with the Earth. Havel offered two scientific contributions from post modern science which could be ingredients in a new moral theology. First, the Cosmic Anthropological Principle which suggests that we are not here by accident or as the consequence of some random event. Second, Gaia theory which tells us that we are part of a larger entity and that inhabit it. So, Gaia in a way gives back that sense of moral obligation that science had stripped away. As Havel said, "We are not here for ourselves alone".

I have spoken as an independent scientist, and it may seem that by stressing the need to take care of the Earth I am indifferent to human needs. Nothing is further from my mind, I want my grandchildren to inherit a world that has a future for them. To make sure that this happens we first need to recognise that human rights are not enough, and to survive we must also take care of the Earth. There is no tenure for anyone on this planet, not even a species.

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Understanding the causes of aging and cancer

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Summary

Cancer, a disease typical of old age, is in large part of degenerative origin. Several factors leading to the development of cancer and other degenerative diseases are discussed. The results of cancer tests in animals have been misinterpreted; they are mainly carried out by using synthetic chemicals, whereas most carcinogenic substances are natural chemicals. Animals have defense systems which prevent them from the carcinogenic effects of both natural and synthetic chemicals.

Key words: cancer, aging, degenerative diseases, oxidative damage, carcinogens

Resumen

Se sabe que el cáncer, enfermedad típica de la vejez, tiene un origen degenerativo. En este artículo se discuten algunos factores causantes del desarrollo de esta enfermedad. Los resultados de estudios sobre el cáncer llevados a cabo en animales se han interpretado erróneamente; se realizan utilizando substancias químicas sintéticas, cuando la mayoría de substancias carcinogénicas son productos químicos naturales. Los animales poseen sistemas de defensa contra los efectos nocivos de las substancias químicas carcinogénicas naturales y sintéticas.

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Introduction

Cancer, like heart disease, cataracts and brain dysfunction, is in good parta degenerative disease whose incidence increases as humans get older. This is under evolutionary control; sixty million years of evolution have lead from a short lived creature with a high cancer rate and a short life-span to a long lived creature with a low cancer rate and a long life span.

Nowadays the fundamental reason for death in non-suckers on good dieta is aging, and this may be related to all those degenerative diseases that arrive with age. An animal's first priority is reproduction, and a lot of its energy is devoted to that activity, the maintenance of the tissues taking a second place. So maintenance is never quite adequate and eventually, with all the trade-offs, it causes death.

Many of the major causes of cancer involve oxidation. Cancer, everybody agrees, is due to mutation. Mutation, a phenomenon which has played a major role in the evolution of life, thus in the maintenance of life on earth, has been also the cause of death.

Causes of aging

Aging appears to be in good part due to the oxidants originated as by-products of normal metabolism (6). These oxidants, such as superoxide and hydrogen peroxide, are the same mutagens that are produced by radiation, and cause damage to DNA, proteins, and lipids. The DNA in each cell of a normal rat receives on average about 100,000 oxidative lesions per day. DNA repair enzymes constantly remove this damage, but they do not keep up: a young rat has about 1 million oxidative lesions in the DNA of each cell, which increases to about two million in an old rat. A human cell receives about ten times less damage than a rat cell, in agreement with the higher cancer rate and shorter life span of a rat (6).

Degenerative diseases

The degenerative diseases of aging such as cancer, cardiovascular disease, cataracts, and brain dysfunction, are increasingly found to have, in good part, an oxidative origin (6). It is argued that dietary antioxidants, such as vitamins C and E and carotenoids, play a major role in minimizing this damage and that most of the world's population is receiving inadequate amounts of them, at a great cost to health. The main source of dietary antioxidants is fruits and vegetables. Humans should eat two portions of fruits and three of vegetables per day, yet only 9% of the U.S. population eats that much. Epidemiological studies show that the incidence of most types of cancer is double among people who eat few fruits and vegetables as compared to those who eat about five portions per day (7). Considerable evidence indicates that oxidative damage is important in cardiovascular disease, cataracts, and brain and immune system dysfunction, and that adequate dietary antioxidants can minimize their incidence (6).

Men with low vitamin C intake have low vitamin C in their seminal fluid and much more oxidative damage to the DNA in their sperm (5, 8). Male smokers are particularly at risk as they have depleted antioxidant pools (cigarette smoke is extremely high in oxidants). A smoker must eat 2 to 3 times as much

vitamin C as a non-smoker to maintain an equal plasma level, yet smokers tend to eat worse diets that non-smokers. Indeed, male smokers may have a higher risk of having children with birth defects and childhood cancer (5).

Causes of cancer

The three main causes of cancer are smoking (2), dietary imbalances (excess fat and calories; inadequate intake of fruits, vegetables, fiber, and calcium), and chronic infections leading to chronic inflammation (hepatitis B and C viruses, *Helicobacter pylori* infection, schistosomiasis, etc.) (6). Chronic inflammation is a major cause of cancer in the world because it releases powerful oxidants which both stimulate cell division and are mutagens. Past occupational exposures might cause about 2% of current human cancer, a major part being asbestos exposure in smokers, and industrial or synthetic chemical pollution causes less than 1%, in my view (9). The age-adjusted cancer death rate in the U.S. for all cancers combined (excluding those attributable to smoking) has been remaining steady since 1950, while life expectancy increases every year. We are the healthiest we have ever been in human history.

Two factors are critical in the formation of mutations: lesions in DNA, formed when DNA is damaged, and cell division, which converts DNA lesions to mutations. Agents increasing either lesions or cell division increase mutations and as a consequence increase cancer incidence (1). Hormones stimulating cell division increase cancer incidence (e.g., levels of estrogen in breast cancer and testosterone in prostate cancer); hormones may be a risk factor in about 20% of human cancer (10).

Misinterpretation of cancer tests

Animal cancer tests, which are done at the maximum tolerated dose (MTD), are being misinterpreted to mean that low doses of the chemicals tested and found positive are thereby relevant to human cancers (1, 9). Animal cancer tests are mainly done on synthetic chemicals and industrial pollutants, yet half of all natural chemicals that have been tested at the MTD are rodent carcinogens. It is argued that the explanation for the high frequency of positive results in animal cancer tests is that high dose animal cancer tests are mainly measuring increases in cell division due to cell killing and compensatory cell division; this is a high dose effect that does not occur at low doses. In any case, 99.9% or more of the chemicals we eat are natural. For example, 99.99% of the pesticides we eat are natural chemicals that are present in plants to ward off insects and other predators (3). More than half of those natural pesticides tested in high dose animal tests are rodent carcinogens. There are about 10,000 or so different natural pesticides in our diet, and they are usually present at enormously higher levels than synthetic pesticides. Cooking food also generates thousands of chemicals. There are over 1000 chemicals reported in a cup of coffee. Only 26 have been tested in animal cancer tests and more than half are rodent carcinogens; there are still a thousand chemicals left to test. The amount of potentially carcinogenic pesticide residues consumed in a year is less than the known amount of rodent carcinogens in a cup of coffee (1, 9).

Conclusion

The reason we can eat the tremendous variety of natural chemical rodent carcinogens in our food is that animals are extremely well defended against all chemicals by many general defense systems (4). These enzymes, e.g., DNA repair and glutathione transferases, which defend against reactive compounds such as mutagens, are all inducible (more of them are made when they are in use). They are equally effective against natural and synthetic reactive chemicals. Thus, animals are extremely well defended against low doses of chemicals. One does not expect, nor does one find, a general difference between synthetic and natural chemicals in their carcinogenicity, and though less well studied, the same would be expected for mutagenicity, teratogenicity, and acute toxicity. The effort to eliminate synthetic pesticides because of unsubstantiated fears about residues in food will make fruits and vegetables more expensive, decrease consumption, and thus increase cancer rates. The levels of synthetic pesticide residues are trivial in comparison to natural chemicals, and thus their potential for cancer causation is extremely low.

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Formation and microbial utilization of amorphous aggregates in the sea: ecological significance

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Summary

Amorphous organic particles, ("marine snow"), are thought to play a vital role in the transfer of carbon and energy from the euphotic layers of the ocean to the deep sea bed. These particles are mainly derived from phytoplankton extracellular release. As 10 to 40% of the dissolved organic carbon consists of colloidal material, colloids may represent a prominent role in these transfer processes. These colloids adsorb charged molecules efficiently and can coagulate further to form the recently described transparent exopolymer particles and eventually the ubiquitous macroscopic marine snow. The other mechanism proposed leading ultimately to marine snow formation is inefficient top-down control of phytoplankton growth by zooplankton. In the senesce
of the phytoplankton bloom the cell surface becomes sticky due to the release of polysaccharides, and single phytoplankton cells then aggregate. Subsequently these aggregates become densely colonized by auto- and heterotrophic microbes, but while the abundance of these microorganisms is from 2 to 4 orders of magnitude higher than in the ambient water, their growth rates are usually not significantly increased in the aggregates. In this review, the reasons for this conspicuous pattern are discussed and recently investigated novel types of particles and their significance in the light of oceanic carbon transfer are presented.

Key words: marine snow, marine bacteria, organic metter in seawater, transparent exopolymer particles (TEP), colloids

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Resumen

Los agregados amorfos de naturaleza orgánica, ("nieve marina"), desempeñan una función esencial en la transferencia de carbono y energía desde los niveles fóticos superficiales del medio marino al fondo del océano. Estas partículas se originan mayoritariamente como consecuencia de la liberación extracelular de productos fitoplanctónicos. El material coloidal, que se encuentra en el agua de mar constituyendo entre un 10 y un 40% del carbono orgánico disuelto, puede desempeñar una función importante en los procesos de transferencia. Estos coloides adsorben eficazmente moléculas cargadas y pueden coagular, formando en primera instancia partículas transparentes de exopolímero y, posteriormente, los agregados macroscópicos que constituyen la nieve marina. Un segundo mecanismo que conduce a la formación de nieve marina está relacionado con un control ineficaz del crecimiento fitoplanctónico por parte del zooplancton. En este caso, la superficie de las células senescentes del fitoplancton que no han sido predadas se vuelve pegajosa debido a la liberación de polisacáridos, con lo que resulta favorecida la agregación. Una vez formados los agregados, tendrá lugar una importante colonización por microorganismos autotróficos y heterotróficos. Sin embargo, mientras que la abundancia de la microbiota en los agregados es entre 2 y 4 órdenes de magnitud superior que en el agua circundante, la velocidad de crecimiento de los microorganismos es semejante o incluso inferior. En esta revisión se exponen las razones que pueden explicar esta importante paradoja y se discute el significado de nuevos tipos de partículas en relación con la transferencia de carbono y energía en el medio marino.

Introduction

It was only two decades ago when Pomeroy (39) revolutionized the traditional picture of the oceanic trophic chain by showing the fundamental role of microorganisms in the energy flux of pelagic marine ecosystems. During the following ten years, the introduction of new methodologies allowed us to quantify microbial processes (18, 26, 54) confirming that the main flux of non-living organic carbon is mediated by bacteria which are, in turn, grazed by protists (the microbial loop hypothesis) (6). Despite this progress in understanding the underlying fundamental processes, we still have a rather limited knowledge on the variability of microbially-mediated processes in time and space in the sea. Most of this variability can be explained by the heterogeneous distribution of organic and inorganic matter in the seawater. From this point of view, particles suspended in the water column are evidently of crucial importance for the understanding of the functioning of pelagic systems.

Particles create spatial heterogeneity in the distribution of organic matter and remineralized nutrients, as well as in the abundance and species composition of microorganisms in seawater. Therefore, they also influence the spatial variability of microbial metabolic rates and the biogeochemical transformation of nutrient species (9). Numerous studies have discussed the importance of oceanic particles as microenvironments for very active bacterial metabolism (13, 16, 33), but the particulate organic matter (POM)-bacteria interactions are only poorly understood. This is probably due to the arbitrary distinction between particulate and dissolved phases (the criterion is based on the filterability). Consequently, a large fraction (from 10 to 40% [53]) of what we consider dissolved organic matter

(DOM) is composed of sub-micrometre particles or colloidal material, which is within the size range of the so-called "dissolved" organic matter but it is clearly "particulate" in nature.

Colloidal particles do not sediment, and belong to what oceanographers consider as suspended particles. Another kind of particles, the sinking particles, sediment to the sea floor and there become the source of energy and matter for deep-sea organisms. Most of these particles originate from phytoplankton production in surface waters, hence phytoplankton nourishes life not only in the euphotic layer but also in the aphotic layers of the oceans. The dominant types of particles in this flux towards the sea floor are fecal pellets or non-grazed senescent phytoplankton cells, which stick one to another and form larger aggregates (36).

Origin and kinds of particles

There is a wide variety of sinking particles, although most of them originate from phytoplankt on near the ocean surface. In stable ecosystems, such as the Pacific eddies, phytoplankton is top-down controlled, indicating that they are effectively regulated by higher trophic levels such as zooplankton. Under these oligotrophic conditions, most of the matter flux towards the sea floor consists of zooplankton-derived fecal pellets (38). Even though the fecal material frequently dominates the flux of POM towards the bottom, flocculent amorphous aggregates, usually called "marine snow" (Fig. 1), are occasionally even more important although they have been scarcely sampled due to their fragile

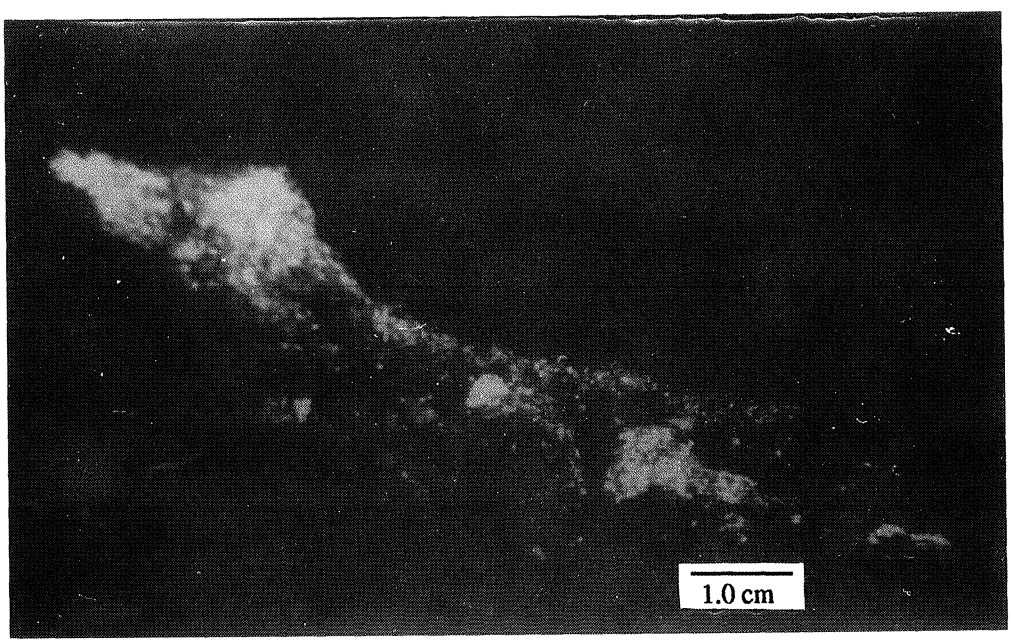

FIG. 1. In situ photograph of marine snow aggregates from the Northern Adriatic Sea; picture taken at 12 m depth on 5 June 1991; largest dimension of marine snow shown here, 10 cm.

nature (21). They consist of a mucopolysaccharide matrix entrapping discrete living and/or dead particles (25). Direct observations by submersibles or scuba diving are usually required to sample and study them, as the sampling devices designed for routine sampling in oceanography create turbulence and destroy these delicate structures (21). Nevertheless, several studies demonstrate that amorphous aggregates are ubiquitous components in the ocean and describe their characteristic features, both in surface waters and in the mesopelagic zone (3).

In neritic environments, the benthos may contribute significantly to the particle abundance. Coral reefs release large amounts of mucus (31) and macrophytes may contribute to the load of suspended particles too. The resuspension of sedimented material can also create macroscopic particles both in neritic and deep-sea environments. Sources of marine snow in the open ocean include discarded appendicularian houses (1), mucus sloughed off by ctenophores (12), fecal material of some tunicates (40) and the mucous feeding structures of pteropods (20); besides this animal-derived marine snow, phytoplankton and other microorganisms may contribute to marine snow formation, although this aspect has not received adequate attention yet.

The flocculent amorphous aggregates of phytoplanktonic origin may develop from two different processes: (i) senescent diatom cells become sticky, aggregate and eventually sink rapidly, and (ii) coagulation of colloidal organic matter released by phytoplankton forms transparent exopolysaccharide particles (TEP) in the micrometre size range, which eventually aggregate further to form marine snow if the turbulent conditions are favourable (Fig. 2).

The first process of marine snow formation by phytoplankton requires a phytoplankton bloom and low grazing pressure by zooplankton. Such phytoplankton blooms, without an effective top-down control, are typical of temperate coastal systems, where nutrients are regenerated during fall and winter, and there are episodic inputs of nutrients due to precipitation or river effluents. These phytoplankton blooms are concluded due to nutrient depletion and result in massive flocculation and aggregate formation. Flocculation of senescent phytoplankton cells is enhanced due to the release of a sticky halo of polysaccharides surrounding the outer cell wall (43). This formation of marine snow by senescent phytoplankton has been described for the north of the Adriatic Sea, where phytoplankton blooms are followed by a succession of various types of marine snow. Initially small flocs (diameter up to 0.3 cm) are formed; if the turbulent conditions of the water column are appropriate, these flocs aggregate further becoming stringers which sediment to the bottom, and if the water column is well-mixed (25) or if it is already stratified, the succession of marine snow further proceeds forming larger aggregates (see Fig. 1) and eventually cloud-type marine snow, the largest kind of marine snow with more than 3 meters in diameter (37).

The second process requires the release of a high amount of colloidal organic matter. As it has been pointed out by Fogg (17), phosphorous depleted primary production could be an important factor—if not the most important—for the release of excessive amounts of colloidal matter, which may coagulate under specific turbulent conditions. Fogg (17) termed this situation of high release rates of colloidal matter during periods of nutrient limitations "overflow reaction of phytoplankton". During a cyanobacterial bloom in the North Adriatic in June 1991, Kaltenböck and Herndl (32) found that these organisms released ca. 80% of the photosynthetically fixed carbon. In addition, the release of organic carbon from phytoplankton is also stimulated by high temperature and intense solar irradiation (55).

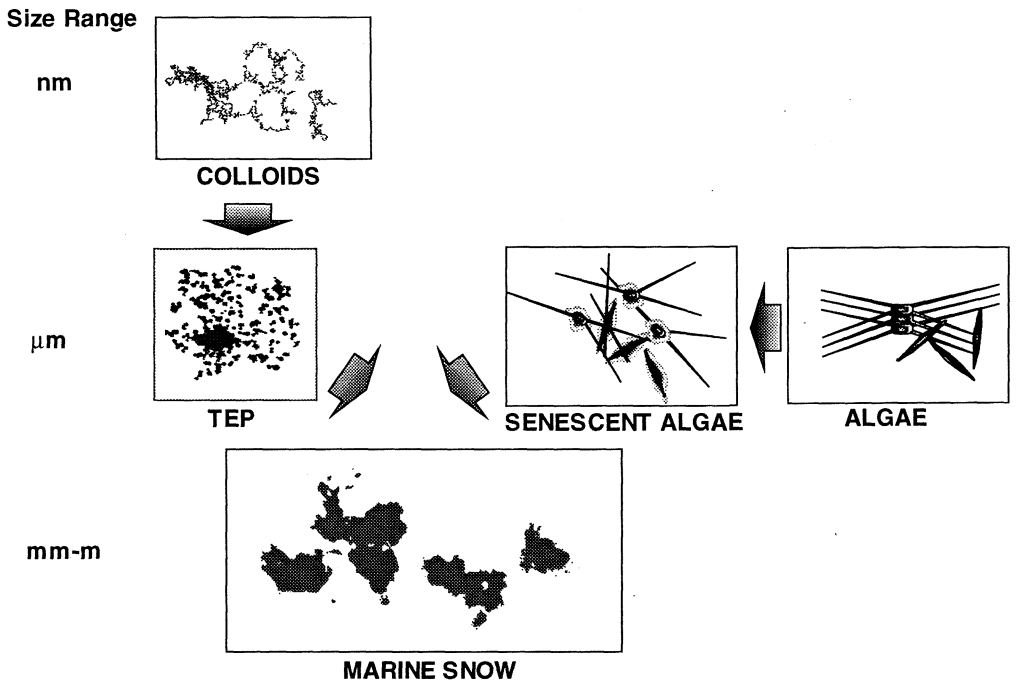

FIG. 2. Proposed pathway of marine snow formation. (i) Coagulation of colloids released by microorganisms leading to transparent exopolymer particles (TEP), which are brought together by differential settling to form marine snow. (ii) Senescent algae surrounded by a sticky halo of colloidal fibrils facilitating clustering. The subsequent microaggregates brought together by differential settling to form marine snow. The drawing size of the different objects does not reflect real relation in size.

Organisms inhabiting particles

Aggregations of organic matter are colonized by a wide variety of organisms, including phytoplankton cells, cyanobacteria, heterotrophic bacteria, protists and even metazoans.

Regarding the phototrophic component of amorphous aggregates, Revelante and Gilmartin (42) studied the species composition of the phytoplankton in marine snow of the North Adriatic Sea during the summer of 1989. They found a predominance of diatom species: *Nitzschia longissima*, *N. closterium*, *Rhizosolenia gracillima* and *R. fragilissima*, and concluded that the species composition inside the aggregates reflects that of the ambient water, and that random attachment of free-living phytoplankton cells to the sticky matrix is the main source of the phytoplankton component in aggregates. In the summer of 1986, coccolithophorids were abundant in marine snow of North Adriatic waters, while in 1987 diatoms of genus *Chaetoceros* became more abundant. On the other hand, in the summer of 1991 the most abundant phototrophs were cyanobacteria (32). The contribution of the primary production associated with aggregates to the total primary production of the water column depends mainly on aggregate abundance. Estimates of primary production associated to marine snow range from a few % to up to 70% for diatom-dominated marine snow (24).

The heterotrophic component in aggregates is dominated by bacteria. Bacterial abundance and biomass are two or three orders of magnitude higher in aggregates than in the surrounding water (12, 37). Considering the total number of bacteria or total bacterial carbon, however, free-living bacteria constitute the dominant fraction in the ocean (29), dominating even over phytoplankton in the oligotrophic surface layers of the ocean (19).

Particle-attached bacteria exhibit several morphological and metabolic characteristics which make them different from their free-living counterparts. Attached bacteria are usually larger (29, 51), and occasionally, their carbohydrate and amino acid uptake rates are higher than those of free-living bacteria (51). It has also been observed that several ectoenzymatic activities, including protease, phosphatase and glucosidase, are higher in aggregates than in surrounding waters (34, 47). Specific growth rates of attached bacteria and free-living bacteria, however, are generally comparable (47, 28, 51).

It was still unknown whether the morphological and metabolic differences between free-living and particle-attached bacteria are due to different genetic expression and growth of free-living bacteria temporarily associated to particles, or whether a specific microbial community, adapted to live on surfaces, develops on aggregates. Recently, DeLong et al. (14) have addressed this question. They analyzed the phylogenetic identity and diversity using amplification of rRNA genes by polymerase chain reaction (PCR), and reported fundamental differences between the free-living and the attached bacterial communities. While most of the bacteria associated to aggregates belonged to *Planctomyces, Cytophaga* and γ-proteobacteria, the dominant types of the free-living bacteria were α-proteobacteria. The most abundant free-living type bacteria was phylogenetically very similar to a group of a proteobacteria quantitatively important in the Sargasso Sea and Central North Pacific (44).

The most abundant phylogenetic types detected by DeLong et al. (14) in bacterial communities associated with aggregates fell within the *Cytophaga/Flavobacteria* group. A very distinct and presumably ancestral phenotypic characteristic of this group is the gliding motility. Moreover, many of the *Cytophaga* and related genera produce one or several exoenzymes which degrade a wide range of polymeric compounds including proteins, polysaccharides, chitin and nucleic acids. The results of DeLong et al. (14) suggest that highly specific bacterial communities may be responsible for the microbial decomposition of particles in the ocean.

Other heterotrophic microorganisms that colonize aggregates are the protists. Among them, nanoflagellates are the most abundant, although ciliates and amoebas can also be detected (46). As bacteria, flagellates exhibit high enrichment factors (abundances of 10^4–10^6 flag·ml^{-1}). There is an important change in the ratio flagellate:bacterial biomass throughout the life-span of the aggregates. While the ratio is very low (<0.1–0.3) in recently formed marine snow, aged marine snow shows ratios higher than 1, which indicates that flagellates also graze on other sources than bacteria associated to aggregates (24). While aggregates sink through the water column, the microbial community associated with the aggregates experiences rapid changes in temperature and pressure. As Turley and Carstens demonstrated (50), some flagellate species colonize the descending aggregates but stay on them only in a very narrow depth range; if pressure conditions become unfavourable for them, they leave the aggregate again and switch to a free-living mode. It is therefore obvious, that microbial successions on aggregates during their sinking have important implications on the decomposition of the aggregates in the water column and at the sediment–water interface.

Finally, the zooplankton can also use both, the mucous matrix and the living components, like phytoplankton or bacteria, of marine snow. High abundances of polychaete larvae (*Nectochaeta*) have been found in marine snow reworking its mucoid matrix (11).

Biogeochemical implications of particle flux towards the sea floor

Sinking particles are responsible for a great part of the transport of matter and energy from the euphotic layer to the sea floor. Therefore, detailed knowledge of the magnitude of the particle flux towards the deep ocean is essential if we want to understand the carbon flux and the role of the deep ocean in the global carbon budget. In current models on the role of the ocean in carbon recycling, the sea floor represents the most important reservoir of DOC (15).

The primary production of phytoplankton is the fundamental source of these particles, either as phytoplankton biomass or transformed into rapidly sinking aggregates and zooplankton feces (4). As the sinking particles have viable metabolically active microorganisms (12, 16, 46), it is reasonable to assume that particles undergo microbial degradation during their descent through the water column. This, together with the potential grazing by zooplankton, leads to a characteristic decrease with depth of the flux of particles (33). This decrease of the vertical flux of POM in the open ocean is not linear, as more than 75% of the net loss of POM occurs within the upper 500 m of the water column (36).

Despite the importance of all these transfer processes of POM for the metabolism of the sea floor, the quantification of the amount of POM sinking to the sea floor is still questionable, due to uncertainties concerning the efficiency of sediment traps (21). Among them, resuspension and lateral advection of particles collected in the sediment traps and changes in the hydrology in the vicinity of the opening of the traps (21) are considered as the main problems.

In order to determine whether microbial (particularly bacterial) decomposition is responsible for the observed distribution of POM below the euphotic layer, Karl et al. (33) determined the ATP content of sinking POM (in situ ATP), as well as the ratio of living particulate carbon:total particulate carbon throughout the water column. The depth-dependent decrease in both, ATP of POM and the living:total carbon ratio, indicated that the sinking particles were generally poor habitats for bacterial growth, making it unlikely that the observed decline in POM was due to remineralization by POM-attached bacteria (33). This conclusion is consistent with the direct measurements of heterotrophic microbial activity from crustacean-derived fecal pellets and from macroscopic organic aggregates, as well as with the long turnover times for POC collected in sediment traps (1500 days) (16).

Karl et al. (33) proposed two alternative processes to explain the net loss of POM as particles sink through the water column. First, the abiotic fragmentation of the sinking particles into smaller non-sinking particles, and finally their complete solubilization. Second, the particle ingestion by zooplankton and micronecton in the mesopelagic zone. In the first process, once the organic matter becomes dissociated from the quickly sinking particles, the microbial community of the upper mesopelagic zone (150–500 m) would be responsible for the oxidation of POM and DOM, and thereby converting organic into inorganic carbon and regenerating nitrate and phosphate (33). Therefore, the site of active microbial growth and decomposition is shifted from bacteria attached to rapidly sinking particles to the microbial communities, which are either free-living or attached to non-sinking POM.

Cho and Azam (13) reported that free-living bacteria were more important decomposers of particles than zooplankton. They suggested that bacterial growth on large sinking particles causes a large-scale generation of non-sinking, small particles (0.3–0.6 μm) in the mesopelagic zone. More than 95% of mesopelagic bacteria were free-living and responsible for almost all the bacterial production observed in those deep layers.

The conclusions published by Karl et al. (33) and Cho and Azam (13) require that a large amount of the sinking POM is transformed into DOM and therefore is made accessible for free-living bacteria. Which mechanisms could explain the large-scale POM solubilization? Attached bacteria could compete with particle-ingesting zooplankton by quickly solubilizing the POM through a hyper-production of hydrolases (5). Bacteria in aggregates have high activities per cell; this characteristic pattern has been shown for a variety of ectoenzymes such as protease, glucosidase, chitinase and lysozyme (47). By using fluorescent substrate analogs, Karner and Herndl (34) found that, independently of the enzyme tested (α- and β-glucosidase, leucine-aminopeptidase), the specific activity per cell of these hydrolases was from 4 to 20-fold higher in the aggregates than in the surrounding water. These high specific activities, together with the high abundances of bacteria attached to aggregates could potentially lead to rapid solubilization of sinking particles. These results agree with the characteristics of the dominant bacterial groups typically found in particles (14).

Attached bacteria usually do not divide at higher rates than free-living bacteria, therefore it seems unlikely that they are important consumers of particle-derived organic matter. Less than 5% of total thymidine incorporation was found to be due to particle-attached bacteria (13). In this way, the low carbon demand of bacteria attached to large particles leads to the release of a large fraction of hydrolyzed organic matter into the ambient water, where it can be utilized by free-living bacteria. Smith et al. (47) estimated that more than 97% of the hydrolyzed combined amino acids are released into the water.

Sinking particles loose nitrogen faster than carbon, so their C:N ratio increases with depth (36), but the mechanism is unclear. According to Smith et al. (47), particulate combined amino acids (PCAA) in aggregates comprise about 15% of the total nitrogen content of the aggregate; this PCAA pool is hydrolyzed faster than the carbon-rich pools such as polysaccharides. This agrees with results from sediment trap studies, which indicate that the concentration of amino acids decreases quickly with depth. Smith et al. (47) observed from 10 to 1000-fold higher protease activity than α- and β-glucosidase activity in aggregates.

The coupling organic matter–bacteria depends on the ability of the resident microbial assemblage to utilize that organic matter. It has been assumed for a long time that in the ocean there is a small pool of DOM which turns over rapidly and supplies most of carbon needed for bacterial growth, while the remaining 90–98% of DOM is old and refractory. Recently, however, the distinction between utilizable and non-utilizable DOM has been shown to be more difficult than hitherto assumed, since Keil and Kirchman (35) showed that there is a considerable interchange between these two pools. These authors (35) observed that when peptides were added to seawater, their degradation rates declined after a short period (hours to days), probably due to condensation with carbohydrates. This agrees with previous results showing that only a fraction of amino acids and peptides in seawater is available to bacterial attack, particularly in deep waters (8).

The biogenic particles are probably dominated by a complex mixture of structural biopolymers (proteins, mucopolysaccharides, chitin and cell wall polymers); their hydrolysis generates high local

concentrations of a chemically diverse mixture of dissolved polymers, oligomers and monomers inside the aggregate matrix. The hydrolysis of structural suprapolymers may produce some slowly degradable DOM. In addition, the microenvironment of the aggregate may support condensation reactions, such as protein glucosylation, to produce that slowly degradable DOM. In fact, an uncoupled solubilization is consistent with the production of slowly degradable DOM in aggregates (47).

Novel types of particles

Over the last years, novel types of suspended particles have been studied, which are invisible to divers. These particles are several orders of magnitude more abundant than the macroscopic amorphous aggregates mentioned above. Among these novel types, probably the most important three groups are described below:

First group. Isao et al. (30) discovered that surface seawater contains about 10^7 organic particles ranging from 0.3 to 1.3 μm in size per ml. More than 95% of the particles were non-living and most of these particles were found in the upper layers of the ocean (50 m). These particles appeared rather fragile and flexible, pointing to a highly hydrated state. Isao et al. (30) presented evidence that it was extremely

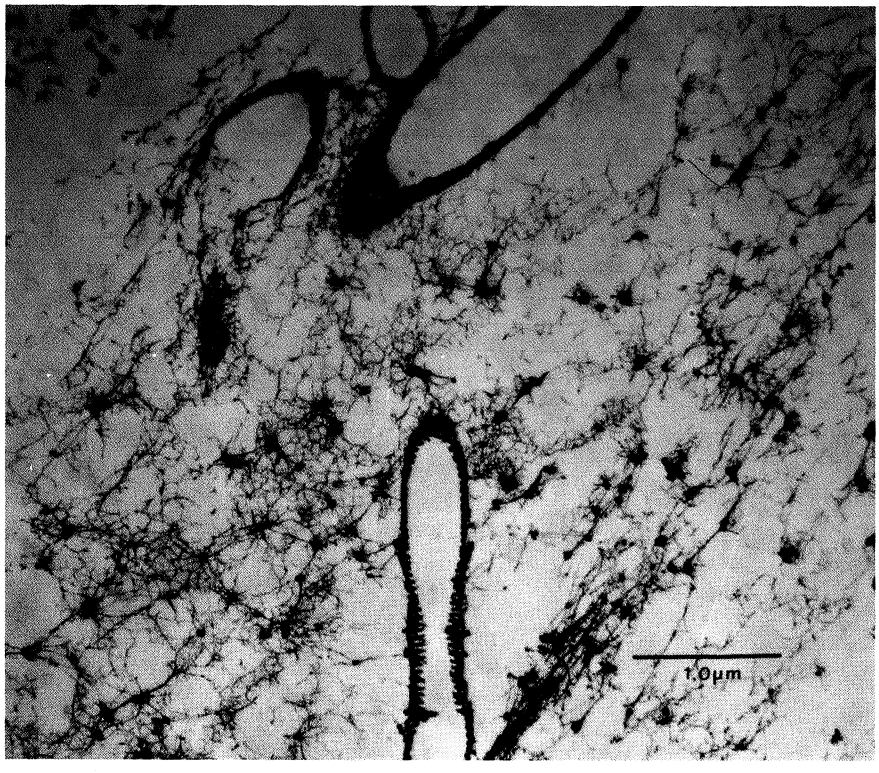

FIG. 3. TEM thin section showing the colloidal matrix of marine snow with organisms (diatoms) embedded.

unlikely that those particles were originated from fragmentation of large aggregates. Furthermore, these authors demonstrated that the production of these small particles requires the presence of bacteria and flagellates (0.6–5 μm size range) in the water; thus, these particles originate probably from flagellate grazing on bacteria. It has been estimated that more than 10% of the "dissolved" organic matter in the surface layers of the open ocean (average concentrations of DOC between 1.0–3.0 mgC l^{-1} [48]) may be constituted of these submicrometre particles (45). Hence, when the chemical and biological processes and optical aspects of the euphotic zone are considered, these small particles should be taken into account, as they are very abundant and provide a large solid-liquid interface (0.2–0.5 cm^2 ml^{-1}) (30). Wells and Goldberg (52) detected colloidal-sized particles (<120 nm) in high numbers (10^7 to 10^{10} particles ml^{-1}) in seawater and pointed out their potential importance as adsorption sites for charged molecules (Fig. 3).

Second group. Recently, another kind of particles has been described in surface waters: the already mentioned transparent exopolymer particles (TEP) which can be stained with Alcian blue, a specific stain for acidic mucopolysaccharides (Fig. 4) (2). TEPs are highly variable in size (from 3 to >100 μm) and their shape varies from sheets, stringers and bundles to filaments. Although they are mainly of phytoplanktonic origin, other microorganisms may also release colloidal fibers leading to the formation of TEPs. Heterotrophic bacteria, for instance, transform low molecular weight material into high molecular weight compounds. Approximately 6% of the incorporated substrates are transformed into refractory high molecular weight compounds and released to the surrounding water (23).

Third group. It has been demonstrated (10) that marine bacteria may respond quickly to inputs of fresh phytodetritus. Phytodetrital particles are rapidly colonized and the attached bacteria give rise to

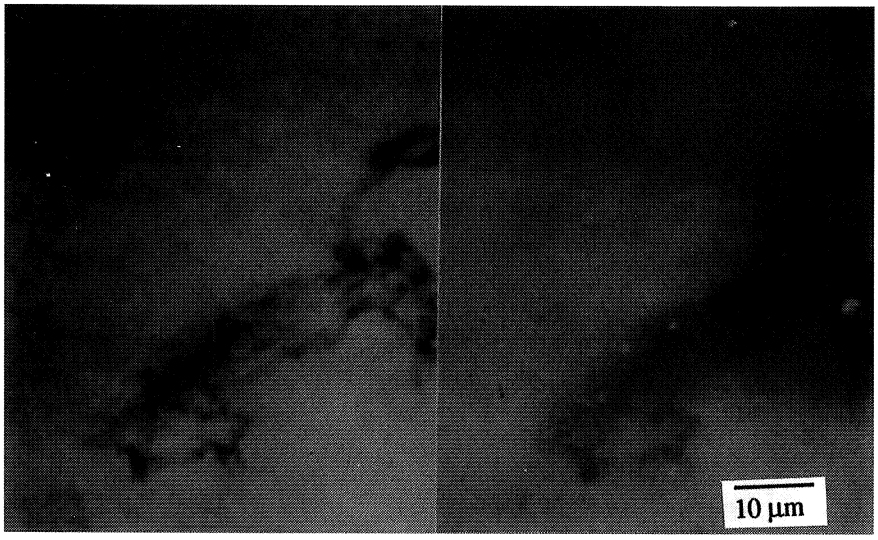

FIG. 4. Transparent exopolymer particle double stained with Alcian blue and acridine orange viewed under the transmission microscope (left) and under the epifluorescence microscope (right) to visualize attached bacteria.

dense communities of relatively large bacteria. These invisible phytodetritus frequently form bacterial aggregates in later stages when incubated in experimental flasks (pers. observ.). Azam et al. (7) propose the hypothesis that these invisible particles, so-called "source" particles, are suitable habitats for bacterial growth.

Attachment to particles: advantage or disadvantage for bacteria?

For a long time, attachment of bacteria to particles has been considered to be advantageous for bacteria. Even before the development of adequate methodology, particles were regarded as zones of high bacterial growth (56). However, since we have reliable measurement techniques available, sinking particles have been shown not to sustain elevated bacterial growth rates (28, 33, 37). Conversion factors of thymidine incorporation into bacterial production rates are also similar (28) for attached and free-living bacteria. However, biomass, inorganic nutrients, dissolved amino acids and monomeric sugars are at least two orders of magnitude higher in the particles than in the ambient water (32, 37).

Several reasons might be responsible for the observed patterns in bacterial growth rates. The high abundance of bacteria accumulating in particles might cause oxygen depletion which, in turn, inhibits growth of aerobic bacteria. Low oxygen and anoxic environments are advantageous for facultative or obligate anaerobic bacteria, as denitrifying or sulfur-reducing bacteria, which exist in anoxic zones inside the aggregates (41). Changes in pH due to acid production by fermentative microorganisms may also inhibit bacterial growth adapted to the slightly alkaline conditions in seawater.

Colloidal matter efficiently adsorbs dissolved trace metals such as Cu, Ni, or Zn, which become part of the aggregate (22); this accumulation of metals in aggregates could significantly retard bacterial metabolism. In addition, those metals may be released and become toxic if the aggregate becomes acidic (see above), or if the organic bonds binding the metals to the cell surface are enzymatically hydrolyzed.

Hoppe (27) reported enhanced extracellular enzymatic activity in particle-attached bacteria. Karner and Herndl (34) and Smith et al. (47) also detected higher extracellular enzymatic activities per cell in bacteria associated to small aggregates than in free-living bacteria. Müller-Niklas et al. (37), however, did not observe any differences in the extracellular enzymatic activity per cell. These contradictory results may be due, among other causes, to diffusion problems. Diffusion could be limited in the particles, creating high gradients of potentially available electron acceptors inside the particle.

Caron et al. (12) found that attached bacteria were not as efficiently grazed by flagellates as free-living bacteria. It might be possible, however, that flagellates associated with marine snow directly consume macromolecules, as has been shown recently for free-living flagellates (49).

Finally, as indicated by Azam et al. (7), the adaptive value of bacterial attachment to aggregates cannot be evaluated without considering also the interactions between bacteria and the surrounding organic matter. Aggregates may at least occasionally turn into hostile environments for bacteria, but when were originally colonized as "source" particles, they might have been sites of active bacterial growth. Bacteria might benefit from other aspects of attachment as well such as enhanced dispersal when ingested by particle-feeding metazoans and/or growing in their feces. Furthermore, high bacterial abundances inside aggregates may facilitate lateral gene transfer among bacteria.

Perspectives

In summary, while substantial progress has been achieved in recent years in the understanding of the ecological role of particles in the ocean, there are still considerable gaps in our knowledge about the formation and degradation of various types of aggregates. We are just at the beginning to understand the complex interactions, on the molecular level, between different chemical components of the DOM pool and the microbes. Resolving these interactions and elucitating the nanoscale patchiness inside aggregates demands novel techniques but should allow new insights into the organization of microbial communities, similar to our current understanding of the interactions between the macroscopic members of ecosystems. It is very likely that findings altering the concept of our knowledge of aquatic microbial ecology, similar to those of Pomeroy (39) and Azam et al. (6), are still ahead of us.

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Analysis of bacterial genomes by pulsed field gel electrophoresis

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Summary

The present article reviews the main achievements reached in bacterial genetics through the application of pulsed field gel electrophoresis. In particular, it summarizes the main subjects where the use of this technique has significant relevance for the analysis of bacterial genomes. With this purpose, it sums up some of the most relevant examples which illustrate the applicability of this technique in bacterial taxonomy, in epidemiological work and in the study of bacterial genome organization (including the assessment of the number of genomic elements and the size and topology of all of them). This technique, which makes it possible to make genomic comparisons in an easy, fast and repetitive way, has been demonstrated to be especially useful for those bacteria where the application of classical genetic techniques has not been possible.

Key words: pulsed field gel electrophoresis, chromosome restriction patterns, megaplasmids, chromosome size, bacterial taxonomy

Resumen

El presente trabajo revisa los principales adelantos obtenidos en el campo de la genética bacteriana mediante la aplicación de la electroforesis de campo pulsado. Concretamente, resume los ejemplos más

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importantes donde el uso de esta técnica tiene una importancia significativa para el análisis de los genomas bacterianos. El artículo ilustra la aplicabilidad de la técnica a la epidemiología, a la taxonomía bacteriana y en el estudio de la organización del genoma bacteriano (incluyendo la detección del número de elementos genómicos y del tamaño y topología de todos ellos). Se ha demostrado que esta técnica, que permite hacer comparaciones genómicas de una manera sencilla, rápida y repetitiva, es de especial utilidad en el estudio de las bacterias en las que no ha sido posible aplicar técnicas genéticas clásicas.

Introduction

A few years ago, the development of genomic studies in the prokaryotic world was dependent on the existence of the only three genetic techniques available: transformation, conjugation and transduction. These methods were only available for some extensively studied species, such as *Escherichia coli*, *Salmonella typhimurium* or *Bacillus subtilis* (87). However, the last decade has seen a revolution in genome/chromosome (genophore) analyses. The introduction of pulsed field gel electrophoresis (PFGE) in its various forms has made it possible to study the whole genome organization of a wide range of microorganisms. PFGE was first described by Schwartz and Cantor in 1984 (73), and remains the only technique capable of physically separating DNA molecules ranging from 50 to 12,000 kb (Fig. 1). This ability to resolve DNA molecules several orders of magnitude larger than those obtained by conventional electrophoresis has had a major impact on the study of genetics.

This article summarizes the fundamental achievements reached in bacterial genetics through the application of PFGE. It focuses on the main subjects where the use of this technique, combined with other methodologies, has significant relevance for: (i) the comparative analysis of chromosomic restriction patterns; (ii) the construction of genetic and physical chromosome maps; (iii) the assessment of chromosome size and topology; and (iv) the analysis of high molecular weight extrachromosomal elements.

Chromosomic restriction patterns: comparative analysis by pulsed field gel electrophoresis

The study of whole bacterial genomes by analyzing DNA restriction patterns was first carried out by using restriction endonucleases which cut genomes into an indeterminate number of fragments. These fragments are then resolved by conventional agarose (19) or polyacrilamide electrophoresis (17, 27) separations. Conventional DNA preparation involved total DNA extraction methods (70) and, consequently, the patterns obtained corresponded to a part of the chromosomic and plasmidic DNA, which led to scanty reproducible results. For that reason such profiles were not a reliable fingerprint of each strain analyzed. The combination of PFGE with restriction enzymes that cut bacterial chromosomes infrequently has now made it possible to obtain chromosomic restriction patterns that are a trustworthy fingerprint of each microorganism. The comparative analysis of these patterns is a really useful tool in the identification of bacterial strains. This kind of approach has been widely

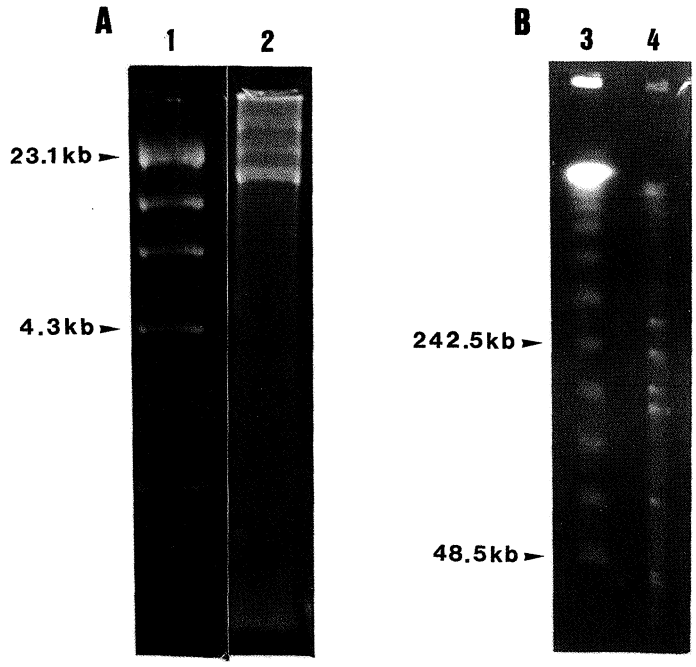

FIG. 1. Comparison of the different sizes of DNA that can be resolved by conventional agarose gel electrophoresis (A), and pulsed field gel electrophoresis (B). (A) Non digested plasmidic DNA from *Chlorobium limicola* DSM 249 (lane 2). Molecular weight marker was Lambda DNA digested with *Hind*III (lane 1). (B) Chromosomic DNA of *C. limicola* DSM 249 digested with *Swa*I (lane 4). Molecular weight marker was Lambda concatemers (lane 3).

used in an extensive number of taxonomic and epidemiological studies. A good example is the study developed in the genus *Pseudomonas* by Grothues and Tümmler (29). In this study 235 strains, belonging to 35 species, were analyzed and a method was developed to recognize chromosomic restriction patterns. These patterns could be used for the identification and classification of pseudomonads at the genus, species and subspecies level. PFGE has also been applied in different taxonomic studies of the genus *Staphylococcus* (77). George and Kloos (26) examined the chromosomal polymorphisms existing within and between *S. epidermidis*, *S. caprae*, *S. capitis* subsp. *capitis*, and *S. capitis* subsp. *ureolyticus*. The results indicated that DNA fragment patterns are unique to each species and subspecies and that they represent a reasonably stable trait of the chromosome.

In the case of epidemiological studies, the analysis of macrorestriction patterns has become an ordinary technique of great value. Many examples can be described to illustrate this kind of application. For example, in the species *Campylobacter jejuni*, the etiologic agent of acute bacterial gastroenteritis, Suzuki et al. (82, 83) have demonstrated that the comparative analysis of chromosomic restriction patterns is a highly effective method to subclassify strains exhibiting the same serotype PFGE was recently used successfully in epidemiological studies of the fish pathogen *Vibrio anguillarum*. A total of 75 serovar O1 strains were studied with regard to their plasmid contents, ribotypes, and PFGE patterns.

From among these three techniques, PFGE had the highest discriminatory power, demonstrating 35 different profiles. The results indicated that strains from different geographic areas belong to different clonal lineages (75). Finally, the studies developed by Khattak and Matthews (34) to differentiate the three medically important *Bordetella* species, *B. pertussis*, *B. parapertussis* and *B. bronchiseptica*, can also be mentioned. The data showed that the three species produce species-specific macrorestriction profiles and that there was some variation between different isolates of the same species.

In our laboratory, this methodology has been applied to check the current classification of several green and purple sulfur bacteria and for the identification of newly isolated strains. In the genus *Chromatium* the comparison of *Ase*I and *Spe*I chromosomic restriction patterns showed very slight differences between *C. vinosum* and *C. minutissimum*, while *C. gracile* presented quite different patterns (25). In the case of green sulfur bacteria, comparative analysis of PFGE profiles has been extremely useful in the identification of *Chlorobium limicola* transformants that could not be phenotypically distinguished from plasmid donor and acceptor strains (49).

Although many other examples could be shown to illustrate the applicability of restriction fragment profiles analyzed by PFGE in taxonomic and epidemiological studies, the approaches explained above are good examples of such usefulness.

Phylogenetic analysis

According to some studies, macrorestriction patterns are a highly discriminatory technique that make it feasible to determine phylogenetic relationships between isolated strains from related species (66, 74). Restriction patterns are an image of the genomic organization and indicate the genetic relationship between strains. Nei and Li developed (53) a mathematical model which analyzed the evolution of restriction sites in mitochondrial DNA. When this model has been applied to the study of prokaryotes phylogeny (66), it has been possible to relate the genetic diversity to the evolutionary distance between strains. Different authors have elaborated phylogenetic trees with the information obtained from the comparative analysis of chromosomic macrorestriction patterns (40, 52). The comparison developed by Rodrigo et al. (66) in the genus *Thermus* between evolutionary distances calculated following the model of Nei and Li (53) and distances obtained by comparison of rRNA 16S sequences (71), showed that phylogenetic trees obtained using both methodologies cluster different strains in a similar way.

Physical and genetic maps and chromosome topology

Physical maps specify the distances between landmarks along a chromosome. The most important landmarks in physical mapping are the cleavage sites resulting from the treatment with restriction endonucleases. By combining restriction patterns with hybridization techniques, physical and genetic maps of more than 40 bacteria have been determined. The compilation of such kinds of maps will make it possible to elaborate a data base of chromosome maps that will help to establish the genetic

relationships between and within different groups of bacteria. Table 1 shows a list of some of the bacterial species that have been mapped. Construction of genetic maps makes it possible to analyze the organization of genes: presence of chromosomic regions where essential genes are accumulated, which could make possible a coordinate regulation of all of them, or aleatory distribution of such genes (31, 60). Comparison of chromosomic maps from different strains of a bacterial species or from different related species enables us to study the mechanisms that have been involved in the differentiation of species and genera and to clarify phylogenetic relationships. Thus, comparative analysis of the chromosome maps from different *E. coli* strains (57) showed the genetic steps that explain deletions, insertion sequences (IS), mutations that originate restriction sites, etc. Genetic maps from *E. coli* and *Salmonella typhimurium* have also been compared (64, 65). The results of this study indicated that both species have a very similar chromosomic structure showing the same gene distribution along the chromosome.

PFGE has also permitted the analysis of archaeobacterial chromosomes. Yamigishi and Oshima (89) described how the chromosomal DNA of the sulfur dependent archaeobacterium *Sulfolobus acidocaldarius* is circular. In another group of archaeobacteria, the halobacteria, López et al. (45) constructed the physical and a partial genetic map of the circular chromosome of *Halobacterium mediterranei*. The extensive analysis of more archaeobacteria will bring knowledge of great interest for the comparison between Archaea and Bacteria regarding their genome organization.

Furthermore, this methodology has made it possible to find exceptions to some classical rules of bacterial genetics, that derived from the generalization of the knowledge accumulated about the genetics of *Escherichia coli* and others. Thus, PFGE has permitted us to determine the first possible exception to the rule "one single circular chromosome per bacterium". *Rhodobacter sphaeroides* may have two distinct circular chromosomes. Digestion of the chromosomal DNA from this bacterium with several rare cutting enzymes and ordering the large restriction fragments resulted in disposition of the

TABLE 1. Some bacteria whose chromosome has been mapped

Microorganism	Reference
Bacillus cereus	37
Chromatium vinosum	24
Escherichia coli	36
Haloferax mediterranei	45
Lactococcus lactis	85
Mycoplasma genitalium	15, 59
Mycoplasma mobile	6
Mycoplasma mycoides	62
Pseudomonas aeruginosa PAO	68
Rhodobacter sphaeroides	80, 81
Salmonella typhimurium	55
Treponema denticola	48
Treponema pallidum	86

fragments in two independent circles, 3046 kb and 915 kb, respectively. Hybridization experiments indicated that the latter structure has two *rrn* loci and some genes which codify for metabolic enzymes. These findings led Suwanto and Kaplan (81) to raise the possibility that *Rhodobacter sphaeroides* contains two chromosomes. However, other authors (38) deny this hypothesis and support the idea that the smaller circular structure could be a megaplasmid. They base their criticism on the theory that plasmids, by definition,are autonomous and nonessential. However, in the case of *R. sphaeroides*, the small chromosome, designated chromosome II (CII), is present in 1:1 stoichiometry with the larger chromosome (CI) and is as stably maintained as the larger replicon; furthermore, attempts to cure either CI or CII have been unsuccessful (13). Whether the structures should both be considered as chromosomes depends on whether they are both required for normal growth.

Additional chromosomes have also been proposed in the species *Brucella melitensis* (50), *Leptospira interrogans* (93) and *Pseudomonas cepacia* (12). On the other hand, PFGE has made it possible to demonstrate that the spirochete *Borrelia burgdorferi* has a linear chromosome in addition to plasmids which are linear and which have covalently closed ends (21). Linear chromosomes have been also described in *Rhodococcus fascians* (16) and in some species of the genus *Streptomyces* (44). Finally, the case of *Agrobacterium tumefaciens* C58 should be remarked because, to date, it is the only known bacterium which has been clearly shown to have two chromosomes, one linear and one circular. The chromosomal nature of the two replicons was suggested because a 16S rRNA probe and gene encoding functions essential to the metabolism of the organism were found to hybridize with both of them (1).

Chromosome size

A succession of techniques have been used to estimate the sizes of bacterial chromosomes. The general temporal order of the principal techniques was: (i) colorimetry, (ii) kinetics of renaturation, (iii) two-dimensional gel electrophoresis of restriction fragments, (iv) summation of the sizes of restriction fragments which produce a unique and complete physical map of the chromosome, and (v) PFGE of macrofragments produced by rare cutting restriction enzymes (38). Depending on the technique used, the computed size of a chromosome varies in some megabases. For example, estimations of the size of the *Myxococcus* chromosome based on autoradiography and colorimetry range from 5,700 to 12,700 kb; the PFGE measurement places the size at 9,450 kb (38). From among all these methodologies, PFGE is regarded as the most precise of the techniques used to estimate sizes of chromosomes. Sizes estimated by the sum of linear macrorestriction fragments detected by PFGE indicate that bacterial chromosomes are commonly from 1 to 9 Mb.

From the known data, it can be inferred that the variation of the genome size of the different strains of a bacterial species is not higher than 20%, although within some species the variation observed is larger. For example, while in the genus *Streptomyces* the deletion mutants often contain amplifications to preserve a characteristic genome size (18), in the species *Bacillus subtilis* the chromosome size can change by 30% (2).

In the case of prokaryotic genomes, the relation between size and quantity of genetic information is still not clear. As an illustrative example can be used the chromosome of *E. coli*, which is about

4,700 kb (36). To date, over 1,000 genes have been mapped and, judging from the size of the genome, it can be inferred that more than 2,000 genes still remain unidentified. If we accept that this relation is nearly constant in the prokaryotic world, the decreasing chromosome size observed in Table 2 must be interpreted as a different amount of total genetic information existing between the different bacterial species. Perhaps this feature could be explained on the basis of a different life cycle or a different metabolic or structural complexity (78). Thus, *Mixococcus xantus* presents one of the largest chromosomes observed in a prokaryote (9,500 kb), and possibly the most complex life cycle in the prokaryotic kingdom. On the other hand, the chromosome of different *Mycoplasma* species, which lack cell wall and are metabolically simple, is ten times smaller. According to this idea, different genera of strictly parasitic bacteria, such as *Rickettsia* and *Chlamydia*, or facultative parasitic bacteria, such as *Borrelia*, have small chromosomes (10). However, in most bacteria the detected variations can not be explained as a consequence of the level of complexity of the life cycle or as a "reflection" of an extremely simple structure. In these cases, the differences could be explained by different metabolic capacities. An example is the genus *Pseudomonas*, in which the chromosome size ranges from 2800 kb in *P. corrugata* to 6800 kb in *P. glathei* (30). While the latter presents a versatile metabolism and is able to use 68 compounds as unique carbon source, the former is metabolically simple (72).

Table 2 summarizes the length (in kb) and structure (circular or linear) of the chromosome of several bacterial species recently analyzed. The reference of the main works are also given.

High molecular weight extrachromosomal elements

PFGE has shown that most analyzed bacteria present high molecular weight extrachromosomal elements in contrast to the idea accepted until now that megaplasmids were rarely occurring genetic elements (8, 28, 46). Some bacterial genera, e.g., *Pseudomonas* and *Rhizobium*, contain extremely large plasmids that specify traits which are species-specific characteristics. Some examples of this kind of extrachromosomal elements are described below. Many of the bacterial genes involved in nodulation (*nod*) and nitrogen fixation (*nif*) are dispersed over the 500-kilobase plasmid pNGR234a of the broad host-range *Rhizobium* species NGR234 (58). In the genus *Pseudomonas* the large plasmids belonging to the IncP2 are of special interest. Some of them carry genes for antibiotic resistance; others, degradative genes or genes for heavy metal resistance (8). In other studies, PFGE made it possible to detect giant linear plasmids in *Streptomyces coelicolor* (35), in *Nocardia opaca* (32), as described above. The linearities of these elements constitute more data to add to the list of linear genetic elements in bacteria. Another interesting trait of bacterial megaplasmids has recently been described. The megaplasmids (up to more than 500 kb) from the halobacteria *Halobacterium mediterranei* are negatively supercoiled, based on their electrophoretic properties. Although the study focused on *H. mediterranei* megaplasmids, different halobacterial species from the genera *Halobacterium*, *Haloarcula* and *Haloferax* carry megaplasmids with similar behavior. It has been suggested that this finding indicates that the occurrence of negative supercoiling megaplasmids may be a general feature in this archaeal branch (46).

TABLE 2. Bacterial chromosome sizes

Microorganism	Size† (kb)	Reference
Myxococcus xanthus	9543	11
Streptomyces ambofaciens	8208	41
Streptomyces livindans	8000*	44
Streptomyces ambofaciens	7793	41
Pseudomonas glathei	6800	30
Pseudomonas cepacia	3400 + 2500 + 900	12
Streptomyces ambofaciens	6540	41
Anabaena sp.	6400	4
Bacillus cereus	5700	37
Pseudomonas aeruginosa	5900	68
Agrobacterium tumefaciens	3000 + 2100*	1
Leptospira biflexa	5040	5
Leptospira interrogans	5033	5
Leptospira interrogans	4500 + 350	93
Escherichia coli	4703	76
Yersinia ruckeri	4592	67
Leptospiras interrogans	4400	92
Yersinia pestis	4102	47
Rhodococcus fascians	4000	16
Rhodobacter sphaeroides	3046 + 900	80
Legionella pneumophila	3900	7
Thiobacillus cuprinus	3700	3
Chromatium vinosum	3674	24
Brucella melitensis	2100 + 1150	50
Sulfolobus acidocalcarius	3100	89
Treponema denticola	3035	48
Haloferax mediterranei	2900	45
Staphilococcus aureus	2822	56, 61
Streptococcus mutans	2819	84
Pseudomonas corrugata	2800	30
Porochlamydia duronomi	2650	23
Lactococcus lactis	2580	42, 85
Propionibacterium freudenreichii	2327	63
Rickettsiella grylli	2100	23
Bifidobacterium breve	2092	9

Continued on following page

TABLE 2.—*Continued*

Microorganism	Size[†] (kb)	Reference
Haemophilus influenzae	1907	33, 43
Thermus thermophilus	1900	66
Thermococcus celer	1890	54
Spiroplasma citri	1780	91
Rickettsiella melolonthae	1720	23
Campylobacter jejuni	1700	55
Campylobacter coli	1700	90
Rickettsiella buthi	1550	23
Chlamydia trachomatis	1450	23
Chlamydia psittaci	1450	23
Mycoplasma mycoides	1240	62
Mycoplasma capricolum	1122	51
Rickettsia prowazekii	1120	20
Campylobacter fetus	1100	69
Treponema pallidum	1050	86
Mycoplasma hyopneumoniae	1011	22
Mycoplasma floculare	988.3	22
Borrelia burgdorferi	950*	21
Ureaplasma urealyticum	900	14
Mycoplasma pneumoniae	800	88
Mycoplasma mobile	784	6
Mycoplasma hominis	747	39
Mycoplasma genitalum	573	79

[†] More than one figure in the size column indicates more than one chromosome.

* Linear chromosome. Those with no asterisk seem to present circular chromosomes.

Concluding remarks

The rapid development of PFGE technology over the past ten years has led to exciting progress in our ability to study genome structure and function in microorganisms. PFGE has been shown to be of particular value for separating and determining the size, topology and number of chromosomes, for making physical and genetic maps of bacterial chromosomes, for obtaining chromosomic restriction patterns which are a fingerprint of each microorganism analyzed and for understanding the until now almost unknown behavior of megaplasmids.

Chromosome sizes estimated by PFGE and shape determined from ordered libraries of restriction fragments indicate that bacterial chromosomes are commonly circular and contain 1 to 9 Mb (38). However, PFGE has also revealed the existence of linear chromosomes and the presence of two different

chromosomes in some bacterial species, which are exceptions to a single circular chromosome (38). In a similar manner, PFGE has greatly increased our knowledge of megaplasmids. While one decade ago megaplasmids were considered to be rare genetic elements regarding to some genera of bacteria, such as *Rhizobium* and *Pseudomonas*, they have now been detected in most of the bacterial groups analyzed by PFGE.

Mapping with PFGE is currently available for virtually all microbial genomes. While construction of a physical map is an achievement in itself, the importance of that achievement will be enhanced by the use of the data made available. Chromosomic restriction patterns thus allow mutations to be readily mapped, make it possible to study the genome rearrangements, and enable us to make genetic maps. Furthermore, comparison of chromosomic maps from different bacteria has a high taxonomic value and can be used to clarify phylogenetic relationships.

Moreover, the known data indicate that comparative analysis of chromosomic macrorestriction patterns obtained by PFGE can be considered as a useful tool in epidemiological studies and for classification and identification of newly isolated strains.

Finally, we can conclude that given the labor-intensive nature of finding, characterizing and using classical systems of genetic exchange in many groups of bacteria, PFGE combined with rare cutting restriction enzymes and hybridization techniques should result in an explosion of genome data that will be of general application to microbial genetics, phylogenetic studies and applied microbiology.

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MICROBIOLOGÍA SEM **11** (1995), 337–342

Effect of cultivation conditions on glycerophosphate oxidase production by a mutant strain of *Aerococcus viridans*

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Summary

The effect of different concentrations of carbon source (lactose) and inducer (glycerol) on biomass and glycerophosphate oxidase (GPO) production by mutant strain of *Aerococcus viridans* 1509 was tested. The best combination of lactose and glycerol concentrations for good enzyme productivity was 0.5% lactose and 2.5% glycerol. Further improvement of GPO levels was achieved after scaling-up of the bioprocess and cultivation of the cells in a 3 liter laboratory bioreactor. Using 4.5×10^{-5} m^3 s^{-1} air flow rate during growth, GPO activity increased 20-times in comparison with cultivation in flasks.

Key words: glycerophosphate oxidase production, growth on glycerol, *Aerococcus viridans*, scaling-up, bioreactors

Resumen

Se ha estudiado el efecto que tienen diferentes concentraciones de la fuente de carbono (lactosa) y del inductor (glicerina) sobre la producción de biomasa y de glicerofosfato oxidasa (GPO) por el mutante *Aerococcus viridans* 1509. Se obtuvo una buena producción de la enzima en el medio que contenía 0,5% de lactosa y 2,5% de glicerol. La producción de GPO aumentó aún más al realizarse el cultivo en un bioreactor de laboratorio de 3 litros. Aplicando una aireación de $4,5 \times 10^{-5}$ m^3 s^{-1} el nivel de la actividad GPO creció 20 veces en comparación con el cultivo en matraces.

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Introduction

The enzyme L-α-glycerophosphate oxidase (glycerol-3-phosphate:O_2-oxidoreductase, E.C. 1.1.3.21) (GPO) is a flavoprotein containing flavinadenindinucleotide (FAD) as the prosthetic group. The enzyme specifically catalyzes the oxidation of L-α-glycerophosphate (GP) to dihydroxyacetonephosphate. Bacterial GPO is an intracellular enzyme and it is isolated only from some genera of lactic acid bacteria, for example *Pediococcus* (9), *Streptococcus* (2), *Aerococcus* (11), *Leuconostoc* (1), *Propionibacterium* (3). The production of GPO in *Aerococcus viridans* is inductible by glycerol (8, 11).

In bacterial cells, GPO plays a significant role in lipid metabolism, where glycerol, the product of triacyglycerol degradation, is metabolized to dihydroxyacetone phosphate through two reactions: phosphorylation, to give L-α-glycerol-3-phosphate, followed by dehydrogenation, to give dihydroxyacetone phosphate. Dehydrogenation generally can be catalyzed either by a NAD(P)-independent enzyme or by a soluble enzyme with NAD as an electron acceptor. GPO utilizes oxygen as the primary electron acceptor.

GPO is used in combination with lipases and glycerol kinase as a diagnostic agent in clinical biochemistry for the determination of triacylglycerols (5), glycerol (6), phospholipids and some enzyme activities in blood serum and other biological materials.

In this study we show the effect of different concentrations of carbon source (lactose) and an inducer of GPO (glycerol) in cultivation medium on biomass and enzyme yields in cells of a mutant of *Aerococcus viridans*, strain 1509. Optimal cultivation conditions of this strain with respect to GPO production under cultivation in a laboratory bioreactor are also documented.

Material and methods

Chemicals. D,L-α-glycerolphosphate disodium salt hexahydrate, SDS, phenol, Triton X-100, horseradish peroxidase (100 purpurogallin units mg^{-1}) were purchased from Sigma (Sant Louis, MO, USA). 4-aminoantipyrine was obtained from Loba Feinchemie Company, Austria. All other chemicals were reagent grade from Lachema Brno Company, Czech Republic.

Organism. GPO was isolated from a mutant strain of *Aerococcus viridans* DBM 1509, prepared by UV light mutagenesis as described previously (7).

Cultivation conditions. Preparations of stock cultures and inoculum were performed as indicated by Suchova et al. (7). We also used the same composition of cultivation medium (except concentrations of lactose and glycerol) and growth conditions of microbial cells cultivated in 0.5 l flasks with 100 ml of medium at 37°C and pH 7.2. Conditions of the experiments studying the effect of different concentrations and ratios of carbon source (lactose) and the inducer (glycerol) are described in Table 1.

Batch cultivation in laboratory bioreactor. To scale up the bioprocess, the cells were cultivated in a 3 liter laboratory bioreactor LH3 (Laboratory Instruments Prague, Czech Republic). Liquid medium was made up to 2.2 liter and its composition corresponded to optimal composition obtained and used

TABLE 1. Biomass yield and glycerophophate oxidase productivity obtained after cultivation in shaken flasks with different concentrations of lactose and glycerol and at different time of cultivation

Cultivation time (h)	Lactose conc. (%)	Glycerol conc. (%)	Cell growth dry weight (g l^{-1}) medium	Productivity of GPO		
				U l^{-1} medium	U g^{-1} dry weight	U mg^{-1} protein
15	2.0	1.75	0.288	15.7	54.6	0.57
15	2.0	2.00	0.258	13.0	50.2	0.48
15	1.0	1.50	0.294	16.7	56.8	0.60
15	1.0	2.00	0.236	17.4	73.6	0.68
15	0.5	2.00	0.235	18.4	78.2	0.69
15	0.5	2.50	0.236	29.0	122.6	0.77
15	0.5	3.00	0.209	23.2	110.9	0.65
15	0	2.50	0.098	9.4	96.6	1.35
15	0	3.00	0.099	13.0	131.7	1.25
15	0	4.00	0.104	13.7	131.9	2.16
20	0	4.00	0.106	16.5	155.6	1.41
24	0	4.00	0.108	16.2	150.6	1.11

during cultivation in flasks. Cells were grown at 37°C and pH 7.2. Biomass production and GPO levels were followed under different air flow rates, ranging from 21 to 5.3×10^{-5} m^3 s^{-1}.

Preparation of crude extract. GPO was extracted into Tris-HCl buffer (pH 7.5) after X-press disruption of frozen microbial cells and removing of the cells debris by centrifugation (10,000 × *g*, 20 min).

Determination of dry cell mass. Cell dry weight was estimated by using a predetermined correlation between optical density at 640 nm and dry cell weight (7, 8).

Enzyme assay. GPO activity was assayed by the peroxidase-linked spectrophotometric method (10). One unit of GPO activity is defined as the amount of the enzyme producing 1 μmol of hydrogen peroxide per minute at 37°C.

Protein assay. Protein concentrations were determined by the method of Lowry et al. (4), using BSA as a standard.

Results and discussion

Mutant strain *Aerococcus viridans* 1509 (prepared by one-step UV light mutagenesis [7]) was used for GPO production and testing the effect of different carbon source and inducer concentrations on the

enzyme production. Our previously published results (8) showed that GPO production under cultivation in flasks reached its maximum in the 15th hour of growth, i.e. at the stationary phase, and that the best carbon source for GPO production, in comparison with other sugars, was lactose. Glycerol was used as an inducer of GPO, but we found that the total enzyme productivity of the cells might be progressively affected by different combinations of lactose and glycerol concentration in the medium. Table 1 shows that increase of the glycerol concentration and simultaneous decrease of lactose concentration ensured higher GPO activity. The enzyme productivity was not proportional to the yield of biomass. Furthermore, higher concentrations of lactose positively influenced cell growth, but diminished the enzyme levels.

In contrast with other authors (11), who used 2% lactose and 1% glycerol for GPO production by *A. viridans*, we showed that 2.5% glycerol and 0.5% lactose is an optimal concentration ratio for good enzyme production by our mutant strain. When glycerol was the sole carbon source, growth was very low, lag and exponential phases were prolonged, and even if the GPO content per gram of dry weight increased significantly, the overall GPO productivity per liter of medium did not reached previous levels. Neither increase of glycerol concentration in medium, nor prolongation of the cultivation time could replace the effect of total lactose absence. The results indicate that low concentration of lactose is necessary for a good start of the process, but after its depletion, glycerol can serve as a suitable carbon source for cell growth and enzyme synthesis. This observation is significant also from the economical point of view, due to the much lower price of glycerol.

Scaling-up of the process and optimization of the cultivation conditions in a laboratory bioreactor showed further improvement in GPO intracellular levels. The reasons for the better enzyme production can be attributed especially to the sufficient and constant supply of air in the bioreactor.

Cultivations in a laboratory bioreactor were carried out under different aeration conditions but without any mechanical stirring. It should be pointed out that *A. viridans* is a microaerofilic microorganism that and it grows without any special needs for high aeration. We found that the value of 4.5×10^{-5} m^3 s^{-1} proved to be a suitable air flow rate for the optimization of the process (Table 2). Comparing the results in Table 1 and 2, it is clear that total GPO activity per liter of medium was 20-times higher after cultivation in bioreactor, although biomass yield was only 5-times higher than during cultivation in flasks.

TABLE 2. Biomass yield and glycerophosphate oxidase productivity obtained after cultivation in laboratory bioreactor at different aeration conditions

Air flow rate ($\times 10^{-5} m^3 s^{-1}$)	Dry weight ($g\ l^{-1}$) medium	Productivity of GPO		
		U l^{-1} medium	U g^{-1} dry weight	U mg^{-1} protein
2.1	0.918	412.1	448.9	1.82
3.7	1.095	548.1	500.5	1.28
4.5	1.100	614.6	558.7	1.32
5.3	1.109	495.6	447.0	1.36

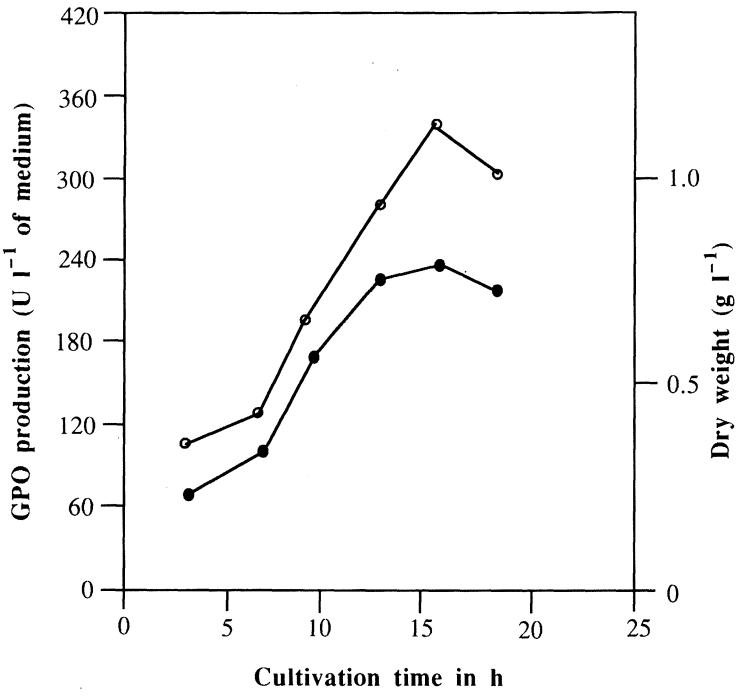

FIG. 1. Growth and GPO production curves of *Aerococcus viridans* 1509 during cultivation in a laboratory bioreactor (cultivation time 18 h, bioreactor volume 2.2 l, pH 7.2, 37°C). m , GPO production; l, dry weight.

The growth curve and the time dependence of GPO synthesis followed the same course both in bioreactor and in shaken flasks. The highest activity of GPO in bioreactor was achieved at the stationary phase of growth in the 15th hour of the process (Fig. 1) and corresponded with optimal time of the enzyme production measured under cultivation in flasks (7, 8).

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MICROBIOLOGÍA SEM **11** (1995), 343–350

Detection of *Bacillus larvae* spores in Argentinian honeys by using a semi-selective medium

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Summary

A semi-selective medium for the detection in Argentinian honeys of spores of *Bacillus larvae*, a pathogen of American foulbrood, was developed. The technique involves dilution of samples (1:2) in phosphate buffer, concentration of spores by centrifugation and heat treatment prior to inoculation. Two media (JNxPa and JNxPb) were prepared from J-agar, to which nalidixic acid and pipemidic acid were added. Both JNxP media were reliable for the isolation of *B. larvae* colonies and, at the same time, prevented the development of other *Bacillus* species which normally develop on the plates before *B. larvae* spores can germinate.

Key words: American foulbrood, *Bacillus larvae*, honey, *Bacillus* spp., culture techniques

Resumen

Se desarrolló un medio de cultivo semi-selectivo para el aislamiento en mieles argentinas de *Bacillus larvae*, agente causal de la loque americana. La técnica consiste en la dilución (1:2) de las muestras en buffer fosfato, concentración de esporas por centrifugación y tratamiento térmico del sedimento previo a la siembra. Los medios JNxPa y JNxPb se obtuvieron a partir del medio J-agar, al cual se añadió ácido nalidíxico y ácido pipemídico. Los medios JNxP resultaron eficaces para el

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aislamiento de colonias de *B. larvae* y, al mismo tiempo, impidieron el crecimiento de otras especies de *Bacillus* que se desarrollan normalmente en las placas antes de que puedan germinar las esporas de *B. larvae*.

Introduction

American foulbrood (AFB) of honey bees (*Apis mellifera* L.) caused by the spore-forming bacterium *Bacillus larvae* is the most serious disease of honey bee brood. It has become established in 11 Argentinian provinces (Buenos Aires, Entre Ríos, Córdoba, Santa Fe, La Rioja, Chaco, Corrientes, Tucumán, La Pampa, Catamarca and Río Negro) since it was first diagnosed in 1989 (1, 2).

The disease spreads when spores are transported on drifting bees, hive parts, clothing, hive tools and contaminated pollen or honey (5). One of the most frequent means of transmission of bacterial spores is honey itself and its occurs when beekeepers allow bees to rob contaminated honey or when bees feed on contaminated honey, no matter it is in the comb, has been extracted or is mixed with cappings (13, 14, 19).

Several methods to study the growth of spores of *B. larvae* on honey have been developed since the first report by Sturtevant (33, 34). Hansen (10, 11, 12, 13) developed a technique to detect *B. larvae* spores by direct inoculation onto J-agar of undiluted honey samples that had been heated to 88–92°C for 5 min. The effectiveness of the Hansen method for the detection of AFB spores in individual honey samples was pointed out by Steinkraus and Morse (32). Shimanuki and Knox (29) reported a method that included dilution, dialysis, centrifugation, resuspension and heat treatment of honey before inoculating onto brain heart infusion agar (BHIA) plates. Hornitzky and Clark (17) described a procedure which involved centrifugation of diluted honey samples, heat treatment of the sediment, and culture onto sheep blood agar plates (SBA) containing 3 mg/ml nalidixic acid (SBANal) to prevent the development of motile colonies of *Bacillus alvei*. Hornitzky and Nicholls (18) reported that J-agar medium was generally better to BHIA or SBA for the isolation of *B. larvae* from bulk honey samples. With the incorporation of nalidixic acid in the culture medium (17, 18), the growth of *B. alvei* is inhibited but other *Bacillus* species overgrow the plates, making it difficult to detect the presence of *B. larvae* in Argentinian honeys due to many false negative results.

Other species of *Bacillus* have been isolated from pollen and bee bread (6, 7), and from digestive tracts of foraging honey bees (8). The investigation of honeys by culturing techniques allows to trace infection sources and disease outbreaks, but Argentinian honeys are highly contaminated with several *Bacillus* species other than *B. alvei*. Thus, the purpose of the present work was to develop a culture technique and a semi-selective medium that would allow isolation of *B. larvae* but would supress the growth of other *Bacillus* species and other contaminating microorganisms.

Materials and methods

Honey samples. 122 honey samples were obtained from different apiaries in 8 Argentinian Provinces: Buenos Aires (105 samples); Río Negro (4 samples); Santa Fe (4 samples); Córdoba (3

samples); La Pampa (2 samples); Mendoza (2 samples); San Luis (1 sample) and La Rioja (1 sample). Within Buenos Aires Province, samples were submitted by beekeepers from 19 different localities (Counties): G. Paz (28 samples); La Plata (22 samples); Villarino (22 samples); Tandil (9 samples); C. Suárez (4 samples); G. Pueyrredón (4 samples); Cañuelas (3 samples); Tornquist (2 samples); and 1 sample each from Castelli, Lincoln, C. Pringles, Luján, Roque Pérez, Patagones, Puán, Saavedra, Magdalena, Brandsen and G. Belgrano.

Development of a semi-selective medium which allows growth of *B. larvae* and inhibits the growth of other *Bacillus* species. J-agar, the basal medium used (9) contains 5 g of tryptone; 15 g of yeast extract; 3 g of K_2HPO_4; 2 g of glucose; 20 g of agar and 1000 ml of distilled water. The medium was supplemented after sterilization with 7 mg/ml of nalidixic acid and 10 mg/ml of pipemidic acid to prepare JNxPa, or with 15 mg/ml of nalidixic acid and 20 mg/ml of pipemidic acid to prepare JNxPb. Antibiotic stock solutions were prepared separately by dissolving the corresponding amount of each antibiotic in 2 ml of 1 N NaOH, diluting to 100 ml with 0.01 M phosphate buffer (pH 7.2) and filter sterilized. Both antibiotic stock solutions were stored at –20°C until used.

Nalidixic acid inhibits the growth of *B. alvei*, *B. polymyxa*, *B. pumilus* and *B. megaterium* (1, 17, 27) while pipemidic acid prevents the growth of *B. circulans*, *B. subtilis* and *B. licheniformis*. *B. cereus*, *B. cereus* var. *mycoides*, *B. apiarius* and *B. pulvifaciens* are inhibited by both antibiotics indistinctly, as previously tested in Mueller-Hinton agar supplemented with different concentrations of each antibiotic according to the standards for antimicrobial susceptibility tests (22, 23, 24). Finally, *B. larvae* strains are resistant to 30 mg/ml of nalidixic acid and 20 mg/ml of pipemidic acid (3).

Evaluation of JNxP media as inhibitors of *Bacillus* species. Strains of *B. larvae* from different geographic areas were isolated from combs with symptoms of AFB. Strains of *B. alvei* and *B. laterosporus* were isolated from EFB-infected colonies. *B. cereus* (ATCC 11778), *B. apiarius* (ATCC 29575), *B. pulvifaciens* (ATCC 13537) and *B. stearothermophilus* (ATCC 7953) were obtained from the American Type Culture Collection. Strains of *B. polymyxa*, *B. pumilus*, *B. cereus* var. *mycoides*, *B. circulans*, *B. subtilis*, *B. licheniformis*, *B. megaterium* and *B. sphaericus* were previously isolated from honey samples.

Mixed spores and vegetative cells of different *Bacillus* species were diluted (to $A_{620} = 0.22$) in sterile distilled water and vortex mixed. Twenty µl drops of each suspension were spread by means of sterile cotton swabs over the surface of JNxPa and JNxPb plates. All the plates (3 replications for each strain tested on both media) were incubated aerobically at 35 ± 1°C for 48 h, or until detection of bacterial growth up to 7 days, with the exception of *B. stearothermophilus*, which was cultured at 55°C. J-agar plates were used as positive controls.

Variations in colony counts of *B. larvae* on J-agar, JNxPa and JNxPb. In order to evaluate the effectiveness of JNxP media in supporting the growth of *B. larvae*, numbers of colonies developed on J-agar, JNxPa and JnxPb plates were compared.

B. larvae cells from 4 days cultures (nearly 100% in the vegetative form) were suspended in phosphate buffer (pH 7.2), vortex-mixed and diluted to $A_{620} = 0.22$. Different amounts (5 µl, 10 µl and

20 µl) per plate were spread with sterile glass spreaders onto each of four J-agar, JNxPa and JNxPb plates, and incubated at 36 ± 1°C for 2 days, and the number of colonies per plate recorded.

A completely randomized design in which values of colony counts were subjected to a logarithmic transformation was employed in the statistical design. Tukey's multiple range test at the 0.05 probability level was used for mean's comparison in the variance analysis.

Treatment of honey samples. Each 15 ml sample was mixed with 30 ml of 0.01 M of phosphate buffer (pH 7.2) and centrifuged for 30 min at 3000 × *g*. Most of the supernatant fluid was discarded to leave approximately 3 ml of fluid per tube. These fluids were vortex-mixed with the sediment (17) and heated at 80°C for 10 min in order to kill non-spore forming bacteria and to activate spores of *B. larvae* to germinate. Twenty ml of JNxPb was poured into each Petri plate (2 replications per sample), and 80 mg of the sediment–fluid mixture was streaked over the plate surface with a standardized platinum loop (32). J-agar plates (2 per sample) were streaked for the detection of other *Bacillus* species. JNxPb plates were incubated at 36 ± 1°C in an atmosphere of 5% CO_2 in air up to 7 days. One replication of J-agar plates was incubated under the same conditions, and the other was incubated aerobically for 2 days or until bacterial growth was detected. Honey samples from colonies with visible signs of AFB were used as positive controls in all experiments.

Recognition of *B. larvae*. Colonies were identified by their shape, margins (25) and microscopic examination of Gram-stained smears. Bacterial cultures were tested for catalase production, Voges-Proskauer reaction, starch hydrolysis and growth in nutrient broth (9). The size, shape and location of spores and the size of vegetative cells were determined. *Bacillus* colonies were maintained on slants of nutrient agar and identified according to Gordon et al. (9) and Priest and Alexander (26).

Results and discussion

Ten out of 122 honey samples (8%) contained *B. larvae* spores. All the positive samples were from Buenos Aires Province, where 60% of Argentinian honey is produced. The isolated *B. larvae* strains were recovered from JNxPb plates, whereas other *Bacillus* species were isolated at the correspondent duplicate J-agar plates (without antibiotics). The 10 samples with *B. larvae* spores contained also spores of *B. alvei* (5 samples), *B. cereus* (2 samples), *B. pumilus* (2 samples) and *B. cereus* var. *mycoides* (1 sample). If only J-agar plates were used, it was not possible to obtain single colonies of *B. larvae* after plating due to the overgrowth of other *Bacillus* species commonly found.

The characteristics of *B. larvae* isolates were the same as previously reported (1, 2, 9, 11, 17, 25). *B. larvae* colonies growing in JNxP plates ranged from whitish to grayish with rough surfaces, about 3–4 mm in diameter and developed after 3–5 days of incubation at 36 ± 1°C. Under low power dry objective, colonies showed irregular edges with typical branches (Fig. 1). All *B. larvae* strains were Gram-positive and unable to withstand serial transfer in nutrient broth. They were catalase and Voges-Proskauer negative and did not hydrolyse starch.

The occurrence of the contaminated honeys is directly related to the occurrence of bee colonies with clinical symptoms of AFB. It should also be pointed out that *B. larvae* was found in two of four examined

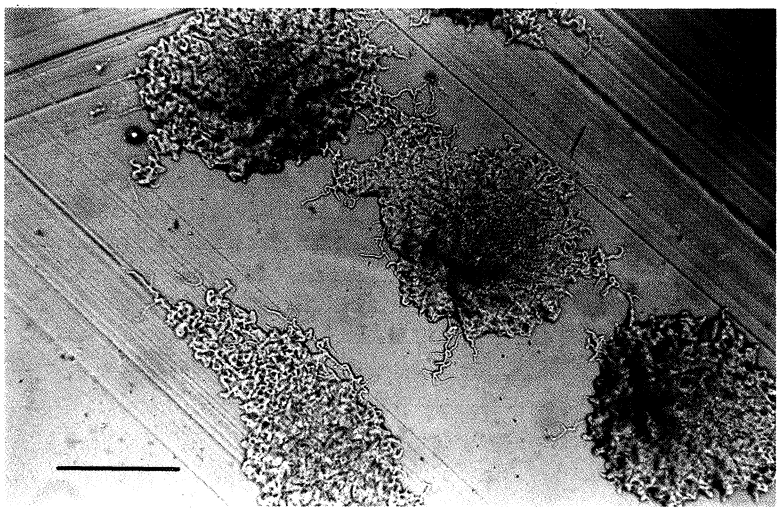

FIG. 1. Colonies of *Bacillus larvae* growing on JNxPb agar plates after 4 days at 36 ± 1°C. Colonies present rough surfaces and irregular edges with typical branches, as seen under low power dry objective (10×). Bar: 2 mm.

Argentinian honeys as early as in 1979–81 (11) and that the bacterium may have been already established in Argentina some years before AFB was first registered in 1989 (1, 2).

Ten species of *Bacillus* other than *B. larvae* were isolated from J-agar plates and identified according to the Priest and Alexander matrix (26).

B. alvei was the *Bacillus* species most frequently (56%) detected in the honey samples, which coincides with previous studies (4, 17). According to Hornitzky and Karlovskis (15), *B. alvei* had been found to be a common inhabitant of hives and had been isolated from 45.8% of the adult bees samples. The presence of *B. alvei* spores was also used as an indicator of European foulbrood (EFB) (16, 17, 30), but, at the same time, this bacterium was recognized as a saprophyte living on the dead remains of larvae (1, 15).

Smith et al. (31) demonstrated that unheated and undiluted honeys showed antibacterial activity against *B. cereus* and *B. subtilis*, while Roth et al. (28) found that the use of 0.1 M phosphate buffer (pH 7.0) to dilute honey completely reduced the natural inhibitor effect against *B. cereus*. *B. cereus* (18%), *B. cereus* var. *mycoides* (2.5%) and *B. subtilis* (1%) were found in some Argentinian honeys. The recovery of these species in J-agar plates could be explained by the dilution of honey samples with phosphate buffer (1:3) prior to centrifugation required to concentrate spores. In nature, bacterial spores present in honey are not inhibited from germinating capacity because honey produces antibacterial effects only on vegetative cells (20, 21).

In relation to the evaluation of JNxP media as inhibitors of *Bacillus* species, not any colony of *B. subtilis, B. cereus, B. apiarius, B. circulans, B. alvei, B. pulvifaciens, B. cereus* var. *mycoides* and *B. megaterium* was recovered from JNxPa plates, while in the correspondant J-agar plates normal growth occurred. Fewer colonies of *B. laterosporus, B. pumilus, B. licheniformis* and *B. polymyxa* observed on JNxPa (with respect to J-agar plates), and their growth was completely inhibited when cultured on JNxPb. Besides, *B. sphaericus* and *B. stearothermophilus* were resistant to high concentrations

of nalidixic acid and pipemidic acid, as seen on JNxPb plates.

Hornitzky and Clark (17) reported a reduction in the number of *B. larvae* colonies when grown on plates containing 6 µg/ml of nalidixic acid.We saw a correlation between colony counts and the sample inoculum volume. The analysis of variance showed a highly significant effect (F** P = 0.01) of culture media and inoculum size on the number of *B. larvae* colonies. Low inoculum volumes (5 µl) produced high differences in colony counts, whereas higher inoculum volumes (10 µl and 20 µl) resulted in similar colony counts on J-agar and JNxPa, and slightly smaller colony counts on JNxPb (Fig. 2). The variation coefficient after logarithmic transformation of values was 1.59%.

There are no reliable methods to make plate counts of *B. larvae*, for not even 10% of the spores produce visible growth on culture media (30). When growing in JnxPb, fewer *B. larvae* colonies are expected. The lower scores for colony counts on JnxPb may be a function of the low concentration of spores in the sample and the high concentration of both antibiotics in the medium.

Whith other isolation techniques, which may involve media such as SBA, BHIA or J-agar, the recovering of single colonies of *B. larvae* after plating is diminished or does not take place due to the growth of other *Bacillus* species.

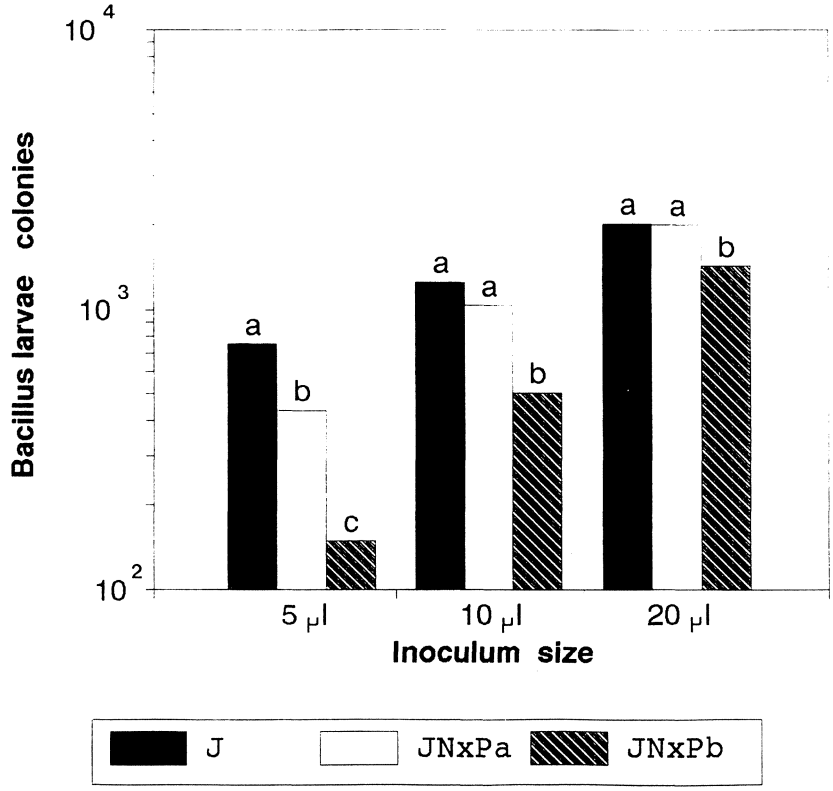

FIG. 2. Colony counts of *Bacillus larvae* in 3 media (J-agar, JNxPa and JNxPb) from 3 different inoculum sizes (5 µl, 10 µl, 20 µl), from a suspension adjusted to A_{620} = 0.22. Bars marked with the same letter do not differ significantly at P = 0.05, according to a Tukey mean separation test.

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Photosynthetic oxidation of MnS and FeS by *Chlorobium* spp.

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Summary

The ability of two species of green phototrophic sulfur bacteria (*Chlorobium limicola* and *Chlorobium phaeobacteroides*) to photosynthetically oxidize several metal sulfides (MnS, FeS, NiS, CuS, ZnS, CdS and PbS) by has been tested in laboratory batch cultures. Both species only oxidized MnS and FeS, which are the ones having higher solubilities (pK_s = 13.5 and 18.1, respectively). The specific oxidation rates were directly related to the solubility of the metal sulfide involved. *C. limicola* oxidized MnS and FeS at specific rates of 11.8 and 0.9 μmol $S^{2-}h^{-1}$ mg $protein^{-1}$, respectively. Specific oxidation rates of *C. phaeobacteroides* for MnS and FeS were 7.1 and 1.8 μmol $S^{2-}h^{-1}$ mg $protein^{-1}$, respectively. The oxidation of both metal sulfides resulted in the release of the free-soluble metal ions in the culture media, but no toxic effect of these cations on the photosynthetic activity of the cells was observed. The anaerobic photosynthetical oxidation of MnS and FeS by *Chlorobium* reveals an adaptation of this bacterial species to sulfide-poor environments, and introduces a new process in the Mn, Fe, and S biogeochemical cycles to be considered.

Key words: *Chlorobium*, green sulfur bacteria, sulfide oxidation, heavy metals, metal toxicity

Resumen

En el presente trabajo se ha evaluado la capacidad de dos especies de bacterias verdes del azufre (*Chlorobium limicola* y *C. phaeobacteroides*) de oxidar fotosintéticamente determinados sulfuros

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metálicos (MnS, FeS, NiS, CuS, ZnS, CdS y PbS). Ambas especies oxidaron únicamente aquellos sulfuros metálicos más solubles, es decir MnS y FeS (pKs de 13,5 y 18,1, respectivamente). Las tasas específicas de oxidación resultaron estar directamente relacionadas con la solubilidad del sulfuro metálico correspondiente. De esta forma, *C. limicola* oxidó el MnS y el FeS con tasas de 11,8 y 0,9 μmol S^{2-} h^{-1} mg proteína^{-1}, respectivamente. Por su parte, las tasas de oxidación de dichos compuestos calculadas para *C. phaeobacteroides* fueron de 7,1 y 1,8 μmol S^{2-} h^{-1} mg proteína^{-1}, respectivamente. Tras la oxidación del MnS y del FeS, los correspondientes iones metálicos aparecieron en el medio de cultivo en forma soluble, sin apreciarse ningún efecto tóxico de dichos cationes sobre la actividad fotosintética bacteriana. La oxidación fotosintética anaeróbica del MnS y del FeS por parte de *Chlorobium* spp. puede considerarse como una adaptación de estas especies a los ecosistemas pobres en sulfhídrico, introduciendo a su vez un proceso adicional dentro de los ciclos biogeoquímicos del Mn, Fe y S.

Introduction

Iron and manganese are commonly found in the hypolimnia of seasonally anoxic lakes, either in dissolved or complexed metallic species. Their distribution in aquatic systems is often linked to the presence of oxic/anoxic transition zones. The spatial and temporal changes of redox boundaries strongly influence the cycling of metallic elements, by determining their mobility along the vertical profile of the lake (1, 2, 4, 22). Phototrophic sulfur bacteria are usually found in the illuminated layers of the anaerobic, sulfide-containing hypolimnia of stratified lakes, forming dense populations either in or below the oxic-anoxic interfaces (8, 11, 13, 15). Therefore, metal and bacterial phototrophic populations may occur together in natural aquatic ecosystems. Garcia-Gil et al. (7) have reported maxima of both *C. phaeobacteroides* and ferrous iron overlapped in the chemocline of two meromictic basins of lake Banyoles. The ability of green phototrophic sulfur bacteria to use amorphous FeS (Mackinawite) as electron donor for anoxygenic photosynthesis has been postulated as a mechanism for thriving in ferrous environments where sulfide concentrations are low (6).

The aim of this work was to determine the ability of two species of green phototrophic sulfur bacteria, *Chlorobium limicola* and *Chlorobium phaeobacteroides*, to photosynthetically oxidize metal sulfides. For this purpose, the oxidation kinetics of both metal-bound sulfide and elemental sulfur (S^0) were monitored in batch cultures and the resulting oxidation rates compared. A second goal was to determine whether the released metal cations after the oxidation is or not harmful to photosynthetic metabolism of these species.

Materials and methods

Bacterial strains and growth conditions. *Chlorobium limicola* and *C. phaeobacteroides* are obligate anaerobic, sulfur phototrophic bacteria belonging to the Chlorobiaceae (18). *C. limicola* strain DSM 249 was purchased at the German Collection of Microorganisms. *C. phaeobacteroides* strain UdG6030 was

obtained from lake Banyoles, located near Girona (NE of Spain) following the isolation methods of Trüper and Pfennig (18) and van Niel (21). Bacteria were grown in 500 ml glass bottles completely filled with standard Pfennig mineral medium (12, 18).

Stirred batch cultures were grown at room temperature under saturating light conditions (30 $\mu Em^{-2}s^{-1}$) provided by two 100 W tungsten incandescent bulbs and maintained under nitrogen pressure to ensure anaerobic conditions. The CO_2/HCO_3^- buffering system of the medium allowed to maintain the pH of the medium fairly constant around 6.7 during the experimental time (ca. 15 h).

Analytical procedures. Sulfide was analyzed by the leuco-methylene blue method (3). Both, photosynthetic pigments and elemental sulfur (S^0) were extracted from the wet cell pellets with 100% methanol after centrifuging 10 ml of bacterial culture for 20 min at 4,500 rpm. Extracts were placed for 24 h at –20 °C to ensure the complete extraction of pigments. After this period, extracts were centrifuged again for 20 min at 4,500 rpm and the clear supernatants were used for the determination of pigments and elemental sulfur (14). Bacteriochlorophyll *c* and *e* concentrations were calculated using the formulae of Takahashi and Ichimura (15) and Montesinos et al. (10), respectively. Protein concentration was determined from the pigment-free pellets according to Lowry et al. (9).

Experimental procedures. *Oxidation of metal sulfides*. The capacity of both bacterial species to use different metal sulfides as electron donors for anoxygenic photosynthesis was tested in culture media containing metal-bound sulfide as unique reductive power source. Stoichiometrical amounts of S^{2-} (as $Na_2S{\cdot}7H_2O$) and metallic salts ($MnSO_4{\cdot}H_2O$; $Fe(NH_4)_2(SO_4)_2{\cdot}6H_2O$; $NiCl_2{\cdot}6H_2O$; $CuSO_4$; $ZnSO_4{\cdot}7H_2O$; $Cd(NO_3)_2$ and $PbNO_3$) were added simultaneously to light preserved cultures. S^{2-} slightly exceeded metal in order to avoid the hypothetical toxicity caused by any excess of metal ion remaining in solution. Metal sulfides were allowed to form for 1 h at the dark. Afterwards, cultures were placed under illuminated conditions as previously described. Samples for sulfide and sulfur oxidation kinetics were taken at 30 min intervals.

Toxicity of soluble metals. Since the only metal sulfides oxidized were MnS and FeS, only free Mn and Fe could be released to the medium and expected to cause a potential harmful effect over bacterial cells. The potential toxicity of these metals was tested by measuring their effect on the elemental sulfur oxidation kinetics by *Chlorobium* cells. For this purpose, $MnSO_4{\cdot}H_2O$ and $Fe(NH_4)_2(SO_4)_2{\cdot}6H_2O$ were added at a final concentration of 100 $\mu mol\ l^{-1}$ to batch cultures immediately after the complete depletion of sulfide, to prevent the formation of undesired metal sulfide. Samples were taken every 30 min.

Results

Photosynthetic oxidation of FeS. In order to ensure that metal sulfides were entirely oxidized by photosynthetic metabolism, a previous experiment was performed; Four culture flasks of *C. phaeobacteroides* with 0.5 $mmol\ l^{-1}$ S–FeS each were incubated under different conditions, as follows: (i) *Chlorobium phaeobacteroides* at light; (ii) *Chlorobium phaeobacteroides* at dark; (iii) sterile medium

at light and (iv) sterile medium at dark. Blackish, FeS containing medium was only cleared (returned to reddish-brown color of *C. phaeobacteroides*) in presence of both, *Chlorobium* and light (i). A new addition of sodium sulfide to the culture—to a final concentration of 0.5 mmol l^{-1}—gave rise to the new formation of FeS, that darkened the medium in a few seconds. This observation indicates that most of the released iron remained in the reduced-soluble form (Fe^{2+}). Flasks numbered 2, 3 and 4, did not become clear. Thus, dark chemotrophic oxidation, non biological photo-oxidation and uncontrolled chemical oxidation were discarded as responsible for FeS oxidation.

Utilization of metal sulfides as electron donors. After the experimental time (ca. 15 h.) only S^{2-} from MnS and FeS was oxidized by both *Chlorobium*, exhibiting identical time-course oxidation patterns (Fig. 1). The sulfur oxidation activity resulted in a concomitant accumulation of S^0 in the culture media. The S^0 oxidation only started when S^{2-} was practically undetectable. The differences between both species were only detected in their respective metal oxidation rates. MnS was faster oxidized (11.8 and 7.1 µmol S^{2-} h^{-1} mg protein^{-1} for *C. limicola* and *C. phaeobacteroides* respectively) than FeS (0.9 and 1.8 µmol $S^{2-}h^{-1}$ mg protein^{-1} respectively). The H_2S oxidation rates in

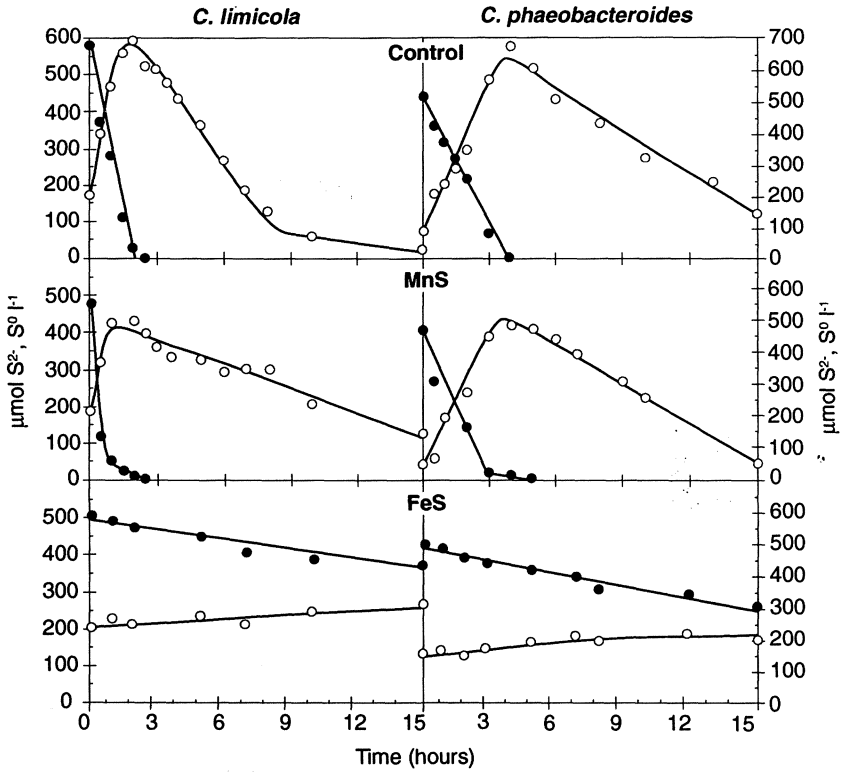

FIG. 1. Time courses of the MnS and FeS oxidation (measured as S^{2-}, full dots) and sulfur (S^0, empty dots) by *C. limicola* and *C. phaeobacteroides*.

the control cultures were 6.7 and 5.7 μmol $S^{2-}h^{-1}$ mg $protein^{-1}$ for *C. limicola* and *C. phaeobacteroides*, respectively. The rest of the metal sulfides tested were not oxidized and therefore, no accumulation of elemental sulfur was detected. In these cases only the S^0 that was already present before the addition of the metal sulfide was oxidized to some extent (data not shown).

The elemental sulfur produced during MnS oxidation was further oxidized to sulfate by *C. limicola* and *C. phaeobacteroides* at rates of 1.4 and 1.8 μmol S^0 h^{-1} mg $protein^{-1}$, respectively. These values were fairly similar to those calculated for control cultures (1.4 and 1.5 μmol S^0 h^{-1} mg $protein^{-1}$, respectively). Elemental sulfur derived from the FeS oxidation, remained unaltered over the experimental time.

Effect of Mn^{2+} and Fe^{2+} on the photosynthetic activity of *Chlorobium*. In order to determine the potential toxicity of these cations, the photosynthetic sulfur oxidation activity of *C. limicola* and *C. phaeobacteroides* was monitored in a culture medium containing 100 μmol l^{-1} of free-soluble manganese and iron. For the concentration tested, no toxic effect of either Mn^{2+} and Fe^{2+} on both *Chlorobium* was detected since bacterial cells could oxidize elemental sulfur after the metal addition. The specific sulfur oxidation rates of both species in the presence of both free-metals in the medium are compared in Table 1. Although the sulfur oxidation activity went on, the oxidation rates were lower than those measured in the control cultures (see table).

Discussion

Metal sulfides have been considered to be inert compounds, since most are highly insoluble. Nevertheless, these compounds can play a significant role on several microbial processes. Particularly, FeS is known to be an electron donor for both, phototrophic and chemolithotrophic bacteria (5, 7).

The ability of *Chlorobium* to photosynthetically oxidize metal sulfides seems to be determined by the solubility of the metal sulfide involved. No oxidation activity of those metal sulfides having $pK_s \geq 19.4$, that is NiS, CuS, ZnS, CdS, and PbS was measured during the experimental time course (15 h). The possibility of a longer term oxidation, however, could not be formally ruled out. In turn, MnS ($pK_s = 13.5$) and FeS ($pK_s = 18.1$) were oxidized, with rates in accordance to their respective solubilities. Torma et al. (16) and Torma and Sakaguchi (17) found the same relationship in metal sulfide oxidation

TABLE 1. Specific sulfur oxidation rates for the two species tested in presence of dissolved Fe and Mn in the culture medium.

Species	Sulfur oxidation rates (μmol S^0 h^{-1} mg $protein^{-1}$)		
	Control	Mn	Fe
C. limicola	1.9	1.3	0.9
C. phaeobacteroides	1.8	1.9	1.3

by *Thiobacillus ferrooxidans*. In *Chlorobium* species this fact appears to be related to the concentrations of free sulfide (in the form of HS^-) by dissociation from the metal sulfide solid phase.

The darkened of the culture medium after the formation of FeS does not seem to be a limitation for its oxidation by *Chlorobium*. The differences observed between the FeS oxidation rates of both *Chlorobium* could partially arise from the fact that *C. phaeobacteroides* is better adapted to poor light conditions than *C. limicola* (10, 11, 15). Moreover, this observation agrees with the sulfide affinities (μ_{max}/K_s) calculated for both species by van Gemerden (19) which are 65 and 120 h^{-1} $mmol^{-1}$ for *C. limicola* and *C. phaeobacteroides*, respectively. The inability to oxidize other metal sulfides, such as NiS, CuS and PbS, that also darkened the medium seems to be related to their low solubility rather than light limitation.

The oxidation of FeS and MnS by green sulfur bacteria can be of ecological significance in aquatic environments where iron or manganese and sulfur cycles coexist and sulfide is present at extremely low concentrations. In these habitats, green phototrophic sulfur bacterial species capable to use those metal sulfides as electron donors for anoxygenic photosynthesis are favored to thrive. Although it can be performed by different mechanisms, metal sulfide oxidation by fungi is related to the capacity of fungal filaments to sorb metal ions (23). Accordingly, the attachment of metal cations to the bacterial cell wall would play an important role on the metal sulfide oxidation. The attached metal cation might act as a trap for the S^{2-} from the environment. In the periplasmic space, sulfide is found under the HS^- form (19, 20). The continuous consumption of HS^- allows the dissociation of S^{2-} from the membrane-attached metal sulfide to the periplasmic space becoming a ready–to–use HS^-. Thus, the overall equilibrium is displaced to the dissociation of metal sulfide, resulting in a steady-state process which continually drives S^{2-} towards the cell. In spite of the fact that Cu^{2+}, Zn^{2+}, Cd^{2+} and Pb^{2+} were also sorbed to cell envelopes (data not shown), their respective sulfides could not be oxidized within the experimental time (see results). This observation suggests that solubility is the critical factor that determines the ability of green sulfur bacteria to photosynthetically oxidize metal sulfides.

Under a biogeochemical point of view, the photosynthetic oxidation of metal sulfides, such as MnS and FeS should be included as a process taking part on the Mn, Fe and S cycles. Oxidation of such solid compounds, either in aerobic or anaerobic conditions, has been traditionally thought to be coupled to respiratory metabolism, that is, to the presence of an external electron acceptor such as oxygen or nitrate. Although further work has to be done in order to elucidate which is the specific importance of the different factors that can affect the photosynthetic oxidation of FeS and MnS, our results supports the idea that this process might be an additional pathway within the biogeochemical cycles of those elements. By this process, sulfur and either Fe or Mn could be re-circulated from anaerobic illuminated compartments of stratified lakes where no electron acceptors are available for chemolithotrophic bacteria.

Acknowledgments

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MICROBIOLOGÍA SEM **11** (1995), 359–368 **PERSPECTIVAS**

Breve historia de la Sociedad Española de Microbiología, I. De 1946 a 1971

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La presente "Breve historia de la Sociedad Española de Microbiología" ha podido escribirse gracias a la utilización de documentos diversos procedentes de los archivos de la SEM —principalmente las Actas de sus Juntas Directivas, así como escritos referentes a los Congresos Nacionales e Internacionales de Microbiología—, de la colección de la revista Microbiología Española *(vols. 1–36, 1947–1986) y de los archivos del Instituto «Jaime Ferrán»* de *Microbiología, en el Centro de Investigaciones Biológicas del CSIC. El manejo de esa variedad de documentos, por parte de una microbióloga, ex-Tesorera y ex-Secretaria de la SEM, cuya dedicación profesional no es precisamente la historia, ha podido dar lugar a algún error u omisión totalmente involuntario. Este artículo es el primero de una serie, que se irá publicando en sucesivos números de nuestra revista.*

Introducción

Este año se cumple el cincuentenario de la Sociedad Española de Microbiología, la SEM. La Sociedad fue fundada oficialmente en 1946, en el Consejo Superior de Investigaciones Científicas (CSIC), aunque su puesta en marcha se acordó un año antes, en 1945. Desde su fundación hasta 1970 la Sociedad recibió el nombre de Sociedad de Microbiólogos Españoles, SME, y, a partir de 1970, por decisión mayoritaria de sus socios, de Sociedad Española de Microbiología, con las siglas SOEMI, SEMIC y, finalmente, SEM.

En la actualidad, la SEM tiene más de dos mil socios y dispone de una sólida organización administrativa, pero su crecimiento y desarrollo no ha sido una tarea fácil. Durante las dos primeras décadas de su existencia no estaba capacitada para organizar grandes actividades científicas, por carecer de infraestructura, fondos e incluso una cierta capacidad estatutaria.

A través de estas paginas se irán describiendo los esfuerzos, no siempre reconocidos y apreciados, de unos cuantos microbiólogos que consiguieron consolidar y expandir nuestra Sociedad. Gracias a ellos, y a los sucesivos cambios llevados a cabo a lo largo de casi cincuenta años de andadura, la SEM ha llegado a ser la activa organización de hoy día.

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Primeros esfuerzos y logros de la Sociedad de Microbiólogos Españoles

El 19 de junio de 1946, en la sede central del CSIC, Juan Marcilla Arrazola, Lorenzo Vilas López, Miguel Benlloch Martínez, Ricardo Salaya León y Pedro Carda Gómez, actuando como Presidente, Secretario y Vocales, respectivamente, constituyeron la Sociedad de Microbiólogos Españoles, de acuerdo con los Estatutos y Reglamento aprobados con fecha 30 de marzo de ese mismo año, y ejecutando así los acuerdos adoptados por los asistentes y adheridos de la reunión gestora celebrada al efecto en 1945. Esta reunión, patrocinada por el CSIC, se celebró durante los días 13 a 15 de julio del citado 1945, con la asistencia de 48 microbiólogos españoles, y en ella se constituyó la Junta Provisional fundadora de la Sociedad de Microbiólogos Españoles, SME.

En la siguiente reunión de la Sociedad de Microbiólogos Españoles, celebrada el día 8 de julio de 1946, se eligió la nueva Junta de Gobierno, al cesar en sus funciones la Junta Provisional. Así, quedaron confirmados en sus cargos los mismos Presidente y Secretario, Juan Marcilla y Lorenzo Vilas, siendo elegidos Gerardo Clavero del Campo como Vicepresidente, Ricardo Salaya León como Bibliotecario y Miguel Benlloch Martínez como Tesorero. Se estructuró la Sociedad en ocho especialidades diferentes, con sus correspondientes Vocalías, que se sumaron a los anteriores cargos en la primera Junta de Gobierno. Estas especialidades fueron: Bacteriología, Valentín Matilla; Inmunología, José García Bengoa; Micología, José de Benito Martínez; Protozoología, Luis Nájera; Virología, Eduardo Gallardo; Microbiología Sistemática, Arnaldo Socías; Microbiología Aplicada, Rafael Ibáñez; y Microbiología Patológica, Pedro Carda. Estas especialidades o ramas se reflejaron en la correspondiente enmienda de los recién aprobados Estatutos.

En esta misma reunión se presentó una comunicación científica seguida de discusión, costumbre que perduró a lo largo de más de dos décadas. Se acordó designar a Juan Marcilla representante de la Sociedad en el Comité de Organización del IV Congreso Internacional de Copenhague de 1947, organizado por la International Society of Microbiology (ISM).* El representante de cada país o sociedad nacional era considerado en aquel entonces como "Miembro Fundador y Vicepresidente" de la ISM, representación que había sido ocupada antes de 1936 por Antonio Ruiz Falcó, por lo que se designó también a este último segundo representante de nuestra Sociedad en la ISM. A este respecto cabe mencionar el intento previo de fundación de la Sociedad Española de Microbiología como sección de la ISM en 1927, por parte de Francisco Murillo, Francisco Tello, Antonio Ruiz Falcó, Guillermo de la Rosa, Servando Barbero, Jerónimo Durán de Cottes, Jerónimo Megías, Gustavo Pittaluga, Pedro González y Juan Peset. En 1930 tuvo lugar en París el I Congreso Internacional de Microbiología, y la representación española corrió a cargo de F. Tello, A. Ruiz Falcó, P. González y A. Socías. Pero

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* La ISM había sido fundada en 1927. En 1933, durante el II Congreso Internacional de Microbiología, celebrado en Berlín, cambió su denominación a International Association of Microbiologists, IAM, y de nuevo, en 1950, durante el V Congreso Internacional de Microbiología, en Río de Janeiro, la organización adoptó la denominación de International Association of Microbiological Societies, IAMS. La IAMS —afiliada a la International Union of Biological Societies, IUBS, como una de sus divisiones— adquirió independencia en 1980 como la actual International Union of Microbiological Societies, IUMS, miembro de pleno derecho del International Council of Scientific Unions, ICSU, con sede en París. En la actualidad, están federadas en la IUMS 103 sociedades nacionales de 60 países. La SEM es una de ellas.

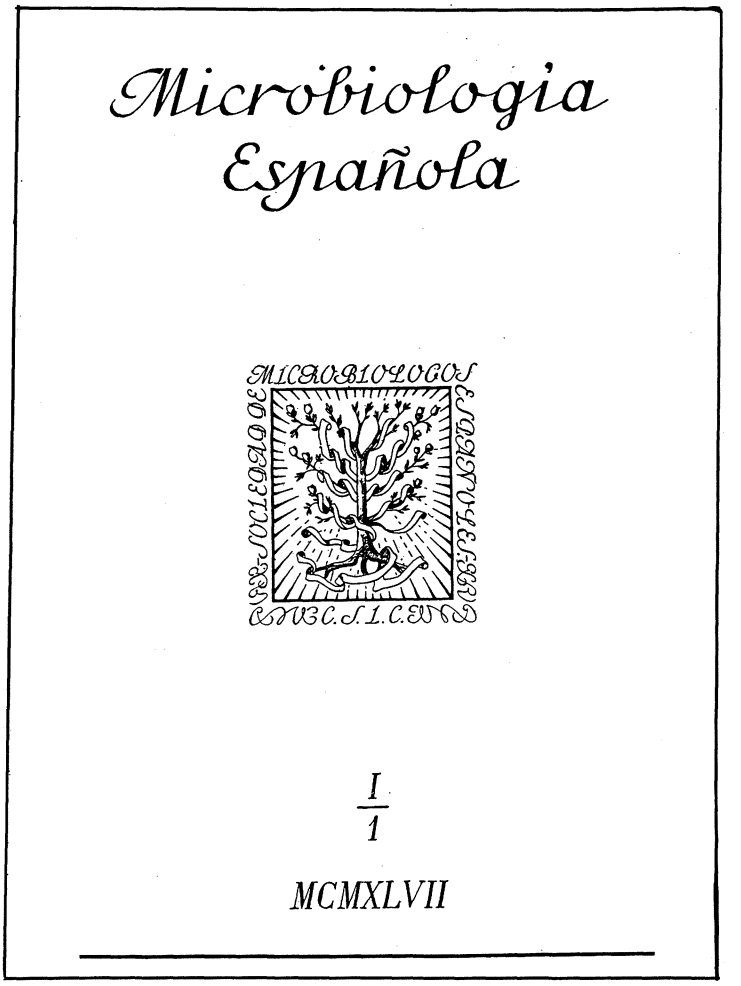

FIG. 1. Portada del primer número de la revista *Microbiología Española*, publicada conjuntamente por el Instituto «Jaime Ferrán» de Microbiología, del Consejo Superior de Investigaciones Científicas, y por la Sociedad de Microbiólogos Españoles.

teniendo en cuenta los avatares políticos de los años treinta en España, y seguidamente los internacionales, no se volvió a intentar la fundación de la Sociedad hasta 1945.

A partir de 1946 la Sociedad celebró reuniones periódicas en las que, a modo de Sesiones Públicas, se presentaron numerosas comunicaciones científicas que se publicaron en la revista *Microbiología Española* del Instituto «Jaime Ferrán» de Microbiología del CSIC (Fig. 1), y que había sido fundada paralelamente a la Sociedad. Continuando con la cooperación internacional se procedió al nombramiento de Socios de Honor a diferentes microbiólogos de reconocido prestigio. A lo largo del primer bienio éstos fueron: E. Ecker, de la Universidad de Cleveland (EE. UU.), A. Bessemans, de la Universidad de Gante (Bélgica), ambos en 1946, y posteriormente el micólogo C. Thom (New York, EE. UU.), en 1947. En 1948, Sir Alexander Fleming visitó el CSIC con motivo de su nombramiento de Doctor "Honoris causa" por la Universidad de Madrid.

El número de socios fue aumentando paulatinamente, siendo requisito indispensable su presentación por dos socios. Dentro de esta línea de crecimiento, se aprobó la creación de la Delegación de Barcelona, que estuvo a cargo de Juan Puiggrós, junto con J. Olivé Suñé y José María Rosell. Continuando con la cooperación con la ISM, se envió la traducción española del Código Internacional de Nomenclatura Bacteriana, para someterla a su aprobación por la Comisión de Nomenclatura y proceder posteriormente a su publicación. El Presidente de la Sociedad fue designado representante español en el V Congreso Internacional de Microbiología a celebrar en 1950 en Río de Janeiro, Congreso al que la ISM invitó expresamente a tres microbiólogos españoles. La representación española por parte del Presidente de la Sociedad en la ISM llevaba implícita la representación en la Comisión Internacional permanente. En este mismo año, Sir Howard Walter Florey (Oxford, Gran Bretaña) y Selman A. Waksman (Rutgers, EE. UU.) que asistían en Madrid a los actos del X Pleno del CSIC, fueron invitados a la reunión correspondiente de la Sociedad y fueron nombrados Miembros de Honor de la misma, juntamente con el científico italiano Giuseppe Penso.

La renovación parcial de la Junta Directiva correspondiente a 1951 dio el siguiente resultado: Presidente, Antonio Ruiz Falcó; Vicepresidente, Gerardo Clavero del Campo; Secretario, Lorenzo Vilas López; Tesorero, Miguel Benlloch Martínez; Bibliotecario, Ricardo Salaya León; Vocales, Genaro Alas Cores, Gabriel Colomo de la Villa, Eduardo Gallardo Martínez, José García Bengoa, Rafael Ibáñez González, Emilio Luengo Arroyo, Florencio Moreno de Vega y Arnaldo Socías Amorós. También en ese año empezaron a publicarse los extractos de los trabajos de investigación de la revista *Microbiología Española* en los *Biological Abstracts*, con lo que empezaron a tener difusión internacional.

Con motivo de una circular de Giuseppe Penso, a la sazón Secretario General de la ISM, la Sociedad Española reivindicó la adopción del español como idioma oficial, que ya había sido reconocido como tal por Bordet, primer Presidente de la ISM, junto con el francés, alemán, inglés e italiano. Igualmente nuestra Sociedad apoyó el cambio estatutario de la ISM para convertirse en la IAMS (International Association of Microbiological Societies), tema debatido durante el Congreso Internacional de Río de Janeiro (1950), pero que no fue efectivo hasta 1967 (véase la nota en la p. 360). Con dicho cambio se daba mayor protagonismo a las sociedades nacionales constituyentes.

En 1952 asistieron como "Invitados de Honor" de la Sociedad a una sesión de la misma los siguientes científicos extranjeros: Olympio da Fonseca (Río de Janeiro, Brasil), Paul Hauduroy (Lausana, Suiza) y Giuseppe Penso (Roma, Italia). En dicha sesión, los dos primeros fueron propuestos Socios de Honor de la Sociedad (Penso ya había sido distinguido anteriormente con el nombramiento). Penso informó sobre las actividades preparatorias del VI Congreso Internacional a celebrar en Roma en 1953, congreso al que también España había presentado su candidatura como posible país organizador, e invitó a la Sociedad a que participara al máximo en dicho congreso.

La Asociación Mexicana de Microbiología distinguió a Antonio Ruiz Falcó y Lorenzo Vilas, Presidente y Secretario de la Sociedad, como "Socios Correspondientes" de dicha Asociación Mexicana. Nuestra Sociedad correspondió con el nombramiento de Socio de Honor al Presidente de la citada Asociación, A. Sánchez Marroquín.

El Prof. Paul Hauduroy, Socio de Honor de nuestra Sociedad, recabó la colaboración española para una Reunión Científica sobre Virología que estaba organizando en Lausana, invitando expresamente al Presidente y Secretario de la Sociedad para que intervinieran durante tal reunión en la posible constitución de una Sociedad Europea de Microbiología. Nuestra Sociedad propuso el nombre de

Federación Europea de Sociedades Nacionales de Microbiólogos, e igualmente propuso que se editase un Boletín de información al efecto. Este acuerdo se elevó también al CSIC para obtener la autorización correspondiente, pues el CSIC, gracias al interés de su Secretario General, José María Albareda, continuaba ejerciendo su mecenazgo sobre la Sociedad, parte de cuyas actividades subvencionaba a través del Instituto «Jaime Ferrán» de Microbiología del CSIC (Fig. 2). También la ISM autorizó en ese momento, reflejándolo en sus Estatutos, la organización provisional de una Agrupación de las Sociedades Microbiológicas de las Naciones Europeas.

En 1954, Kenneth Smith, Director del Departamento de Virus de Plantas del Instituto Molteno de Cambridge (Gran Bretaña) asistió a la correspondiente Reunión de la Sociedad en la que se le nombró Socio de Honor. Ese mismo año se renovó parcialmente la Junta Directiva. Fueron elegidos: Tesorero, Miguel Benlloch Martínez, Bibliotecario, Ricardo Salaya León, y Vocales, Eduardo Gallardo Martínez, José García Bengoa, Juan Manuel Martínez-Arroyo Núñez y Arnaldo Socías Amorós.

Una vez más, Giuseppe Penso, en nombre de la IAMS, propuso en 1955 que nuestra Sociedad se encargase de organizar el VII Congreso Internacional de Microbiología, en 1958. Pero nuestra Sociedad tuvo que declinar tal invitación, al no cristalizar las ayudas económicas. Se encargó de celebrar dicho Congreso la Sociedad Sueca en Estocolmo.

Correspondiendo una nueva renovación de la Junta Directiva, y habiendo fallecido su Presidente, ésta tuvo lugar en 1956, resultando elegidos los siguientes socios: Presidente, Arnaldo Socías Amorós; Vicepresidente, Gerardo Clavero del Campo; Secretario, Lorenzo Vilas López; y Vocales, Genaro Alas Cores, Gabriel Colomo de la Villa, Emilio Luengo Arroyo y Florencio Moreno de Vega y Soler.

FIG. 2. Edificio del actual Centro de Investigaciones Biológicas, cuando era sede del Instituto «Jaime Ferrán» de Microbiología, CSIC, al principio de la década de los sesenta.

Las gestiones realizadas por el Prof. Paul Hauduroy en favor de la creación de la Sección Europea de la Asociación Internacional de Sociedades de Microbiología habían dado su fruto en la Reunión celebrada al efecto en 1957 en Dôle (Francia), ciudad donde nació Louis Pasteur. Allí fueron aprobados los correspondientes Estatutos por los representantes de las Sociedades Europeas, asistiendo J. Oliver Suñé, de la Delegación de Barcelona, como representante español.

De nuevo correspondió la renovación parcial de la Junta Directiva en 1958, habiendo quedado vacante la Presidencia por fallecimiento de Arnaldo Socías en 1957. Tras las correspondientes votaciones fueron elegidos: Presidente, Gerardo Clavero del Campo; Tesorero, Miguel Benlloch Martínez; Bibliotecario, Ricardo Salaya León; y Vocales, Eduardo Gallardo Martínez, José García Bengoa, Juan Manuel Martínez-Arroyo Nuñez y Miguel Rubio Huertos.

En 1960 se nombró al Presidente de la Sociedad Española representante de la misma en la IAMS; también se le nombró representante en la Sección Europea recién fundada. Román de Vicente Jordana fue nombrado Delegado para la organización del VIII Congreso Internacional de Microbiología en España. A pesar de las gestiones realizadas, dicho congreso no pudo realizarse en nuestro país y tuvo lugar en Montreal (Canadá) en 1962. También en 1960 Julio Rodríguez Villanueva fundó la Colección Española de Cultivos Tipo (CECT), en el Instituto «Jaime Ferrán» de Microbiología del CSIC, fusionando la colección de hongos de Carlos Ramírez con la de levaduras de Enrique Feduchy y juntándolas con las cepas procedentes de la Estación de Fitopatología de Oeiras (Portugal), traídas por él mismo y después por Juan Antonio Leal Ojeda. La CECT ofrecía a los microbiólogos españoles las cepas que requirieran. En 1962, J. Rodríguez Villanueva organizó en Madrid el I Curso de "Bioquímica de Microorganismos" y la I Reunión Científica de Microbiólogos Españoles.

La I Reunión Científica de Microbiólogos Españoles

La ceremonia de apertura de la I Reunión Científica de Microbiólogos Españoles tuvo lugar el 22 de noviembre de 1962 en el Salón de Actos del edificio central del CSIC (calle Serrano, 127, Madrid). La introducción estuvo a cargo de Julio Rodríguez Villanueva y de Lorenzo Vilas, como Organizador de la Reunión y Secretario de la Sociedad, respectivamente. Pronunció unas palabras de apertura el Director General de Sanidad, J. García Orcoyen. Las sesiones del congreso se dividieron en tres Secciones: (i) Morfología, Sistemática, Nutrición, Química Microbiana y Microbiología Industrial; (ii) Microbiología de Alimentos, Microbiología Clínica, Virus y Protozoos; y (iii) Microbiología del Suelo y Fitopatología. Asistieron más de un centenar de microbiólogos de toda España, que presentaron 80 comunicaciones orales. Esta primera reunión de microbiólogos españoles sirvió de germen de los posteriores Congresos Nacionales. Alcanzó un gran éxito científico, y fue el inicio de unas fructíferas relaciones entre la universidad, el CSIC y la industria (Fig. 3).

El período 1963–1969

El período que se reseña corresponde al tiempo comprendido entre la I Reunión Científica de Microbiólogos Españoles y el II Congreso Nacional de Microbiología, celebrado en Madrid en

FIG. 3. Julio Rodríguez Villanueva y su equipo de investigación durante la I Reunión de Microbiólogos Españoles (Madrid, 1962). De izquierda a derecha, Juan Antonio Leal, Gregorio Nicolás, Concepción García Mendoza, Raúl Margarida, Emilio Muñoz, María del Carmen Romero, Isabel García Acha, Julio Rodríguez Villanueva, Antonio Martín González, Encarnación Estévez, Carlos Hardisson, María José Rodríguez Aguirre, Cándida Carreño y Santiago Gascón.

noviembre de 1969, en el cual se acordó celebrar estos congresos nacionales cada dos años. La Sociedad de Microbiólogos Españoles continuó con sus actividades habituales, reanudando, durante las reuniones periódicas de la Junta Directiva, las Sesiones Públicas de presentación de comunicaciones orales. Éstas continuaron publicándose en *Microbiología Española*. También organizó el II Curso de "Bioquímica de Microorganismos" (Madrid, 1964) dirigido por Julio Rodríguez Villanueva, quien continuó esta labor en los cursos que siguieron, realizados ya en la Universidad de Salamanca.

Una vez más, la Sociedad intentó organizar el X Congreso Internacional de Microbiología, obteniendo en 1963 la correspondiente autorización del Ministerio de Educación Nacional. Y aunque comenzó a planificar su celebración para 1970, el congreso, finalmente, tuvo lugar en México.

En 1964 la Sociedad tuvo que adaptar sus Estatutos a la nueva Ley de Asociaciones, y aplazó la renovación de su Junta Directiva hasta la aprobación de los mismos. Ese año se procedió al nombramiento como Socio de Honor de H. J. Bunker (Gran Bretaña). El CSIC continuó apoyando las actividades científicas de la Sociedad y así designó a su Secretario, Lorenzo Vilas, representante en el Congreso Mexicano de Microbiología, en 1964.

Con motivo de la celebración del IX Congreso Internacional de Microbiología, en Moscú, en 1966, la Sociedad organizó en Madrid una Reunión de todos los microbiólogos de habla hispana asistentes al mismo, así como un simposio sobre "Bacterias Fitopatógenas". Durante el mismo año se procedió al nombramiento de Socios de Honor a los siguientes microbiólogos hispanoamericanos: Luis

Felipe Bojalil, Carlos Casas-Campillo, Adolfo Pérez Miravete, Enriqueta Pizarro, Manuel A. Rodríguez y Gerardo Varela, de México; Antonio L. Briceño Rossi y Otto Núñez Montiel, de Venezuela; Fernando Montero Gei, de Costa Rica; Armando Parodi.y Casimiro Rechniewski, de Argentina; y Manuel Rodríguez Leiva, de Chile.

La renovación correspondiente de la Junta de Gobierno dio como resultado la siguiente constitución: Presidente, Gonzalo Urgoiti Somovilla; Vicepresidente, Román de Vicente Jordana; Secretario, Miguel Rubio Huertos; Tesorero, Enrique Feduchy Mariño; Bibliotecario, Álvaro Zugaza Bilbao; y Vocales, Ángel Fernández Nafría, Dimas Fernández-Galiano Fernández, Sebastián Miranda Entrenas, Julio Pérez Silva, Agustín Pumarola Busquets, Benito Regueiro Varela, Julio Rodríguez Villanueva y Fernando Ruiz-Falcó López.

Continuando con la cooperación internacional, y con motivo de la asistencia de Miguel Rubio, Román de Vicente y Lorenzo Vilas al IV Congreso Latinoamericano de Microbiología, celebrado en Lima en 1967, se acordó que se discutiera con los asistentes al mismo la posibilidad de constituir una Federación Latinoamericana de Sociedades de Microbiología, a la que perteneciera nuestra Sociedad. En esta misma línea de proyección internacional, Julio Rodríguez Villanueva, Catedrático de Microbiología de la Universidad de Salamanca desde 1967, donde trasladó la CECT, estaba organizando allí el "III International Symposium on Yeast Protoplasts" para 1972, para lo cual solicitó el patrocinio de la Sociedad.

Durante 1968 el Secretario de la Sociedad, Miguel Rubio, miembro entonces de la Comisión de Revistas del «Patronato Ramón y Cajal» del CSIC, ofreció a la Junta de Gobierno de la Sociedad su colaboración para tratar de potenciar la revista *Microbiología Española*. Tal como se había propuesto en anteriores reuniones, se decidió la creación de un Comité de Selección de los trabajos a publicar en la revista, con objeto de mejorar su contenido y ampliar sus horizontes. Este Comité de Selección quedó constituido por las siguientes personas: Julio Pérez Silva, Julio Rodríguez Villanueva, Miguel Rubio, Fernando Ruiz-Falcó, Luis Sánchez, Gonzalo Urgoiti, David Vázquez, Román de Vicente y Lorenzo Vilas.

Paralelamente, Álvaro Zugaza propuso, y así se aprobó, el desarrollo de un curso teórico-práctico sobre "Antibiogramas", a cargo de Jorge López Tello, patrocinado por la Sociedad, que se impartiría conjuntamente en el Centro de Investigaciones Biológicas y en los laboratorios de Antibióticos, S.A., de Madrid. Se acordó igualmente, a requerimiento de W. J. Nickerson, de la Rutgers University (EE. UU.), que la Sociedad colaborara en un homenaje a Selman A. Waksman, Socio de Honor de la misma, con motivo de su octogésimo aniversario. El homenaje consistió en la publicación de un artículo en *Microbiología Española* sobre su persona y trayectoria científica. Igualmente se decidió que se publicarían sendas semblanzas sobre los también Socios de Honor Howard W. Florey y Paul Hauduroy, recientemente fallecidos.

Ante la necesidad de celebrar una Reunión Científica anual para presentar los distintos trabajos de investigación realizados, cada vez en mayor número, se decidió organizarlas sucesivamente en distintas ciudades españolas. En estas fechas, la IAMS pidió la colaboración de las sociedades constituyentes, entre ellas la nuestra, para modificar su estructura debido al activo crecimiento de sus Secciones y Comités y, decidió que la reestructuración final tuviera lugar en 1970, durante el X Congreso Internacional de Microbiología en México. Por otro lado, la IAMS había solicitado de la

Sociedad el nombramiento de representantes en las Secciones de Higiene y Microbiología Alimentaria y de Virología, para lo cual se designaron a P. Alonso Carrión, para la primera, y a Florencio Pérez Gallardo, Fernando Ruiz-Falcó, Ángel García Gancedo, Miguel Rubio Huertos y Dionisio López Abella, para la segunda. Finalmente se acordó que la Sociedad celebrara la ya previamente acordada II Reunión Científica con anterioridad al Congreso Internacional de México, al cual había sido invitada nuestra Sociedad. Se decidió que dicha reunión, considerada ya Congreso Nacional de Microbiología, el segundo, tuviese lugar en Madrid en noviembre de 1969, y que fuera organizada por el entonces Secretario de la Sociedad Miguel Rubio Huertos.

Así mismo la Sociedad intervino en la discusión de los Estatutos de la recién constituida Federación de Sociedades Latinoamericanas de Microbiología, en cuya gestación había participado activamente, aceptando la invitación recibida para asistir en 1971 al V Congreso Latinoamericano, en Uruguay.

El II Congreso Nacional de Microbiología

El II Congreso Nacional de Microbiología se desarrolló desde el 20 al 22 de noviembre de 1969 en la sede central del CSIC. La sesión de apertura estuvo a cargo del Director General de Sanidad y del Presidente de la Sociedad. Se pronunciaron cuatro conferencias plenarias, por parte de Julio Rodríguez Villanueva, Lorenzo Vilas, Rafael Gómez Lus y Gonzalo Piédrola Gil. Se presentaron más de un centenar de comunicaciones orales repartidas en las siguientes Secciones: (i) Fisiología Microbiana y Biología Molecular; (ii) Virus; (iii) Microbiología General; (iv) Inmunología y Microbiología de Patógenos; y (v) Antibióticos. Durante el II Congreso Nacional se decidió que estas reuniones se celebrasen con carácter bianual, acuerdo que se ha venido cumpliendo hasta la fecha.

En la Asamblea General celebrada durante el II Congreso Nacional, contando con el apoyo mayoritario de sus Socios, se cambió el nombre de Sociedad de Microbiólogos Españoles por el de Sociedad Española de Microbiología. Se renovó parcialmente la Junta Directiva, siendo elegidos: Vicepresidente, Agustín Pumarola Busquets; Secretario, Julio Pérez Silva; y Vocales, Vicente Callao Fabregat, Andrés Chordi Corbo, José Manuel Gómez Jiménez de Cisneros, Antonio Portolés Alonso y Juan Santa María Ledochowski. Se decidió, además, crear Secciones de trabajo dentro de la misma Sociedad. Dicha organización, junto con el cambio de nombre, requerían una modificación de los Estatutos.

Continuidad de logros de la SEM

Continuando sus actividades habituales, se aprobó entonces la propuesta de Jorge López Tello, en nombre del Comité Organizador del I Simposio Internacional sobre "Antibiogramas", para que la Sociedad tuviera una participación activa en estos simposios. Estando también próxima la celebración del X Congreso Internacional de Microbiología, en Méjico, la Sociedad nombró representantes en dicho Congreso a Gonzalo Urgoiti Somavilla, Agustín Pumarola Busquets y Julio Rodríguez

Villanueva. Igualmente se nombró representantes españoles en el Comité Permanente de Documentación Microbiológica e Inmunológica a Fernando Baquero Mochales, Antonio Portolés Alonso y Román de Vicente Jordana.

La enmienda de Estatutos anteriormente citada, relativa al cambio de nombre de nuestra Sociedad y a la creación de Secciones de trabajo dentro de la misma, fue sometida a aprobación en la correspondiente Asamblea General Extraordinaria celebrada en Madrid en abril de 1970. Las modificaciones propuestas fueron aceptadas por unanimidad, excepto en lo referente a las siglas SEM. El cambio de organización por el cual la Sociedad creaba Secciones de trabajo especializadas llevaba implícita la multiplicación de las actividades científicas, a la vez que daba lugar a la creación de las correspondientes Juntas Directivas, compuestas por Presidente, Vicepresidente, Secretario y Vocales. Paralelamente a estos acuerdos, en la Asamblea Extraordinaria se solicitó la constitución de un Comité de Redacción para la revista *Microbiología Española*, así como la introducción de cuantas modificaciones se considerasen oportunas, incluyendo Normas para la publicación de artículos y particularmente el mantenimiento de su periodicidad, con objeto de aumentar su prestigio internacional. En aquellos momentos la revista actuaba como órgano difusor de la Sociedad, de tal forma que se habían comenzado a publicar, junto con los trabajos originales de investigación, las Actas de la Reuniones celebradas bajo su patrocinio, tales como el "I Simposio sobre Aplicación de la Espectroscopía Infrarroja en Biología Microbiana". Por otro lado, ante el aumento de actividades previstas, el entonces Presidente de la Sociedad, Gonzalo Urgoiti, sugirió la necesidad de publicar un Boletín Informativo independiente de la revista.

La financiación de la representación de la Sociedad en el X Congreso Internacional de Microbiología (Méjico, 1970) corrió a cargo del CSIC, del Ministerio de Asuntos Exteriores y del Instituto de Cultura Hispánica. Durante el mismo tuvo lugar la ya anunciada división de la IAMS en las Secciones de Bacteriología, Virología y Micología, así como la reestructuración de las secciones y comités preexistentes. Se aprobó la organización de Comités, Comisiones y Federaciones, denominadas COMCOFs; todos ellos tenían atribuciones para organizar sus propias actividades científicas de forma independiente.

Prosiguiendo sus relaciones con las Sociedades Latinoamericanas de Microbiología, nuestra Sociedad nombró a Antonio Portolés representante español en el Comité de la Revista Latinoamericana de Microbiología, así como a Julio Rodríguez Villanueva representante de la Sociedad en el anteriormente citado Congreso Latinoamericano de Microbiología (Punta del Este, Uruguay, 1971). En 1971 se nombraron Socios de Honor a Luis J. Archer (Oporto, Portugal), John L. Ingraham (Davis, California, EE. UU.) y Roger Sohier (Lyon, Francia).

En 1971 nuestra Sociedad organizó en Granada, junto con la Sociedad Española de Óptica, el "II Simposio sobre Aplicación de la Espectroscopía Infrarroja en Biología Microbiana", cuya reseña apareció en el volumen correspondiente de *Microbiología Española*. Según el acuerdo adoptado durante el II Congreso, correspondía ese año la celebración del III Congreso Nacional de Microbiología, que fue organizado por Agustín Pumarola Busquets, en Barcelona.

Y hasta aquí, la primera entrega de esta narración, los primero veinticinco años. En sucesivos artículos se continuará este sucinto, personal, a veces involuntariamente inexacto o impreciso —por lo cual, pido disculpas—, pero siempre entrañable intento de hacer una breve historia de nuestra querida Sociedad Española de Microbiología.

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Plasmid transfer during growth and survival of *Escherichia coli*

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Key words: plasmid transfer, *Escherichia coli*, aquatic systems

This study will address the influence of physiological state of parental cells on gene transfer between *Escherichia coli* strains by examining the effect of growth phase and survival in freshwater on conjugal plasmid transfer using several plasmids and hosts.

Various reasons of sanitary and ecological interest highlight the importance of studing and quantifying gene transfer processes in aquatic systems. Among these reasons we can point out the high number of bacteria harboring R plasmid found in aquatic environments and the increased use of genetically engineered microorganisms (GEMs) in human activities such as agriculture or wastewater treatment.

The risk of GEMs accidental release has raised questions about the spread of novel genetic information among aquatic microbial communities and the impact such releases may have on these natural communities. Conjugation certainly seems to be the most important gene transfer mechanisms in nature. Thus, several authors have demonstrated the existence of plasmid transfer by conjugation in aquatic systems (1, 6, 7). However, transfer frequencies calculated in aquatic systems are generally lower than those observed in laboratory or microcosms (6, 7).

We know very little about the mechanisms which promote or impede plasmid transfer in aquatic systems. Not only is this a very complex phenomenon indeed, but it is also very difficult to study, mainly because of the great number of factors which influence the results. In this way, some works (4, 5, 10) have studied the influence of some biotic and abiotic factors. Recently, Muela et al. and Arana et al., studing growth and survival of *E. coli*, have shown that the number of transconjugants formed is greatly influenced by the physiological state of the donor.

The physiological variations undergone by donor cells during growth in culture media have significant influence on plasmid transfer. As we can see (Fig. 1) transfer frequency varies significantly along the growth curves of donor strains.

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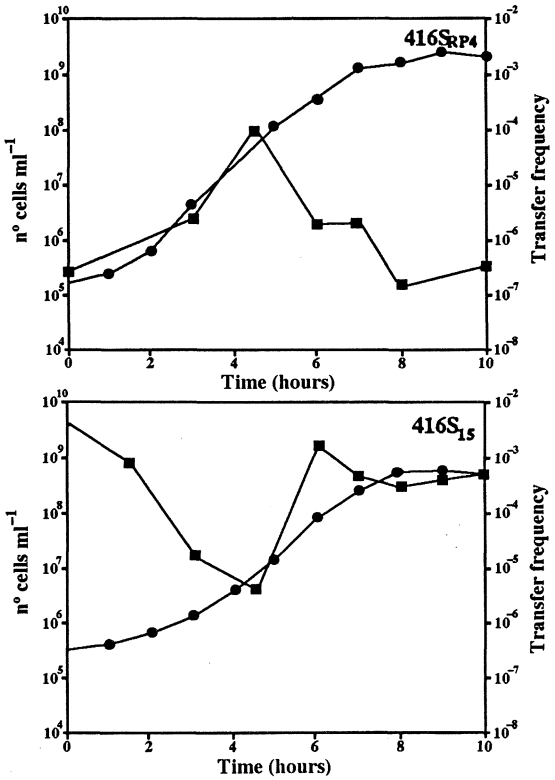

FIG. 1. Changes in transfer frequency (■) and total number of donor cells (●) during the growth of donor strains 416S$_{RP4}$ (upper panel) and 416S$_{15}$ (lower panel).

However, transfer frequency does not evolve in the same way along the growth curve of all the strains studied. In strains 416S$_{RP4}$ and 416S$_{AR4}$, transfer is favoured during the exponential phase. On the other hand, in strains 416S$_{pSU2007}$, 416S$_{15}$, J62$_{15}$ and EC$_{15}$ the highest transfer frequency values appear in the stationary phase and in the lag phase. Thus, the transfer frequency of plasmid material 15 presents a single evolution pattern which does not depend on the plasmid-bearing strain. Moreover isogenic strains (serie 416S) present different behaviour as donors depending on the conjugative plasmid material that they bear. The whole of our results leads us to the conclusion that the evolution pattern of the transfer frequency along the growth of the donor strain is regulated by the plasmidic content and not by the host strain.

As we have seen, transfer of some plasmids becomes relevant under conditions unfavorable for population growth. In bath cultures these conditions might arise several generations before the onset of the stationary phase because of a reduction in the availability of specific nutrients. Extrapolating this fact to other unfavorable situations, plasmid transfer between allochthonous bacteria in aquatic systems would acquire particular relevance. In this way, we have studied the plasmid transfer during long-term survival assays of all the donor strains cited in Table 1.

However, the results obtained during survival in river water do not confirm the hypothesis stated above. Thus, the number of transconjugants formed in all matings decreases from the beginning of the experiments (Fig. 2). In this case, and unlike the results obtained in growth studies, we cannot find different responses among the strains assayed. Fig. 2 suggests that strains harbouring plasmid material 15 are more strongly affected by environmental stress than the others. Thus, with strain 416S$_{15}$, we do not detect transconjugants at the end of the experiment while in the case of strain 416S$_{RP4}$ the number of transconjugants remains high. We can conclude that strain 416S$_{15}$ has lost the conjugal ability whereas strain 416S$_{RP4}$ has just a reduced capacity to conjugate. Nevertheless, a more adequate interpretation of the results will take into account the initial number of transconjugants as well as the final one. Following this analysis, strain 416S$_{RP4}$ loses more than 99% of its conjugal transfer ability during one week in river water. This change pattern is close to that shown by strain 416S$_{15}$.

On the other hand, other variables analyzed in the survival assays (total number of cells, number of culturable cells, glucose uptake rate and percentages of glucose respirated and assimilated) remains constant throughout incubation. According transfer, although we cannot detect any other variations in their physiological state. Moreover,

TABLE 1. Relevant characteristics of *Escherichia coli* strains

E. coli strains	Source	Relevant phenotype*	Plasmid bands
416S	Streptomycin-resistant mutant of STCC 416	$Lac^+Gm^sKm^sTc^sNalA^sSm^r$	No
J62	Laboratory K12 strain	$Lac^-Gm^sKm^sTc^sNalA^rSm^s$	No
EC_{15}	Environment	$Lac^+Am^rGm^rTc^sNalA^sSm^s$	Yes
$416S_{15}$	$EC_{15} \times$ 416S matings	$Lac^+Gm^rKm^sTc^sNalA^sSm^r$	Yes
$J62_{15}$	$EC_{15} \times$ J62 matings	$Lac^-Gm^rKm^sTc^sNalA^rSm^s$	Yes
$416S_{pRP4}$	$HMS174_{pRP4} \times$ 416S matings	$Lac^+Gm^sKm^rTc^rNalA^sSm^r$	Yes
$416S_{pSU2007}$	$D1210_{pSU200} \times$ 416S matings	$Lac^+Gm^sKm^rTp^rNalA^sSm^r$	Yes
$416S_{AR4}$	Exogenus isolation	$Lac^+Km^sTc^sHg^rNalA^sSm^r$	Yes

* Abbreviations: Lac, lactose; Am, ampicillin; Gm, gentamicin; Km, kanamycin; Tc, tetracycline; Tp, trimethoprim; Hg, $HgCl_2$; NalA, nalidixic acid; Sm, streptomycin; s, sensitive; r, resistant.

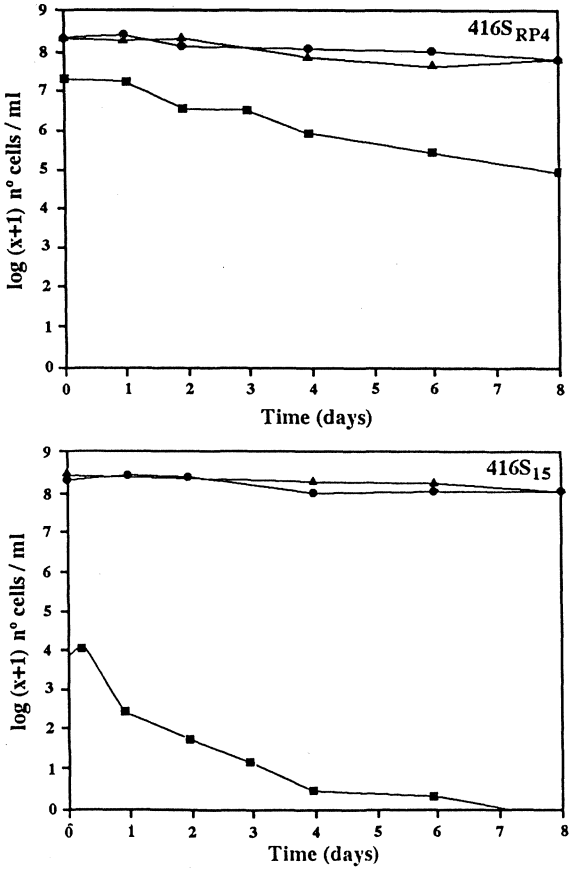

FIG. 2. Changes in colony forming units during long-term survival in sterile river water of donor strains. (●), CFU on TSA; (▲), CFU on TSA supplemented; (■), transconjugants.

this failure in the transfer process cannot be attributed to a total loss of plasmids, since throughout the survival period we verified the culturability of cells on media supplemented with marker antibiotics. In other words, the maintenance of plasmids by donor cells during survival in freshwater does not necessarily imply their transference. Molecular studies on conjugative transfer of IncP plasmids have discovered control circuits which limit the transfer of gene expression and which may provide a switch between genes for vegetative replication and conjugative transfer. In the same way, earlier studies observed that a loss of F pili was linked to the loss of donor ability in F^+ and Hfr strains. This could simply be a means of ensuring efficient maintenance of the transfer apparatus under unfavorable situations without overburdening the host with excess gene expression.

If we consider the physiological variations undergone by *E. coli* in responding to environmental stress (2, 3), it is conceivable that not only donors ability but recipients ability might also be affected. However, according to our results, the physiological state of recipient cells does not seem to influence the number of transconjugants ren-

dered. Thus, we do not detect significant differences in transfer frequency along the growth cycles of the two recipient strains, 416S and J62, studied. In the same way, and unlike the results obtained with donor cells, the capacity of recipient cells to receive and express plasmids does not vary during one week in river water.

The variety of the results presented prevents the description of a unique pattern of behaviour in the plasmid's ability to be transmitted from cell to cell. To explain the behaviour variability, some authors (8) have pointed out the external energetic disponibility and the physiological state of the donor cells (internal energy). In our opinion, there would be other factors inherent to the plasmid material that also regulate the transfer process during growth and survival. Anyway, when it comes to evaluating the risks of dissemination of genetic material as a consequence of GEMs release to the environment, we think it should not be generalizated and each case should be studied individually. Moreover, we must not forget that although the capacity to donate decreases as time passes, it does remain for a reasonably long period, during which recipient cells retain their capacity to receive and express plasmids.

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Regulation mechanisms of the gene expression involved in ciliate cryptobiosis

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Key words: gene expression, ciliate cryptobiosis, *Colpoda inflata*

Cryptobiosis is a very complex cell differentiation process which appears in both prokaryotic and eukaryotic systems. In ciliates, the cell differentiation that involves resting cyst formation (cryptobiosis) is called "encystment" and the opposite process, the emergence from the cryptobiotic state (resting cyst) is named "excystment". Both processes constitute a cycle—the E-E (encystment-excystment) cycle—which presents two stable differentiated states, the vegetative and the cystic or cryptobiotic one. This facultative cycle is connected to the growth-division cycle of the ciliate, which can "jump" from one cycle to another when the inducer conditions appear. This "leap" involves the "switch on" of the genetic program involved in the macronuclear biosynthesis for the cryptobiotic state formation (2). During ciliate encystment, extensive structural and physiological changes take place. They can be summarized in the following points:

(i) A drastic decrease of cellular volume takes place, which is a consequence of the loss of intracellular water.

(ii) Partially permeable barriers (cyst wall) are produced. The distinct cyst wall layers are derived from different precursors, which are formed in the precystic cytoplasm (1, 4, 8). Cyst wall polypeptides have been studied by using different methods (3).

(iii) Organelles cluster as a consequence of cytoplasmic dehydration (8).

(iv) Ciliate encystment depends on both RNA and protein synthesis (2).

(v) A high autophagic activity is present during ciliate encystment. By means of this autolytic process the starved cell probably maintains a basic metabolism that helps to synthesize the necessary macromolecules to form the cyst, and to eliminate some cellular elements unnecessary in the resting state (e.g. in the majority of ciliates the cortical structures, involved in the cell mobility and/or the uptake of food, are totally or partially destroyed.

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Also, in some ciliates, like colpodids, macronuclear extrusion bodies takes place during encystment, which is destroyed by an autophagic activity).

(vi) A high macronuclear chromatinic condensation is produced in the majority of encysting ciliates. This nuclear condensation may involve the transcriptional inactivation of the ciliate genetic system in the cryptobiotic state.

For more than ten years, we have studied the encystment process on the ciliate *Colpoda inflata*, therefore this discussion will be mainly focused on this ciliate.

According to a generalized ciliate encystment model (2); the exogenous inducer (nutritional deficiency), triggers the "turn off" of some vegetative genes involved in the growth-division cycle and the "turn on" of encystment genes. This activation of encystment genes involves the appearance of some new transcripts, which are necessary to synthesize the cystic elements (such as cyst wall proteins). Therefore, we actually think that a specific gene expression takes place during the ciliate encystment.

How many genes are involved in this process? How is their expression regulated? These and other basic related questions are still unknown.

In the last few years, we have tried to answer some of these questions using the *C. inflata* encystment model. Actually, we believe that, at least, three different regulation mechanisms of gene expression might be involved in the ciliate encystment.

The first one involves DNA macronuclear methylation pattern changes during the encystment process (5). DNA demethylation during cell differentiation of higher eukaryotic cells has been reported (6), and many studies have established a correlation between undermethylation and unimpeded gene expression.

Restriction patterns of vegetative cells and resting cysts have shown some differences with regard to HhaI and MspI enzymes, indicating that resting cyst macronuclear Ma-DNA is demethylated in those sequences compared to the vegetative stage. Experiments with 5-azacytidine (a potent demethylating agent) experiments corroborate this hypothesis. From these results we conclude that, as in other eukaryotic cell differentiation processes (e.g. encystment process of the myxomycete *Physarumflaviconum* [9]), a specific genomic DNA demethylation process could be involved in the activation of ciliate encystment genes. Also, a methylation of some unnecessary genes (like ribosomal DNA) has been detected by using a specific 18S rDNA probe.

Summarizing, we have, for first time, experimental evidence involving DNA demethylation and methylation during ciliate encystment. We believe that activation of some encystment gene promoters is probably achieved by a specific Ma-DNA demethylation, and the inactivation of vegetative genes unnecessary for encystment occurs by DNA methylation. Likewise, DNA methylation could be involved in the chromatin reorganization that takes place, at Ma-DNA level, during encystment.

The second control mechanism involves variation in transcript abundance during encystment. For first time in ciliate encystment, we have detected a specific transcript accumulation into the resting cysts of *C. inflata*, which is probably synthesized during encystment. The specific cDNAs we obtained from mature resting cysts shows the presence of poly(A)$^+$RNA in the dormant stage. After hybridization on cDNA populations from precystic and vegetative cells, by using a probe originated from a resting cyst cDNA polymorphism, the presence of this transcript has been detected in precystic and vegetative cells, but it is accumulated in resting cysts.

We still do not know if this transcript is involved in the encystment or excystment process. But, in any case, a control of transcript number is probably present in the encystment of *C. inflata*.

The last control mechanism that we wish to consider here that is involved in ciliate encystment is chromatin condensation. In the last phase of encystment, the Ma-DNA undergoes a high condensation, which probably results in macronuclear genome inactivation. This is a usual characteristic of any microbial cryptobiotic form. It can be obtained by a double mechanism; as a consequence of the cytoplasmic dehydration and/or a specific biosynthesis of basic nuclear proteins.

By using both, the spreading chromatin method and standard transmission electron microscopy we have studied the Ma-chromatin changes during the encystment of *C. inflata*. Active chromatin has been mainly detected in vegetative cells. For the second time in ciliates (7), a very special structural organization of the Ma-chromatin (hexagonal or polygonal lamellaes) has been observed in the resting cyst macronucleus. These polygonal chromatin forms, especially abundants in resting cysts, might represent a highly condensed transcriptionally inactive state of the Ma-chromatin, indicating that the mature resting cyst is a transcriptionally inactive stage.

These three control mechanisms of gene expression might be present in different phases of the encystment process. Methylation pattern changes could occur during the initial phases of encystment, after the cell response to the exogenous inducer. The control of the abundance of some specific transcripts probably is developed during encystment, with their accumulation in the resting state involving the presence of some protective structure against ribonuclease activities. Finally, the Ma-chromatin condensation is only present in the definitive dormant form or last phase of encystment. This microbial eukaryotic differentiation process must be highly controlled at both the levels of transcription and DNA conformation.

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Protein kinases and phosphatases during the developmental cycle of myxobacteria

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Key words: protein kinase, phosphatase, myxobacteria

Myxobacteria are rod-shaped Gram-negative bacteria that show several striking and unique features that make them different from the other bacteria (2). They are able to degrade a wide variety of complex molecules and even living organisms; they glide on the surface of a solid medium or in the interface air–water; and they show a spectacular social behavior and morphogenetic potential. Myxobacteria are always found as communities of cells known as swarms, preying on other microorganisms or on complex macromolecules.

Under starvation conditions, cells start to glide to certain points where they pile on top of each other to eventually construct macroscopic structures known as fruiting bodies. Inside the fruiting bodies cells differentiate to originate myxospores, which are resistant to several stress conditions such as desiccation, sonication, and UV irradiation. Myxospores are usually shorter than the vegetative cells and are covered by a thick coat.

To undergo this spectacular cell cycle cells must somehow communicate with one another. Attempts to explain the mechanisms of intercellular communication in myxobacteria are currently carried out in several laboratories.

The cooperative feeding and development of myxobacteria require the coordination of many cells. This coordination is possible by the exchange of several extracellular signals. Five different types of signal-defective mutants have been isolated. These mutants are unable to sporulate when they are plated alone, but sporulation can be rescued just by adding wild-type cells. The mutants have been referred to as *asg*, *bsg*, *csg*, *dsg* and *esg*, and the extracellular signals to which they are dependent on as A, B, C, D and E factors (1, 3).

Although there is much information about the signals that act during development and the genes whose expression depends on the transmission of these signals, we do not know the pathways used by myxobacteria from signals to gene expression. In other words, we do not know how the signals are

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transduced through the membrane to regulate gene expression.

The social behavior of myxobacteria and the similarities of their developmental cycle to that of *Dictyostelium* led to speculate that they could use signal transduction systems similar to those reported in eukaryotic cells consisting of receptor-G protein-effector and protein kinase. One attempt to identify a eukaryotic-like signal transduction pathway in *M. xanthus* was focused in the detection of protein kinases (4). By polymerase chain reaction (PCR) using primers designed according to the conserved regions of eukaryotic protein kinases and the entire *M. xanthus* chromosome as a template, three different products were amplified. Analysis of the sequence of these three products revealed a high similarity to protein kinases. Further hybridization analysis showed that *M. xanthus* may possess 26 different protein kinases (7). Protein kinases with sequences similar to those of eukaryotic cells had not been identified in bacteria previously.

By using the PCR products as probes, the entire genes that encode these protein kinases have been cloned. These genes are referred to as *pkn1*, *pkn2* and *pkn3*. *pkn1* encodes a protein of 693 residues (4). The amino terminal portion of this protein contains the eleven subdomains conserved in the eukaryotic protein kinases, while the C-terminal portion shows no similarity to any other known protein. *pkn1* has been expressed in *E. coli* cells and it has been shown that Pkn1 autophosphorylates at both serine and threonine residues, but not at tyrosine. *pkn1* is developmentally regulated to be induced at the onset of sporulation, reaching a maximum at 50 h and staying at a nearly constant level thereafter (4). In order to investigate the function of Pkn1, a strain harboring a deletion in the *pkn1* gene has been constructed. This strain is able to aggregate and sporulate; however, the fruiting bodies are smaller and less compact than those obtained with the wild-type strain. The yield of myxospores of the mutant is only 35% (4).

pkn2 encodes a protein of 830 amino acid residues. Pkn2 consists of an amino terminal portion with similarities to the catalytic domain of eukaryotic protein kinases and Pkn1, and a C-terminal portion with no similarities to any other known protein (5). In the middle of this C-terminal portion there is a typical transmembrane domain consisting of 19 hydrophobic residues. Analysis of *phoA* fusions with *pkn2* has shown that Pkn2 is indeed a transmembrane protein, with the kinase domain located in the cytoplasm and the C-terminal domain located outside the cytoplasmic membrane. Pkn2 has been overproduced in *E. coli*, and it has been found that it is able to autophosphorylate at both serine and threonine residues. Pkn2 is also able to phosphorylate β-lactamase but only at threonine residues. Phosphorylation of β-lactamase shifts its apparent molecular weight from 29 to 44 kDa, and prevents its secretion to the periplasmic space.

pkn2 is expressed only during vegetative growth, obtaining a peak at the beginning of the stationary phase. Expression then drops sharply to a basal level. Expression also drops to a basal level just after starvation. However, studies with a strain harboring a disruption mutation of *pkn2* have shown that the mutant shows no phenotype on rich medium; while during the developmental cycle, fruiting bodies appears loosely packed. The yield of myxospores of the mutants is approximately 30–50% of the level of the wild-type strain.

The finding of eukaryotic-like protein kinases involved in the regulation of the developmental cycle of myxobacteria implies the existence of other proteins able to dephosphorylate the substrates phosphorylated by the protein kinases, phosphatases. In myxobacteria it has been reported several proteins with phosphatase activity, some of which are induced during the developmental cycle (6). However, we do not know whether

they are the counterpart of the protein kinases. We do not know either the specificity of these phosphatases. Are they eukaryotic-like protein phosphatases or are they similar to unspecific acid, neutral and alkaline phosphatases? In order to elucidate these questions, we have cloned in our laboratory four genes that encode proteins with phosphatase activity. Characterization of these genes will help to clarify the role that phosphorylation/dephosphorylation play in the regulation of the developmental cycle of the myxobacteria.

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Intraspecific diversity in Bacteria. The case of *Escherichia coli*

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Key words: bacterial species, ribosomal RNA, *Escherichia coli*

The study of intra-species diversity is an essential condition for understanding how evolution takes place in Bacteria. This is of paramount importance to predict the future behaviour of these microorganisms in critical issues such as chemotherapy resistance or their pathogenicity and virulence. The models developed from the population genetics of multicelular organisms with sexual reproduction cannot be applied here. However, the problem has received very little attention in the past. Even so, during the last 20 years some information has accumulated, fundamentally from multilocus enzyme electrophoresis (MLEE) data. This method consists simply of detecting the various alleles existing for a determinated enzyme by their different mobility in starch gel electrophoresis, which separates raw proteins by their isoelectric point, after which the protein's position in the gel is located by an enzyme-specific developing system. Thus the different alleles (different sequences) migrate in distinct ways and can be directly detected. In this way up to 40 different enzymes can be processed, the majority of them belonging to the central (house keeping) metabolism of the cell. It has been shown that many bacteria, e.g. *Escherichia coli*, have a relatively undisturbed, clonal mode of propagation maintaining strong linkage disequilibrium of genetic markers (specific combinations of alleles are maintained for important sections of the population) showing that recombination between clonal lineages is low (10). Other species such as *Neisseria gonorrhoea* seem to behave differently, with highly frequent recombination that leads to a population structure described as panmictic by some authors (11). More recently, important information has been obtained by sequencing the same gene in representative isolates of the same species (1, 2, 8, 9). Nevertheless many questions still remain unsettled and different authors express almost opposite interpretations of the same results (6, 11). In particular, as to what degree the purely

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clonal model is altered by horizontal gene transfer and what is the situation in different groups of bacteria. MLEE has the disadvantage that only a relatively small section of the bacterial genome can be studied, normally house keeping enzymes, and besides, many allelic variations can pass unnoticed. With regard to sequencing, the number of strains-sequences that can be studied with a reasonable amount of time and effort is relatively small.

Molecular methods allow extremely fine strain typing that can be used to establish the population structure of bacterial species. The recently developed technique of random amplified polymorphic DNA (RAPD) provides an effective method for obtaining genetic markers in all sorts of organisms. The RAPD method identifies polymorphisms that are detected as DNA fragments amplified by the *Taq* polymerase chain reaction (PCR). Generally short oligonucleotides (about 10-mer) of arbitrary nucleotide sequence are used with a relatively low annealing temperature that allows priming of sequences to which partial matching exists. The result is a pattern for each primer, of amplified bands that often are present in one but not another individual or strain. In this sense RAPD is a methodology of total genome typing comparable to PFGE but with the advantage of being simpler, and that makes it very suitable for populations genetics and evolutionary studies because many individuals can be assayed, as is normally necessary in this kind of study. The number of different primers that can be used is virtually unlimited and therefore so is the amount of information. It has been shown that each RAPD band is a reliable genetic marker (a specific allele at a certain locus) the absence of the band in a strain is interpreted as an allelic variation. That has been shown, for example, by the overlap found between MLEE and RAPD in a paper in which clonality of populations of *Trypanosoma cruzy* was ascertained by RAPD analysis. When sufficient number of random primers is used, RAPD can typify a bacterial strain to the level of defining individual clones (only individuals sharing a very close common ancestor show identical RAPD patterns), as has been found for macrorestriction patterns.

The 16S rDNA-23S rDNA spacers are short stretches of DNA located in the rRNA operons and most of which represent non-coding DNA of unknown function. As such these sequences are relatively variable and variation in restriction patterns can be a good marker of major intraspecies lineages, in fact its discriminatory potential is similar to that of ribotyping.

Finally, the presence or absence of G adhesins in *Escherichia coli* as well as the typer of adhesin (three types are known) can be shown by PCR amplification followed by digestion with restriction enzymes. The possession of one type or other of adhesin genes has been considered as a trait that can be easily won or lost, and horizontally transferres. We have used the techniques described above to characterize a collection of uropathogenic *E. coli*. A total of 80 strains have been studied, obtained from three hospitals located in geographically distant Spanish towns, some representative of the ECOR collection and other reference strains have been included.

As expected, a wide diversity was shown by RAPD and only in the case of strains isolated from the same individual, an obvious case of relapse, were identical patterns found. Furthermore, the distribution of RAPD band frequencies shows that less than half the bands were present in more than 50% of the strains. This picture has been found previously by methodologies such as PFGE. It illustrates the extremely high genetic diversity of *E. coli* and other bacteria. In fact, in a study carried out with descendants of the original strain K12 obtained over a span of 30 years, of 150 RAPD bands 8 were polymorphic (3). Therefore, it is to be expected that the number of clones with shared ancestry old enough to have diverged significantly

to give different RAPD patterns is so high that the probability of finding the same clone twice by a merely stochastic process is virtually null. That is true even considering a subpopulation of the species such as the strains able to induce urinary tract pathology (4).

The RFLP of the 5S–16S rRNA spacer appears to be a good and reproducible methodology for typing the *E. coli* strain studied, as shown for other species (5, 7). As expected, a number of strains shared the same spacer RFLP, the largest group was composed of 24 strains with identical pattern. In this characteristic PCR-ribotyping has a discriminatory capability similar to MLEE. Both RAPD and spacer restriction patterns originated similar clusters of strains showing a consistency in the evolution of the global genome with the sequence variation of the ribosomal spacers. In both cases two main clusters that we have named α and β appeared well defined. The consistency of the two groups regardless of the markers or group of strains used is very high, indicating that this grouping actually reflects a natural subdivision of the species. This again suggests that the total genome is evolving synchronized with the ribosomal operon, and strengthens the general impression of linkage disequilibrium that characterizes *E. coli* even within the two main RFLP lineages i.e. horizontal genetic exchanges is rare even inside those two groups taken separately. The references strains appear equally distributed in the two groups showing that both groups have a wide host distribution, the ECOR representatives used were isolated from humans (ECOR 10, 45, 35 and K12), cougar (ECOR 44), orang-outang (ECOR 52) and lion (ECOR 58).

Although some special characteristics should be required to produce the urinary tract pathology, the clustering of the strains with the different ECOR representatives that are a sample of faecal *E. coli* of very different origin seems to indicate that most faecal clone lineages have representatives capable of causing UTI.

The adhesin type correlated with the spacer polymorphism as well, most of the strains having adhesin G corresponded to a single rRNA spacer group with only a few exceptions that could well correspond to the putative horizontal transfer of these genes. It is remarkable that the presence of G adhesin and the involvement in upper urinary tract infections is tightly coupled to the α group (of 7 pyelonephritis cases detected in the course of this study, 6 were caused by strains of Group α while of 10 instances of cystitis detected 7 were produced by strains in Group β). This illustrates that in spite of the proven transferability of the adhesin genes, this happens rarely between strains of the α and β groups and that could be true of the other horizontally transferable markers. In fact, the differences found between α and β could be explained by a barrier to horizontal genetic transfer between the two groups—due, for example, to a different restriction modification system, sensitivity to phages, or recognition by sexual fimbriae. In fact α and β groups could well be populations on their way to becoming new species if the stablishment of recombinative barriers is part of the speciation process in bacteria, as has been suggested (6).

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Genetic bases of the chromosomal polymorphism of industrial yeasts

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Key words: chromosomal polymorphism, industrial yeast, Ty and Y' elements

Several industrial *Saccharomyces* strains including bakers', wine, brewers' and distillers' yeasts have been characterized with regards to their DNA content, chromosomal polymorphism and homologies with laboratory strain genes. Results showed a great polymorphism both in the number and size of the chromosomes and the presence of multigenic families. The variations observed indicated adaptation to specific industrial environments and seem to be caused by chromosome reorganizations during meiosis, mediated by Ty elements which seem to undergo multiple transpositions, and Y' elements which give rise to asymmetrical homologous recombination.

Traditionally, industrial yeasts have been characterized according to their physiological properties such as fermentation and assimilation of carbon and nitrogen sources (2). However, the high variability found in these parameters made it necessary to develop new techniques for strain identification such as chromosome electrophoresis (6). This technique has shown that almost every industrial yeast displayed a characteristic pattern due to the extensive polymorphism existing within and between different industrial yeasts (9). When comparing bakers', wine, distillers' and brewers' yeasts we have also found that polymorphism in the chromosomal patterns was so enormous that each strain could be individually identified. Although the length and number of chromosomes was very variable, no specific changes related to a particular group were observed.

Furthermore, some authors have detected interchromosomal changes (translocations) in adittion to intrachromosomal ones (deletions and duplications) as well as the presence of a variable number of chromosomes with high or low homology in these industrial yeasts when compared to those of haploid reference laboratory strains (6, 9). This homology is such that, although chromosomes from brewers' yeasts have been shown to complement laboratory auxotrophic strains, sequence divergence of homologous DNA was sufficiently high to prevent genetic recombination (7). On the other hand, wine and laboratory strains seem to be genetically quite distinct from bakers' yeast, since there is an apparent incompatibility between nuclear and mitochondrial genomes from

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different sources (4).

In this study, hybridization using different probes from laboratory strains was very strong, indicating that all industrial strains tested possess a high degree of DNA homology with laboratory yeasts. Probes such as *URA3*, *CUP1*, *LEU2*, *TRP1*, *GAL4* or *ADC1* demonstrated the presence of several bands, specially in bakers' strains. This indicates either the presence of several copies of different length of some chromosomes or, less probably, translocations to different chromosomes, following duplication of fragments containing the genes. Results also indicate that these genes are located on the same chromosome both in laboratory and industrial strains. In addition, translocations seem to have occurred in distillers' strains judging by the location of the *CUP1* probe.

Industrial *Saccharomyces* yeasts have been considered for many years as microorganisms which possess an unknown chromosomal constitution and a great deal of genetic heterogeneity (1). Aneuploidy and/or polyploidy among industrial yeasts is so widespread that the maintenance of an unbalanced chromosome set has been suggested to be advantageous (9). The reason is that increasing the number of favourable genes might give rise to more vigorous strains (8). For instance, a good bakers' yeast requires high potential glycolytic activity and high invertase activity. Any chromosome change resulting in increasing growth rate, for instance, the duplication of invertase genes, will be favoured by the artificial selection exerted on these bakers' yeasts, since they are grown in molasses where the main carbon source is sucrose (8). The facts are that the bakers' strains have undergone a selective pressure by mankind favouring those phenotypes with the highest growth rates and productivities in molasses.

When using a *SUC2* probe in this study, results indicate a very widespread presence of this gene only in bakers' and distillers' strains, clearly suggesting gene amplification as an adaptive mechanism allowing greater fitness to their specific industrial conditions. This suggestion has been supported by the following facts: (i) the specific invertase activity detected in the industrial yeasts varies enormously. However, under derepressed conditions bakers' yeasts possess an average of 11 times more invertase than laboratory strains, this increase varying between 7 to 20 fold. Wine yeasts only have a two-fold increase in invertase activity with regards to laboratory strains, data varying from 1 to 5 fold, according to the wine strain. Some distillers' yeasts, which also seem to possess multiple copies of *SUC* genes, have 11 times more invertase activity than laboratory strains. (ii) When grown in molasses, the laboratory strains tested reduced their growth rate to 80% of that in laboratory media; industrial yeasts other than bakers', such as wine yeasts, reduced their growth rate to 45%. However, all the bakers' yeasts tested grow almost at the same growth rate or even faster in molasses than in laboratory medium.

It therefore seems that chromosomal polymorphism can be attributed to the fact that nuclear genes code for genetic functions required for optimal fitness in each industrial environment and, thus, that much of the nuclear genomic polymorphism is under strong selection pressure. Some authors (5) suggested that large-scale rearrangements are invariably the result of the movement of Ty elements into or out of the DNA. In this study, both Ty1 and Ty2 transposable elements were found on many chromosomes in all strains tested. However, both sequences seem to be more frequent in bakers' and to a lesser extent in laboratory yeasts than in wine and brewers' yeast and some of the distillers' strains.

Results also indicate unequal distribution among industrial groups and among different chromosomes of the Ty1 and Ty2 elements. The presence of so many copies of Ty1 and Ty2 elements in most bakers' yeasts is in line with the

great polymorphism found in these strains as compared to other industrial groups. Furthermore, DNA-length polymorphism of sporulation-deficient mutants was described as generated by the loss or addition of 100 kb DNA during meiosis or sporulation and by the presence of Ty elements (5).

Telomeric sequences have also been implicated in generating polymorphism in some organisms. Furthermore, evidence for a human factor in the evolution of *Saccharomyces* derives from the presence of a large number of mainly small chromosomes and the clustering of fermentation markers at chromosome ends, which reflects selection pressure for high fermentation rates and hence, for duplication and crossing-over events (3). When telomeric Y' probes were tested in this study, large differences in both the number of hybridization signals and their intensity among the strains were observed. The telomeric Y'16 probe was very scarce in wine and laboratory yeasts. Y'29 was much less represented in the yeast populations, appearing in most chromosomes of bakers' strains only.

It has been suggested that homologous recombination within subtelomeric repeat sequences contributes to chromosome size differences (3). In this study, data on Y' distribution together with those of the Ty1 and Ty2 elements supports, the high degree of polymorphism found for chromosome pattern in bakers' yeasts and the high copy number found of polymeric gene families such as *SUC2* gene. The widespread presence in the strains of Ty1 and Ty2 elements, and of Y' subtelomeric sequences, is probably the reasons for the inter and intrachromosomal changes detected. This suggestion has been supported by data obtained from meiotic analysis of some of the bakers' yeasts. When complete tetrads were subjected to molecular analysis, results confirmed in some cases segregation of homologous chromosomes of different sizes. However, the presence of chromosomal bands absent in the parentals or the disappearance of bands present in the parental strains was frequently seen. As before, the result was attributed to the presence of Ty transposable elements which seem to undergo interchromosomal translocation, together with amplification, giving rise to the differences in the size of the chromosomes. Multiple Y' subtelomeric regions, also give rise to asymmetrical homologous recombinations. While these phenomena seem to occur with very high frequency during meiosis, mitosis is very stable as judged by the electrophoretic pattern shown by the parental strains in successive generations.

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MICROBIOLOGÍA SEM **11** (1995), 386–388

The yeast *Phaffia rhodozyma* as an industrial source of astaxanthin

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Key words: astaxanthin, carotenoids, *Phaffia rhodozyma*

An important factor affecting consumer acceptance of cultivated salmonids or crustaceans is the color of their flesh or shell. The natural pink color is due to the carotenoid astaxanthin (3,3'-dihydroxy-β,β-carotene-4,4'-dione) a pigment common to some extent in the animal kingdom (e.g. flamingos, salmon and trout, among others) but rarely found in eukaryotic microorganisms (e.g. fresh-water and marine microalgae and the yeast *Phaffia rhodozyma*). This red-pigmented fermenting yeast was isolated in the sixties from exudates of different trees, first named as *Rhodozyma montana* and later (8) renamed after Professor Herman Jen Phaff. Since then the species has been included in the deuteromycetous group but is considered to most likely have a basidiomycetous origin due to its cell wall composition. Most recently, however, Golubev (3) demonstrated the formation of basidiospores when the yeast is grown in the presence of polyalcohols, and went on to propose the genus *Rhodomyces* as the sexual form of *Phaffia*. As indicated before, this peculiar yeast is strikingly different from other basidiomycetous pigmented yeasts, such as *Rhodotorula*, in that it synthesizes and accumulates the carotenoid astaxanthin as the principal carotenoidic pigment, an end product of a putative four-step bioconversion from beta-carotene (7).

For the last two decades there has been much interest in the use of microorganisms as a biological source of astaxanthin for diet formulation in aquaculture. Besides *P. rhodozyma* the microalga *Haematococcus* has been reported as a good source of this carotenoid and recently *Halobacterium salinarium* also demonstrated the ability to readily accumulate the ketocarotenoids 3-hydroxy-echinenone and all-trans astaxanthin. The yeast *P. rhodozyma*, because it exhibits a chemoheterotrophic metabolism, has advatanges over *Haematococcus*. It grows faster (despite it being a moderate psychrophilic microorganism) and gives

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higher biomass yield when growing in glucose or fructose as the sole carbon source and it may be grown in classical batch fermentors. On the other hand *H. salinarium* has as an important advantage over *P. rhodozyma* since it lacks cell wall so the process of astaxanthin extraction is facilitated to a great extent.

P. rhodozyma can be produced at very low cost, using grape must (very abundant in Mediteranean countries) as the raw material. This juice, without any further treatment or chemical addition, is in fact an excellent substrate for yeast growth and for astaxanthin production. It has been shown, however, that unless the yeast is mechanically (6) ruptured or the cell walls enzymatically digested, the carotenoids are not available to either chickens, salmon or rainbow trout. So, the overall cell wall analysis reveals the presence of β-glucose (in the form of 1,3-β-D-glucan and 1,6-β-D-glucan), α-glucose (as 1,3-α-D-glucan) and a quite complex heteropolysaccharide formed by L-arabinose, D-xylose, D-glucuronic acid and D-galactose. This peculiar cell wall makes *P. rhodozyma* resistant to enzymic digestion with zymolyase or any other 1,3-β-D-glucanase including snail juice, being partially digested with complex lytic media from the bacterium *Bacillus circulans* WL-12 (4, 5) or from the filamentous fungus *Trichoderma viride*. It was previously known that after feeding rainbow trout or salmon with undigested *P. rhodozyma*, the animals were able to take up but a little fraction of astaxanthin; that led to the obvious conclusion that the yeast cells had to be previously either mechanically broken down or digested with enzymes, so that the animals would be able to readily take and deposit the carotenoid. This approach is precluded because the carotenoid pigments of yeast are extractable in neutral solvents only after acid hydrolysis and astaxanthin is very sensitive to acids.

Thus, *P. rhodozyma* grown in a 50-liter fermentor until early stationary phase, was heat-killed and inoculated with either *B. circullans* WL-12 or *T. viride*. Both microorganisms grew at the expense of cell wall material of the yeast, as evidenced by electron microscopy observation and by the liberation of high quantities of non-dialyzable material. After 22 h of bacterial growth or 60 h of fungal growth, yeast physical integrity was almost lost and all the astaxanthin was readily extractable by either acetone or ethanol. The degraded yeast biomass was directly used in rainbow trout feeding or for astaxanthin-rich oil production. Feeding trials were in all cases positive. Recently we have achieved astaxanthin crystalization starting from the same degraded yeast biomass.

Most *P. rhodozyma* strains currently used do not exhibit the sexual state. Cell wall analysis, though, reveals its relationship to the basidiomycetes. It is now known, after carrying out an unambigous karyotyping study (1) that the number of chromosomes may vary between 9 and 17 depending on the yeast strain. This, and the reports on isolation of auxotrophic mutants plus the Golubevs discovery on sexual activity (3) opens up new ways of trying to initiate a genetic approach to the problem of astaxanthin synthesis and an eventual cloning of the genes involved.

Despite all this we have been able to obtain new strains (2) by the combined use of benomyl and ethyl methanesulfonate. After HPLC analysis of their carotenoidic content, the mutants could be classified into five groups: (i) hypopigmented strains (E7, E2, B1 and BE2) producing lower concentrations of total carotenoids but higher amounts of β-carotene than the wild-type strain of *P. rhodozyma* and considerably reduced trans-astaxanthin content; (ii) hypopigmented strains (E3 and BE7) producing higher concentrations of total carotenoids than the wild-type strain (this increase was due, however, to increases in the intermediate carotenoids, while the trans-astaxanthin content decreased as compared to the wild-type strain; (iii) Hypopigmented strains

(E4, BE1, and BE4) with a remarkable decrease in the production of trans-astaxanthin but an overaccumulation of β-carotene, which increased the total carotenoids content; (iv) hyperpigmented strains (B10, BE10, BE6, BE3, BE11 and BE5) exhibiting an increase in total carotenoids content as compared to the wild-type strain due to higher amounts of intermediate carotenoids as well as trans-astaxanthin; (v) hyperpigmented strain BE9 showing higher amounts of total carotenoids than the wild strain, 60% being trans-astaxanthin.

Strain BE2 was further treated with benomyl, allowing colonies to grow for a month in the presence of the carbamate. After this time had elapsed very red pipillae had become aparent. These were subsequently picked and spread onto fresh medium. The new strains (PAP series) showed greater genetic stability than the BE strains. The carotenoids were HPLC-quantified as before and the PAP strains could be placed into four groups: (i) hyperpigmented strains that showed an increased production of total carotenoids, in which the trans-astaxanthin content was that of the wild-type but the content of intermediates was far higher; (ii) hyperpigmented strains that showed an increased production of total carotenoids, mainly due to the overproduction of astaxanthin; (iii) hyperpigmented strains with higher content of carotenoids than the wild-type due to the hyperproduction of both β-carotene and trans-astaxanthin; and (iv) hyperpigmented strains that produced high levels of cis-astaxanthin.

From the results reported here it seems apparent to us that this peculiar yeast has industrial interest for exploitation as a source of astaxanthin. In fact, industrialization has already occurred and has been commercialized as "Natupink".

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MICROBIOLOGÍA SEM **11** (1995), 389–392

Survival strategies of enteric bacteria in aquatic systems

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Key words: bacterial survival, enteric bacteria, aquatic systems

The comparative survival of *Escherichia coli* and *Enterococcus faecalis* is studied in two aquatic media. The negative effects of visible light and osmotic stress upon cells cause a progressive loss of activity and of culturability. The predation exercised by the natural microbiota affects the survival of the two bacteria.

Survival of bacteria in aquatic environments has been defined as the series of changes resulting from their stay in a hostile environment. Traditionally, bacterial survival has been studied through culturable population evolution during a certain period of time. Thus, the most used parameter to evaluate this survival was the T_{90} or the necessary time to decrease 90% of the culturable population. Usually, the survival was studied according to the bacterial death definition proposed by Postgate (7), according to which cellular death was interpreted as loss of culturability. In this context, viability was the same as culturability.

The development of measuring techniques for activity at the cellular level showed that many dead cells, according to the definition of Postgate (7), presented physiological activity. Based on these techniques, in 1987, Roszak and Colwell (8) defined two cellular stages: viviforms, which are formed by cells which present some kind of activity, whether culturable or not, and by those which do not present activity but keep their cellular integrity; and dead ones, where bacterial death should be understood as loss of cellular integrity. These authors also made a characterization of cells in survival stages of progressive dormancy based on enumeration or detection methods.

Enteric bacteria form a suitable group for the study of this dormancy process. These bacteria tend to disappear more or less slowly in natural systems and experience a process of continuous loss of activity which lets us detect the cellular groups described by Roszak and Colwell (8).

The enumeration methods currently employed allows us to detect: (i) the total number of cells (AODC), (ii) the number of culturable cells (CFU) and (iii) the number of active cells (DVC). There are mainly two types of methods to quantify this last group of cells based on the functionality of the

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electrons transport system or the elongation of cells in the presence of nutrients and inhibitors of cellular replication. Based on these countings, different cellular groups can be determined (see below).Among the abiotic factors of aquatic systems, visible light seems to be the main regulator of enteric bacteria survival. Thus, Barcina et al. (2) showed that light has a negative effect upon the survival of *E. coli* and *Ent. faecalis*. Under illuminated conditions, these bacteria experiment a process of dormancy or of progressive loss of cellular activity and culturability.

Fig. 1 shows an example of the gradual formation of intact, viable nonculturable and

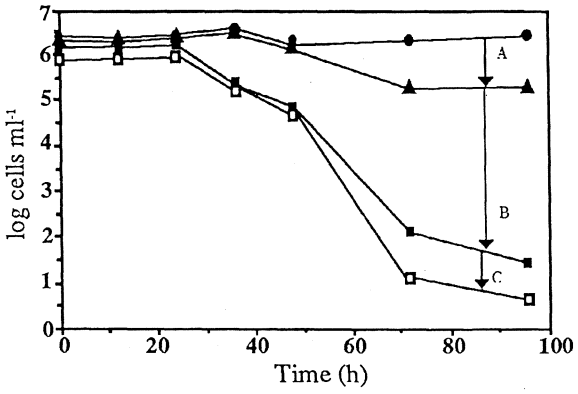

FIG. 1. Different counts for *E. coli* in illuminated fresh water system. ●, total number of cells; ▲, metabolically active cells; ■, CFU on TSY; ❑, CFU on EMB.

culturable cells as the exposure time to light is increased. A sharp decrease in the uptake of glucose as well as a clear decrease in the incorporated glucose density, due to an increase in the respired percentage as opposed to the assimilated percentage, is detected. This behaviour a decrease in the metabolic activity, in this case the glucose incorporation, and the greater use of the incorporated substratum to obtain energy to survive is a typical answer to stress situations. The behaviour of *E. coli* in seawater is very similar to that in fresh water. *Ent. faecalis* presents a similar behaviour to the one described for *E. coli*, although the decreases in the cellular activity and culturability in both systems are less than the ones detected for *E. coli*.

In the non-illuminated systems, *E. coli* presents a much higher capacity to incorporate glucose in fresh water than in seawater (Table 1). This fact could be a reflection of the osmotic stress of seawater on *E. coli* and explains the lower survival of this indicator in the marine system than in fresh water (2). The glucose incorporations both in fresh water and in seawater, obtained for *Ent. faecalis* are very similar (Table 1). These results show a greater resistance of the *Enterococcus* to the negative effect of visible light and to the osmotic stress of seawater and, therefore, a greater survival capacity of this indicator with respect to *E. coli*.

TABLE 1. Percentage of total [^{14}C]glucose uptake (I) and percentages of assimilation (A) and respiration (R) with respect the total uptaken in non-illuminated systems

	In seawater						In freshwater					
	E. coli			*Ent. faecalis*			*E. coli*			*Ent. faecalis*		
Time (h)	I	A	R	I	A	R	I	A	R	I	A	R
0	2.72	28.12	71.88	12.81	58.18	41.82	56.57	87.07	12.93	11.24	37.91	62.09
12	1.65	28.91	71.09	9.73	49.16	50.84	58.26	88.72	11.28	8.16	18.00	82.00
24	1.99	32.88	67.12	9.21	54.03	45.97	63.69	86.57	13.43	8.27	19.80	80.20
36	2.08	35.62	64.38	7.54	53.92	46.08	65.12	80.69	19.31	9.32	26.89	73.11
48	2.05	38.68	61.32	8.51	45.16	54.84	56.10	74.89	25.11	11.56	44.42	55.58

Arana et al. (1) showed the role of the photochemically generated hydrogen peroxide in the loss of culturability of *E. coli* by studying the mechanism through which visible light provokes dormancy in cells.

With respect to plasmid transfer by donor and recipient strains under nonilluminated and illuminated conditions, Muela et al. (6) report that the donor strains lose the ability for plasmid transfer before any other physiological variations are detected (Fig. 2). Nevertheless, no variation in the ability of recipient cells to receive and express plasmid material was observed. In addition, these authors state that visible light affects very negatively the ability to transfer plasmids.

Enteric bacterial survival is negatively affected by biotic factors and/or the natural microbiota. In all the microbial populations that form the natural microbiota there coexist three main groups that may influence allocthonous bacterial survival: heterotrophic bacteria, lytic organisms such as phages and *Bdellovibrio*, and protozooplankton.

Traditionally, the predation effect performed by protozoa has become evident by the simultaneous decrease in the number of *E. coli* or *Ent. faecalis* culturable cells and the increase in the protozoan population density. Barcina et al. (3) demonstrated that, in the presence of the natural microbiota, *Ent. faecalis* survival was superior to that of *E. coli*. Protozoal growth in the presence of *Ent. faecalis* was also inferior to that detected in the presence of *E. coli*. In these experiments, the results show a preference by protozoa for *E. coli* than for *Ent. faecalis*.

By means of a direct method in which enteric bacteria were marked with fluorochromes and added to microcosms of river water, predation by protozooplankton was demonstrated by the presence of labelled bacteria in the interior of the protozoa digestive vacuoles. The ingestion rates as well as the total ingestion and digestion data

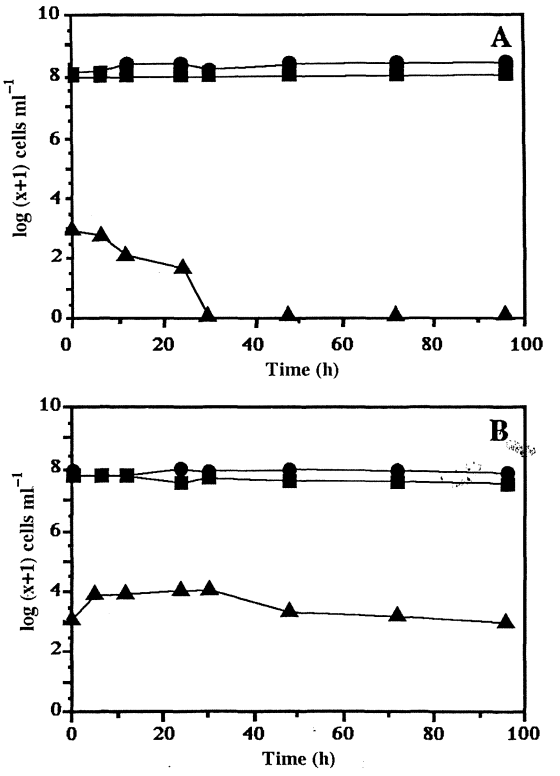

FIG. 2. Changes in bacterial counts during long-term survival in sterile river water of donor strain (A) and recipient strain (B), in a nonilluminated system. ●, total number of cells; ■, CFU on NA; ▲, transconjugants.

corroborated that *E. coli* is predated more efficiently than *Ent. faecalis* (45 and 29%, respectively) (5). The low elimination rate of *Ent. faecalis* shows a scarce predation efficiency of this bacterium and explains the higher survival rate of this indicator with respect to *E. coli* in the presence of the natural microbiota.

E. coli and *Ent. faecalis* survival using two kinds of cells, FLB (fluorescence-labelled bacteria) and RSB (rhodamine-stained bacteria), was studied to determine the impact of phages and lytic bacteria on these enteric populations. RSB cells are active, even culturable and suitable for elimination by protists as well as by lytic organisms while FLB cells are heat-inactivated cells only

suitable for predation by protists. Thus, the different numbers of FLB and RSB cells in the presence of the natural microbiota lets us determine the possible effect of the lytic organisms(4).

For *Ent. faecalis*, the authors did not detect differences between FLB and RSB cells in the two aquatic systems studied. However, for *E. coli*, significant differences between the decrease in FLB and RSB populations were observed. Those differences can be attributed to the effect of lytic organisms. However because of the higher biovolume of RSB cells, the authors can not discrimate between the effect of lytic predation and selective predation as a function of the greater biovolume.

The results show that predation by protozooplankton and perhaps by lytic organisms effectively eliminates enteric bacteria from aquatic systems. The abiotic factors only provoke loss of culturability. This fact suggests a sanitary risk because the methods traditionally used to detect enteric bacteria are based on culturability.

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The survival of enteric viruses in the water environment

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Keywords: enterovirus, virus survival, water pollution

Introduction. Human pathogenic viruses enter the water environment primarily through the discharge of treated and untreated sewage into surface waters. The critical question which arises, however, is whether or not these viruses can survive long enough and in high enough concentrations to cause disease in individuals who are in contact with polluted recreational water or who consume contaminated water or seafood.

Virus persistence in fresh water. In a 17-month study conducted at five sampling stations along the Duero river, we could verify the occurrence of self-purification processes in this river water (2). Based on our data, the river did not appear to be highly contaminated before entering the urban area of Soria. The discharge of a highly contaminated sewage outfall added a high level of faecal contamination to the river. However, a decrease in virus levels was already observed 50 m downstream. This decrease in virus load was even more pronounced 5 km from the discharge of the sewage outfall. Opposite conclusions could be drawn from a study performed on the heavily polluted Besòs river (4), where the viral load steadily increased as the river moved towards its mouth. Natural self-purification mechanisms are ineffective in the Besòs river because of the high level of urban and industrial pollution.

Virus persistence in marine water. Studies were conducted on the influence of temperature, suspended solids, and origin and type of water on the persistence of enteric viruses in seawater. Among the many factors affecting virus survival in the marine environment are temperature, virus association to solids and the presence of microbial flora (5, 6, 8, 10). We investigated the comparative survival of poliovirus 1 (PV) and hepatitis A virus (HAV) at 5°C and 25°C in marine water (Fig. 1). Little inactivation of any virus was observed at 5°C after 30 days. No significant differences in the decay rate of both virus strains were observed at this temperature. At 25°C, HAV persisted significantly ($p < 0.05$) longer than PV. As expected, virus survival was higher at low temperature (5°C) than at high temperature (25°C). The presence of marine sediment enhanced virus survival in seawater at 25°C. The protective effect of solids was particularly significant ($p < 0.05$) for PV after 30 days in marine water (Fig. 1).

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It seems reasonable to assume that environmental factors and the compositional make up of seawater may be substantially different from one geographical location to another. We studied the distinct persistence of PV when suspended in water from the Atlantic ocean at North Carolina, or in water from the Mediterranean sea at the Catalan coast (Fig. 1). The decay rates were significantly (p <0.05) more pronounced in Mediterranean water than in Atlantic water. Supposedly, all marine waters contain a variety of potential antiviral factors, and the overall antiviral action generally results from the most dominant factor(s) present in the water source.

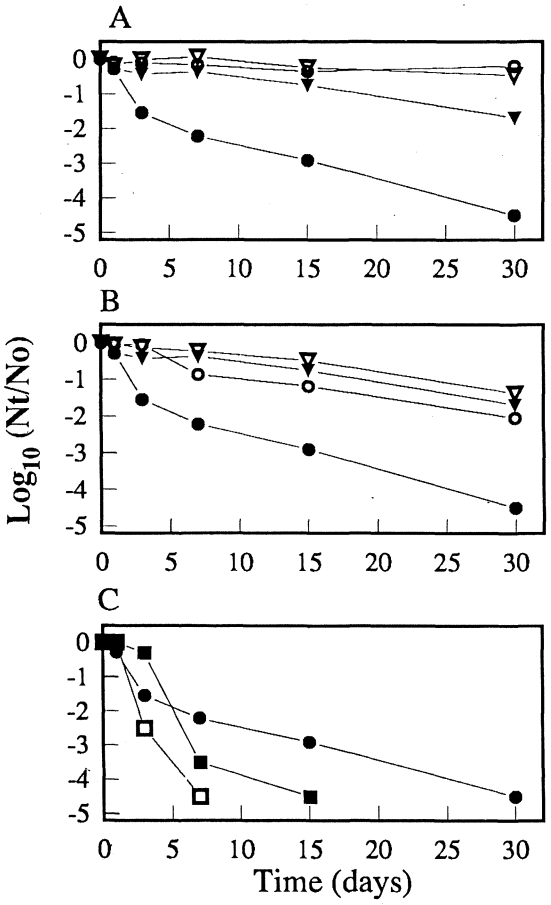

FIG. 1. Stability of poliovirus 1 (PV) and hepatitis A virus (HAV) in seawater under different conditions. (A) Effect of water temperature on virus survival (○, PV 5°C; ●, PV 25°C; ∇, HAV 5°C; ▼, HAV 25°C). (B) Effect of the presence of sediment on virus survival at 25°C (●, PV; ○, PV sed; ▼, HAV; ∇, HAV sed). (C) Influence of the source of water on poliovirus survival at 25°C (●, PV NC; ❑, PV Gava; ■, PV Pl Aro). NC: Atlantic Ocean at North Carolina; Gava: Mediterranean sea at Gavà beach (Barcelona); Pl Aro: Mediterranean sea at Platja d'Aro (Girona).

Autochthonous bacteria were shown in previous studies to be the principal virus inactivating factor in water from Gavà beach (7). Marine bacteria were isolated showing virucidal activity specifically against PV, but not against other enteric viruses. In the present studies, a pronounced decrease in the titre of echovirus 1 (EV), another member of genus *Enterovirus*, was observed when the virus was suspended for 7 days at 25°C in water from Gavà beach (5). The antiviral effect was removed, totally or partially, after autoclaving the water, or after treatments with antibiotics or trypsin. In contrast to the anti-PV activity (8), the virucidal activity could be separated from the bacteria by filtration through 0.22 µm, and was not confined to EV but was also effective against PV, coxsackievirus (CV), human rotaviruses (HRV) and HAV. From our data we may assume that the antiviral factor(s) must be of bacterial origin, of a proteinaceous nature and temperature sensitive.

Concentration of the actual anti-EV substance(s) was not achieved by filtration through CX-30 Millipore immersible units. This observation leads to the conclusion that the molecular weight of the cell-free inactivating factor(s) must be below 30,000 Da. Attempts to isolate the bacteria with anti-EV activity were not successful until after 64 subcultures in seawater, when one isolate with virucidal properties was obtained. This isolated bacterium, strain 7-2A, grew at 20, 25, 30 and 37°C on TSA with 2% NaCl or normal salt concentration, on marine agar and on McConkey agar. This bacterium was subcultured for over one year, at weekly intervals, without losing its antiviral activity. Gram staining revealed

Gram-negative bacilli, negative for the oxidase test, and positive for the oxidation/fermentation test. Electron microscopy showed rod-shaped bacteria measuring approximately 1.5 μm × 0.5 μm.

Virus disinfection in water. Chlorine is widely used for the routine inactivation of pathogenic microorganisms in water and particularly swimming pool waters. However, chlorination has been found to contribute to the formation of numerous chlorinated organic compounds in water hazardous to human health (3). Electrolytically generated copper and silver (C:S) ions have been alternatively introduced as a relatively safe and odourless method for water disinfection. The efficacy of C:S ions, in combination with low levels of free chlorine (FC), was evaluated for the disinfection of HAV, HRV, human adenovirus (ADV) and PV in water (1). HAV and HRV showed little inactivation in all conditions (Fig. 2). PV showed more than 4 $\log_{10}$ titre reduction in the presence of C:S combined with 0.5 mg/l of FC, or 1 mg/l of FC alone (Fig. 2). ADV persisted longer than PV under the same treatments, although significantly less than HRV or HAV. The addition of 700:70 μg/l of C:S did not enhance the inactivation rates after the exposure to 0.5 mg/l or 0.2 mg/l of FC, although on some occasions it produced a level of inactivation similar to that induced by a higher dose of FC alone (Fig. 2). Virus aggregates were observed in the presence of C:S ions, although not in the presence of FC alone. From our data, the use of C:S ions in water systems may not provide a reliable alternative to high levels of FC for the disinfection of viral pathogens. Additionally, poliovirus does not appear as an adequate model viral strain to be used in disinfection studies.

Conclusions. The significance of the health threat posed by the presence of human pathogenic viruses in the environment partly relies in the ability of the viruses to persist under natural or disinfection conditions. The phenomenon that self purification processes are more pronounced in

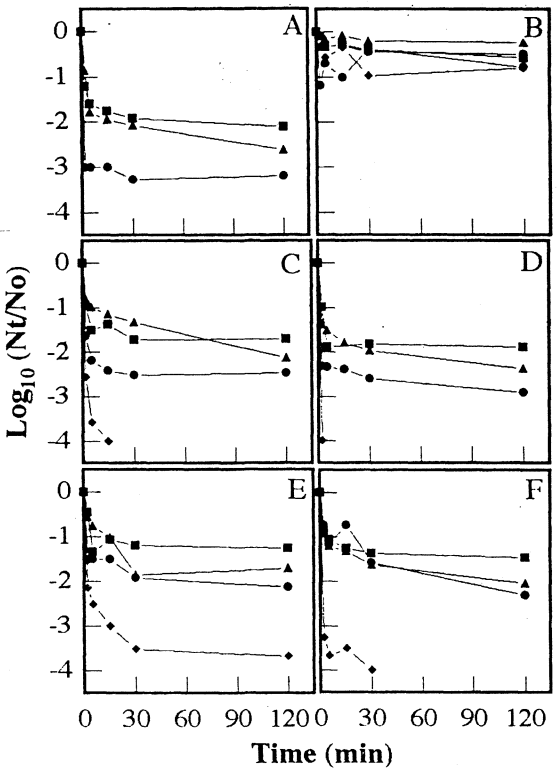

FIG. 2. Inactivation of human enteric viruses by free chlorine (FC), and copper and silver (C:S) ions. (A) 1 mg/l FC. (B) Control without disinfectants. (C) 0.5 mg/l FC. (D) 0.5 mg/l FC + 700:70 μg/l C:S. (E) 0.2 mg/l FC. (F) 0.2 mg/l FC + 700:70 μg/l C:S.

seawater than in river water has been reported by several authors (7, 9). However, there is no consensus on the nature of the factor(s) responsible for the virucidal capacity of seawater. Several observations demonstrate the potential involvement of native marine microorganisms in the inactivation of viruses in marine habitats, although data on the successful isolation of microorganisms with virucidal properties are scarce (5, 8, 10). Additionally, the ability of bacteria to inactivate viruses is usually lost while subculturing the bacteria in the laboratory. The use of the receiving waters for drinking, shellfish harvesting, agricultural or recreational purposes calls for a thorough understanding of the behaviour of human enteric viruses

in the water environment. Effective disinfection of water is required to prevent the threat posed by microorganisms of significant health importance.

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MICROBIOLOGÍA SEM **11** (1995), 397–399

Bacterial survival mechanisms in microbial mats

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Key words: stress factors, spirochetes, microbial mats

Microbial mats, laminated benthic communities, thrive in a wide variety of environments (2, 9). Those studied here are located at the Ebro Delta (Catalonia, Spain). The physico-chemical factors, the species composition and the spatial and temporal variations of the communities have been extensively studied (7). We described the microorganisms living in the photic zone in a well-developed microbial mat by electron microscopy and pigment analysis. Communities in microbial mats are living under many stresses, among others: nutrient depletion and extremes of light, temperature, pH and salinity; desiccation; high concentration of sulfide and toxic metals. So, microorganisms must be able to adapt their metabolism or to migrate vertically in response to the diurnal changes of environmental conditions. Prokaryotes have evolved mechanisms of resistance to stress conditions. Some authors consider how and why air drying can induce water stress in prokaryotic cells and how and why some prokaryotic cells tolerate that stress. Mucous or slime and other exopolymers act as highly hydrated water storage reservoirs that surround the cells. Many microorganisms may exhibit an inherent ability to form resting stages, e.g. cysts and spores (5).

On the other hand, some bacteria possess metabolic versatility in order to cope with fluctuations in chemical conditions. In phototrophic microbial mats, where light is the most important factor, purple sulfur bacteria exhibit metabolic diversity. Poly-β-hydroxyalkanoates (PHA) accumulate as a product of the photoassimilation of acetate in those bacteria, but may also be formed as a consequence of the oxidation of storage carbohydrate during the process of endogenous respiration. This process was always observed in cells incubated or living in darkness or under light limitations.

Finally, cells that live in hypersaline habitats request a high osmotic pressure in their cytoplasm to balance the external osmotic pressure. A variety of organic osmotic solutes have been detected in different halophilic microorganisms (8).

Resistance of purple sulfur bacteria to light limitation. Accumulation of intracellular storage

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polymers is a strategy of bacteria that increases survival in a changing environment. PHA serves as an endogenous source of carbon and energy during starvation, for encystment and sporulation, and as a regulator of the oxygen content of the bacterial environment during nitrogen fixation. It is also used as an electron donor. Purple sulfur bacteria in pure cultures and in lakes accumulate PHA during dark periods by consumption of glycogen and production of sulfide.

In order to determine the accumulation of PHA in single cells of purple sulfur bacteria from the purple layer in Ebro Delta microbial mats, a quantitative ultrastructural study was performed (3). For measurements and data processing, between 30 and 50 ultrathin sections were processed from every sample. TEM images enlarged 40,000 ×, to obtain accurate measurements. Numerical density, which is the area occupied by the intracytoplasmic inclusions, was calculated considering the cross section area.

Ultrathin sections of the deepest pigmented purple layer in Ebro Delta microbial mats revealed: cells of *Chromatium gracile*, which have been identified from axenic cultures as motile rods (1 μm × 2.5 μm), with an intracytoplasmic membrane system of the vesicular type; cells of *Thiocapsa* sp., spherical non-motile bacteria surrounded by a capsule and showing vesicles inside; and two unidentified purple sulfur bacteria. The first one is a filamentous segmented bacterium with a lamellar photosynthetic membrane system. The second one is ovoidal and exhibits phenotypic characteristics similar to *Chromatium* cells, but the major difference between *Chromatium* and this bacterium lies in the nature of the intracellular membrane system (vesicles and lamellae, respectively).

All these bacteria accumulate PHA intracellular granules inside when they are growing in their natural habitat (Fig. 1). The average percentage of the cell profile area occupied by sulfur

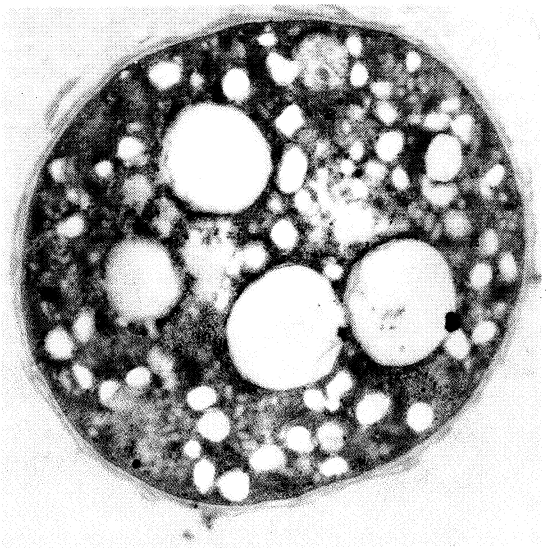

FIG. 1. Unidentified purple sulfur bacteria from Ebro Delta, showing intracytoplasmic inclusions. (Courtesy, D. Ceballos.)

globules was 16.8% and by PHA granules was 6.8%. These results were compared with others obtained in pure cultures of *C. warmingii* and *C. vinosum* at different conditions of growth and in Lake Cisó in a dyel-cycle (Esteve et al., in preparation). In all studies purple sulfur bacteria increased the concentration of PHA when living under light limitation.

Resistance of spirochete populations to desiccation. Because of tidal exposure and alternating meteoric rain water and flood water, desiccation plays a determinative role in the ecophysiology of bacterial communities that comprise microbial mats. Curiously, despite its intrinsic importance and ecological significance, desiccation of bacteria as a major stress parameter seems to have continually escaped the critical attention of bacteriologists (1).

Spirochetes are defined as helically-shaped, motile, walled bacteria in which the flagella are internal to the outer membrane. No spirochete greater than 0.5 μm in diameter bearing more than 20 flagella has ever been cultivated.

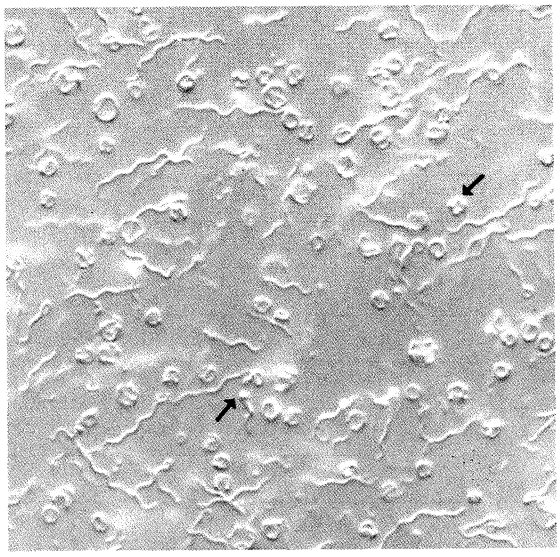

FIG. 2. Refractile bodies prominent in swollen live cells of *Spirosymplokos deltaeiberi*, from Ebro Delta.

Spirosymplokos deltaeiberi, a large (up to 100 μm long), loosely coiled, free-living spirochete with variable diameters (from 0.4 to 3 μm) (Fig. 2) was observed in mud water and enrichment media from highly specific habitats in intertidal evaporite flats in Ebro Delta microbial mats and in other localities, e.g. Laguna Figueroa (Baja California, México) and Sippewisett salt marsh (Woods Hole, MA, USA). Associated with *Microcoleus chthonoplastes*, the large spirochetes from Spain display phototaxis and a composite organization. The ultrastructural characteristics of this bacterium and its requirement for growth, have been described (4).

The large spirochetes are studied in order to determine their resistance to desiccation. Spirochetes became swollen on exposure to air. The onset of erratic, slower swimming, swelling, and withdrawal appear developmental. Within a few hours while they continued to move, from one to four refractile bodies formed in nearly all cells. These became visible after the protoplasmic cylinders were withdrawn. Refractile bodies prominent in swollen live spirochetes correspond to membranous structures in electron micrographs (6).

As a consequence of all the above mentioned, a high biodiversity is often found in microbial mats. Probably a vast array of different microorganisms correlate with specific environmental conditions. This would result in a highly flexible community. Although microscopical analysis often reveal the presence of different morphotypes, investigators are still uncertain whether these represent different genotypes.

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MICROBIOLOGÍA SEM **11** (1995), 400–402

Ciliated populations in activated sludge plants: structure, dynamic and indicator value

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Keywords: water treatment plant, ciliates, indicator microorganisms

The biological treatment of waste water by the activated sludge system is supported by the development of a mixed culture of microorganisms with protozoa being one of the most important groups. Ciliates contribute to clarification of the effluent in two different ways: (i) stabilization of bacterial populations by depredation and, (ii) flocculation. Moreover, species and groups of ciliates can be used as indicators of the efficiency of the biological process.

Comparative statistical studies at several plants are scarce (1, 9, 10), so the aim of this work is to study the abundance of ciliates at different activated sludge plants in the Community of Madrid, determining the most representative groups in this biological process and analyzing, by statistical treatment, the relationships between physico-chemical/operational parameters, and those of biological significance.

Ten activated sludge plants located in Madrid were selected according to the type of inputs and the design of the biological reactors. Samples were collected from the mixed liquor monthly for a year at each station. The counting of each sample was done in triplicate according to the method proposed by Madoni (6, 7). Identification of the species was carried out "in vivo", using bright field and phase contrast illumination, and by means of the silver impregnation technique of Fernández-Galiano (4, 5). Physico-chemical parameters were determined following the Standard Methods (2). The statgraphics 5.0 program was used for statistical analysis.

We determined the distribution in the four categories of abundance established according to Madoni (7), and total occurrence frequency of ciliate genera appearing in activated sludge plants of this study (Table 1).

In general, the genera found by us have been described as typical of waste water plants commu-

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TABLE 1. Frequency of occurrence and distribution into the categories of abundance

Genera	Percentage of ocurrence in the categories of abundance				Total
	Scarce	Moderate	Abundant	Dominant	
Acineria	10%	7%	10%	7%	34%
Aspidisca	19%	15%	19%	11%	64%
Carchesium	1%	1%	6%	1%	9%
Chilodonella	7%	6%	6%	1%	20%
Cinetochilum	–	1%	1%	–	2%
Coleps	4%	2%	7%	–	13%
Colpidium	2%	2%	–	3%	7%
Epistylis	10%	14%	27%	34%	85%
Euplotes	4%	2%	1%	–	7%
Litonotus	17%	12%	8%	–	37%
Loxophyllum	1%	–	–	–	1%
Opercularia	1%	1%	–	–	2%
Pseudochilodonopsis	–	3%	1%	1%	5%
Tetrahymena	4%	2%	1%	–	7%
Trithigmostoma	3%	–	–	–	3%
Vorticella	10%	9%	33%	41%	93%

nities. However, the genera *Acineria* and *Pseudochilodonopsis*, frequently reported in our study are not considered as typical ciliates of the system. In our opinion, these genera, *A. uncinata* and *P. fluviatilis* can be missidentified "in vivo" as *Trachelophyllum pusillum* and *Chilodonella uncinata*. Therefore, we consider that more accurate data obtained from silver impregnation specimens are required for correct identification of these species. Other genera, such as *Loxophyllum*, occasionally appear, but can not be considered within the stable communities of these plants.

The ciliates from activated sludge plants have been placed into three functional groups by Curds (3) and Madoni (7, 8): swimming ciliates that are not related to the floc, and stalked and crawling ciliates, both related to the floc. We propose a new group: swimming-crawling ciliates, to describe those ciliates that are only temporarily associated with the floc.

The correlation analysis mainly relates the abundance of stalked ciliates to the high volumetric load values and also to the low effluent BOD values, so this group can be used as an indicator of both accurate design and management of the plants. Moreover, swimming ciliates are related to the high effluent BOD values and the low sludge age. Therefore, this group is associated with a decreased efficiency of the depuration process, or in some cases, to a short retention time of the sludge in the reactor so that the groups which constitute the stable community are not allowed to develope.

Through factor analysis, six factors derived by Varimax rotation were selected explaining the 85.9% variability of the process (Table 2). The first factor, called as the process control factor, groups together the massic and volumetric load. The biological importance of this factor is represented by *Vorticella striata*, the species indicating low efficacy in the depuration process, and

TABLE 2. Relationships between the species of ciliates and the physico-chemical/operational plant parameters

Variables	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
Aspidisca cicada	–0.475	–	–0.704	–	–	–
Acineria uncinata	–	–	–	+0.914	–	–
Litonotus lamella	–	–	–	–	+0.892	–
Epistylis spp.	–	–	+0.853	–	–	–
Vorticella convallaria	–	+0.890	–	–	–	–
Vorticella striata	+0.737	–	–	+0.401	–	–
Influent $N\text{-}NH_3$	–	+0.830	–	–	–	–
Effluent $N\text{-}NH_3$	+0.535	+0.745	–	–	–	–
SVI	–	+0.654	–	–	0.458	–
Sludge age	–	–	–	–	–	+0.969
Effluent BOD	+0.776	+0.468	–	–	–	–
Massic load	+0.815	–	–	–	–	–
Volumetric load	+0.898	–	–	–	–	–
Variance explained (%)	36.7	16.1	11.9	9.7	6.8	4.7
Accumulated variance (%)	36.7	52.8	64.7	74.4	81.2	85.9

Aspidisca cicada, which is related to stability conditions. The second factor considered is the nitrification factor, which relates the species *Vorticella convallaria* to the existence of nitrification in the biological reactor. Biological factors (3rd and 4th) oppose *Aspidisca cicada* and *Epistylis* spp. and associate *Acineria uncinata* with *Vorticella striata* which means that these species compete or cooperate against each other in the same niche. The fifth factor, quality of sludge, relates the species *Litonotus lamella* to the volumetric index, indicating the low quality of the sludge.

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MICROBIOLOGÍA SEM **11** (1995), 403–404

Molecular identification of pathogenic actinomycetes: a new approach

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Key words: actinomycetes, 16S rRNA, nucleic acid probes

The term actinomycetes is an informal designation for bacteria belonging to the order Actinomycetales, characterized by having a high % of G+C. This group include genera such as *Mycobacterium, Corynebacterium, Nocardia, Rhodococcus*, etc., which have many species responsible for important infections both in humans and animals.

Bacterial infections treatment recquires the rapid and accurate detection and identification of the bacteria obtained from clinical samples. However, in infections caused by actinomycetes the identification of the etiological agent is sometimes hindered by several factors, such as difficulty of isolation (some mycobacteria do not grow in culture media or grow very slow), or difficulty in the classification and identification based on conventional morphological, physiological and biochemical properties. The difficulty to reach an accurate identification is especially marked when rapid commercial micromethods are used. On the other hand, quimiotaxonomic criteria, which have been very useful for the classification and identification of actinomycetes, are not easy to apply as a routine diagnostic procedure.

Molecular techniques have represented an alternative to the traditional microbiological methods. Among the different techniques proposed for the detection and identification of pathogenic microorganisms, the polymerase chain reaction (PCR) and the nucleic acid probes have been most widely used. The target regions have usually been the bacterium's pathogenicity or virulence genes.

The sequencing of rRNA molecules (mainly 16S, but also 23S) has been successfully applied in bacterial taxonomy for establishing phylogenetic relationships (1). A large database of rRNA sequences from approximately 2000 bacteria has been created (6). In addition to this application in bacterial taxonomy, the accumulation of large numbers of rRNA sequences has contributed to use their in microbial ecology studies and in clinical microbiology for the routine detection and identification of pathogens (5).

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rRNA molecules have several characteristics that make them ideal targets for diagnostic purposes: (i) universal distribution, (ii) the high number of copies (>10^4 copies/cell), (iii) their primary structure which is composed of regions with highly conserved sequences together with other regions composed of variable or hypervariable sequences ("signatures"). The latter sequences have a special interest for identification.

There are basically two strategies for using the sequence of the 16S rRNA molecules for the identification of actinomycetes. An easy, fast and accurate approach, for those actinomycete isolates that can not be identified by phenotypic methods, consists of the extraction of their DNA, PCR amplification and sequencing of the 16S rRNA gene, followed by a further comparative analysis with those 16S rRNA sequences available in the databases. This comparative analysis allows the matching of the sequence of the unknown actinomycete isolate with an existing retrieved sequence or identifying it as new. Although there have been reports of some problems in the identification of some bacteria based on rRNA sequencing data (6), the correlation of the data with phenotypic results of conventional biochemical data permits a reliable identification (7).

Alternatively, the comparison of a new 16S rRNA sequence with the rRNA databases allows the directed design of complementary oligonucleotides, specific for a certain species or a determined group of bacteria. These primers can be used with PCR (specificity at amplification stage), nucleic acid probes (specificity at detection stage) or a combination of both techniques for the detection and identification of clinical isolates of actinomycetes. This approach is especially useful with slowly growing or non-cultivable mycobacteria.

The 16S rRNA molecule has been applied for diagnosis purposes to only a few groups of actinomycetes, mainly mycobacteria (2). The imperfect classification of this phylogenetic group of bacteria reflected by its taxonomic changes, and the relatively low number of taxa within the actinomycete group with known 16S rRNA molecule sequences may explain the limited use of the 16S rRNA as target diagnostic molecule in this phylogenetic group. Fortunately, most of the genera with species of medical or veterinary significance are being completely sequenced (3, 4). This will facilitate the identification of actinomycetes by the analysis of their 16S rRNA molecules and the development of specific probes for their routine detection and identification.

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MICROBIOLOGÍA SEM **11** (1995), 405–406 **OPINION**

Restoring good manners in research*

John Maddox

Editor, Nature, *London, United Kingdom*

Is there an intrinsic conflict in the principles on which the scientific enterprise is based? Everybody agrees that science is a cumulative enterprise in which people stand on others' shoulders "to see further"; Copernicus and Galileo were among "the giants" on whose shoulders Newton stood, but there were Galileo's lens grinders and polishers whom he did not mention. Now, the cumulative character of the enterprise means that spectacular discoveries (say the structure of DNA) are rare, but that lesser discoveries are necessarily precursors and are therefore equally estimable. Good science is good science, whatever its importance, and its practitioners are equally deserving of respect. International interdependence flows from that.

Second, publication is of the essence. A discovery has no meaning unless an account of it is generally available. Paradoxically, equipment is more vulnerable to the passage of time than an account in words of the use made of it. But the function of publication is more than the transmission of the news to others. It is the only means by which the record of discovery can be authenticated in the long run; critical readers pick holes in the logic, others test for consistency by telling whether some field can be welded into a coherent whole. (One of the problems yet to be solved in electronic publications is that of telling which is the authentic text.)

Third, the traditional and, until recently, the sole reward of the working scientist has been his or her bibliography. It is a curiously insubstantial proof that a lifetime's science has been well done, but when the deepening of understanding is a cumulative business, even a handful of good papers rightly engender pride. The other traditional component of the profession's reward structure is the joy of teaching students. It is impressive how often senior people list the names of erstwhile graduate students who are now senior and productive people. The solicitude of the profession for its students is its most appealing attribute.

Where, in this account of an altruistic research profession, can there be conflict? If publication is essential to the authentication of discovery and also relevant to a researcher's self-esteem (and to the esteem accorded him or her by others), how can (and how strongly should) the temptation to improve the lustre of a personal bibliography be resisted? Succumbing to temptation distorts, even corrupts, the record. That is why there had emerged in the old days (and people will differ over how long ago they were) a code of conduct, an etiquette, to which people adhered more or less scrupulously.

Good manners were simple. A person should write and talk openly about past accomplishments and even future plans, answering intelligent questions even from competitors. Research data and materials should be shared with serious peo-

* Gist of the inaugural address to the 8th International Conference of Science Editors, given at the University of Barcelona on 9 July 1995. Published in *Nature*, vol. 376, p. 113 (13 July 1995). With permission.

ple, at least after the relevant papers had been published. Peripheral contributions to a piece of work should be acknowledged for what they are, and not dignified by co-authorship. Although Sir William Ramsay's plea a century ago that people should not follow up a discovery without the permission of the discoverer asks too much of science as well as its practitioners, to fail to give full credit to pathfinders in a field is not merely discourteous, but a way of robbing them of their own place in the record of discovery. And people established in a field have a duty to comment on the work of others in the capacity of referees.

Fifty years ago, these principles were widely followed, even in busy and productive university departments. The principles are still understood and still widely followed. Journals are especially conscious of what this entails. *Nature*'s demands on its referees are clamant and onerous; it is a constant source of wonder that so many offer such careful, judicious and constructive reports on manuscripts people working in fields related to their own interests are anxious to have published. Good manners still obtain.

But there is evidence in the hands of all journals that bad manners increasingly coexist with good. Self-advertisement is more common now than it used to be. People refer to their own publications when others' would be more apt. When reference to competitors is unavoidable, they may refer to a minor paper, ignoring a more important work. The tendency to overlook publications in other languages, or even by colleagues in other countries, is a growing source of angst. So to is the use of the word "first", as in "We have for the first time shown that Ohm's Law is valid in spacecraft beyond Jupiter (or Neptune, or Pluto, as the case may be)".

The perils of honorary co-authorship are now well documented, not least by Feder and Stewart's investigation of the circumstances in which John C. Darsee, at Emory University and the Harvard Medical School, was routinely able to recruit distinguished people as fellow-authors. The difficulty is that people with responsibility for administration and fund-raising are supposed to be more effective at those tasks if they can also boast of a research record.

There are also lapses from good manners by the gallant company of referees. As long ago as 1967, a paper on the metallurgy of austentitic steel was returned from a referee with the opinion that it did not deserve publication; the referee enclosed a paper of his own saying much the same thing, not manifestly more compellingly. Cases in wich the sight of an authors paper has stimulated referees to publish their own parallel work elsewhere have been recorded. The assumption that referees always treat a manuscript as confidential seems not always to be valid.

What can be done to bring back good manners? Journals have a responsibility to be more severe on offending manuscripts, and to make public flagrant trangressions of the unwritten rules. They have a particular duty to fight against that form of obscurity that stems not from a poor grasp of language, but from a wish to shelter from clarity in case it should be more vulnerable to criticism. It may make sense to ask corresponding authors to vouch for the collective responsibility of all co-authors.

But academic institutions and grant-making agencies have the chief responsibility. The temptation to magnify an individual bibliography, always present and usually resisted, is magnified by the pressure to win promotion and research funds, where decisions may hang on bibliometric indices of some kind. Because the importance of a discovery may seem, at least in its authors' mind, to be magnified by reports in the general press and thus by being first, anxiety over priority mounts.

Yet little thought has been given to mechanisms for decreasing the pressure to publish and to be first—especially in the Unites States where they are strongest, and which sets the tone for the rest of science. Until that is done, good manners will remain under threat.

MICROBIOLOGÍA SEM **11** (1995), 407 **OPINION**

Science, Culture & Communication for the 21st century *

Paolo Fasella

Director General for Science, Research and Development (DG XII)
European Commission, Brussels, Belgium

This conference (IFSE-8) addresses at the first sight two different categories of issues. The first is related to the problems issuing from the communication within the scientific community; the second with the communication of science to the public.

It would be erroneous to consider these two categories of issues as completely separated. In fact, they are deeply interrelated. Moreover, it is at their interface that appears what constitutes maybe the main issue in this field. What I have in mind is the problem of the quality of scientific information released to the public, a basic aspect of what we can call the "ethics of scientific information".

More and more often, scientific results are communicated to the public before any real peer evaluation. This trends is particularly clear in the field of biomedical research. New therapies and drugs are presented by the daily papers and the media, of which the efficiency has not yet firmly been established.

The reasons behind this are clear: the media like scoops and the scientists are seeking publicity. The point I would like to stress is therefore the following: one can not say that the responsibility, here, is only with the media; it is shared, and the scientists are, at least, accomplices.

In a field so close to everybody's concern like health, the development of this practice is particularly harmful. It is deeply incorrect to create false hopes among the sick and their close relatives.

More visible in the field of medical research, the problem is not limited to this area. The famous examples of "cold fusion" or "water memory", are there to remind us the danger, for the scientists, to release information before having obtained what represent the best guarantee of quality: the control by the peers. This behaviour is dangerous and to condemn, because it helps to create in people's mind an incorrect picture of what science is, and to undermine the public confidence in science.

I think that this issue should be addressed, by the scientists and the scientific journalists together, more in depth and more systematically than it is at present. As responsible of European research, I would like to add that it would be advantageous that this issue be addressed at European level. There are many reasons for this. Because research is carried out more and more by European teams and networks; because the scientific journalists will the more and more cover not only the scientific achievements occurring in their country and United States, but also in other European countries; and because the traditions of scientific information and the situations in the different European countries are not exactly the same and that, in this field too, real possibilities do exist to learn the ones from the others.

* Address to the 8th International Conference of Science Editors (IFSE-8), held at the University of Barcelona from 9–13 July 1995.

Microbiología y genética molecular

Josep Casadesús (ed.)

Coedición de la Sociedad Española de Microbiología
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MICROBIOLOGÍA SEM **11** (1995), 409–410

Maria Therezinha Martins (1936-1995) and the Seventh International Symposium on Microbial Ecology

Victoriano Campos,[1] Gary A. Toranzos[2]

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Maria Therezinha Martins, one of the most renowned and loved Latin American environmental microbiologists, passed away on August 16, 1995. Although her early death has left a great void in the Brazilian scientific community, which will be hard to fill, her scientific humanistic legacy lives on with the many students, colleagues and friends she left behind.

Therezinha, who had been originally trained as a dentist, eventually left her practice to dedicate her life to environmental microbiology and microbial ecology. She was one of the pioneers in the field of environmental microbiology in Latin America, having published several key papers on the evaluation of processes of water and sewage control in South America. She also studied the ecology of *Vibrio* spp. in South America, several years before the large cholera outbreak.

Her more than 110 publications are but a token of what Therezinha was. Her dedication to science contributed to the development of environmental microbiology in Latin America, and especially in Brazil, where she trained many students at all levels (B.S., M.S. and Ph.D.). Her scientific legacy will be passed on by these students and trainees.

Her presence was a mainstay at microbiological societies meetings. Always with an entourage of students and colleagues wherever she went, she was a most accessible environmental scientist, always ready to answer questions or give advice.

Her expertise was sought after by international agencies such as the World Health Organization and the Pan-American Health Organization, for which she served as a consultant on several occasions. Therezinha was also a member of the editorial board of *Standard Methods for the Examination of Water and Wastewater* (16th, 17th and 18th editions), *Environmental Toxicology and Water Quality* (as Regional Editor), and *Revista de Microbiologia* (the oficial journal of the Brazilian Microbiology Society), for which she was later appointed Editor-in-Chief, and several others.

She had presided the Brazilian Microbiology Society, and was one of the founding members (and president for several years) of the Latin-American Committee for Environmental Microbiology (CLAMA). Terezinha died only two weeks before the celebration of the 7th International Symposium on Microbial Ecology (ISME-7), to which she devoted the last few years of her life as chairperson of the Organizing Committee.

Those who had collaborated with her in the organization of the ISME-7 were heavily distressed by such a sudden death, but decided to work even harder for the success of the meeting, which would be a way to honor Terezinha. We would like to express our sincere condolence to her colleagues, and hope they keep working to further develop microbial ecology.

Petra S. Sanchez took the leadership of the ISME-7, which was held, as scheduled, from August 27 to September 1, 1995, in Santos, State of São Paulo. The meeting gathered more than 800 researchers from 61 countries, and the scientific program included a wide array of topics, ranging from the use of new technologies in basic research in microbial ecology to the latest trends and applications of microbes in industry, health programs, pest control, waste treatment, bioremediation, pollution control, et cetera. The program of the ISME-7 consisted of 20 symposia, 21 sessions of contributed papers, around 500 posters, and 3 plenary lectures. The topics chosen for the two inaugural lectures dealt with issues of current major significance: microbial biodiversity (by Erko Stackebrandt, DSM, Braunschweig, Germany) and global environmental change (by David M. Karl, University of Hawaii, USA). The closing lecture referred to a topic of great interest in the geographical area where the ISME-7 was held: endophitic fungi and their roles mainly in tropical plants (by João Lúcio Azevedo, University of São Paulo, Brazil).

The first International Symposium on Microbial Ecology was held in Dunedin, New Zealand, 1977, with the support of ICOME (International Committee—or Commission, as it was then called—on Microbial Ecology), IUMS (International Union of Microbiological Societies), IUBS (International Union of Biological Siences), and the UNEP/UNESCO/ICRO Panel for Microbiology. Since then, not only have the Symposia on Microbial Ecology grown in size, but ICOME, which is the main organizing entity of the Symposia, has changed from a Commission to a Committee of IUMS. ICOME aims to promote worldwide interest in microbial ecology by organizing the ISME three-yearly meetings, by publishing the series *Advances in Microbial Ecology* (Plenum Press), and by sponsoring or co-sponsoring other scientific activities. After Dunedin, the venues of the ISME were Warwick, UK (1980), East Lansing, Michigan, USA (1983), Ljubljana, Yugoslavia (1986), Kyoto, Japan (1989), and Barcelona, Spain (1992).

MICROBIOLOGÍA SEM **11** (1995), 411–412

Revisión de libros

Guía de los lagos y humedales de España

Santos Casado, Carlos Montes

J. M. Reyero Editor, Madrid, 1995. 256 pp. ISBN 84–605–3109–0

En las páginas de la presentación de esta *Guía de los lagos y humedales de España,* los autores ponen de manifiesto que los procesos de degradación están afectando los humedales a un ritmo más rápido que el de la concienciación sobre su valor ecológico. Porque es cierto que la preocupación ambiental y el respeto por la naturaleza, aún sin alcanzar los niveles que serían deseables, se está introduciendo paso a paso en la sociedad, que está descubriendo la necesidad de contribuir con sus hábitos y actitudes al mantenimiento del medio en que se desenvuelve. Es de esperar que de todo ello resulte una mayor presión para su conservación.

La preservación de un sistema no significa aislarlo de la actividad humana, sino más bien controlar que aquéllas que lo puedan afectar se realicen con criterios racionales. En este sentido, no son defendibles ni las actitudes radicales de quienes proponen un aislamiento a ultranza de todos los sistemas naturales, apartándolos de cualquier presencia humana, ni mucho menos las de quienes desprecian el peligro de destrucción de unos espacios de indudable valor, sea por favorecer intereses propios, por ignorancia o por una actitud de simple indiferencia ante algo que les resulta ajeno.

La *Guía de los lagos y humedales de España* puede contribuir a que, a través del conocimiento de esos espacios que todavía resisten en diferentes puntos del país, surja el interés y la voluntad de participar en la conservación de lo que representa un importante patrimonio natural.

Son varios los motivos por los que saludamos con el mayor entusiasmo la llegada de esta *Guía.* Uno, es el profundo conocimiento que tienen los autores sobre los sistemas y lugares descritos. Otro, nuestra experiencia personal de su solvencia científica, que provoca ya la confianza en el rigor del texto. Finalmente, que la obra ofrece una parte determinada de una larga y continuada investigación que desde hace años desarrolla el equipo de Carlos Montes en el Departamento Interuniversitario de Ecología de las Universidades Autónoma y Complutense de Madrid. Que el esfuerzo humano y material comprometido en esa investigación llegue tanto al especialista como al gran público y que éste pueda beneficiarse directamente, con textos como el que se presenta, constituye una experiencia muy positiva. Es una práctica que, por otro lado, sigue la tradición de nuestro recordado Fernando González Bernáldez, maestro y amigo también de los autores, a quien éstos citan y dedican su *Guía.* Evocamos aquí la última aportación del Prof. González Bernáldez *Los paisajes del agua: Terminología popular de los humedales*, publicado poco después de su muerte, por José Manuel Reyero, también editor

de esta *Guía* y de otros interesantes libros de ciencias de la naturaleza.

La introducción de la obra ofrece los elementos para el entendimiento de estos ecosistemas, con explicaciones sobre su funcionamiento, comunidades que lo habitan, la necesidad de su conservación —una vez que se pone de manifiesto su valor como componentes esenciales de la biosfera— También se tratan las actividades humanas y sus efectos sobre los humedales, datos históricos, y datos relativos a su conservación y desaparición, entre otras explicaciones de considerable interés.

Siguen ocho capítulos distribuidos en las tres siguientes partes: Los lagos y humedales de alta y media montaña, los humedales de las cuencas sedimentarias, y los humedales costeros. Completan la guía una toponimia de los humedales, una bibliografía clasificada en temas de interés general, y en los temas específicos de cada una de las partes, y un índice temático. Se incluye un mapa con las localizaciones lacustres y palustres más significativas que aparecen a lo largo del texto. Hay que destacar, igualmente, las excelentes y numerosas fotografías que, con detalladas explicaciones, ilustran y embellecen el texto, así como la esmerada presentación editorial de la obra.

No cabe duda de que el lector que se acerca a cualquier tipo de guía de los diferentes aspectos de la naturaleza, tiene ya un interés específico en ellos y, en este sentido, no dudamos en recomendar el libro a todo el público interesado en el tema de los ecosistemas acuáticos continentales. Pero sería una gran labor contribuir también a su difusión entre un público cuyo interés puede no estar definido, y que conviene despertar si queremos contribuir a la conservación de estos espacios. Es el caso de los estudiantes de enseñanza media y, por supuesto, de los cursos iniciales de las carreras de, biología, ciencias ambientales, geología, etc.

Con detalle y rigor, impregnado de una gran belleza descriptiva, los autores examinan las diferencias, similitudes y diversidad en suma de los múltiples tipos de humedales españoles — y también de aquéllos que ya han desaparecido o se encuentran en peligro de desaparición— para establecer los criterios que permiten su concepto y definición. Distinguen los rasgos aparentes y visibles de aquéllos que hay que deducir en razón del tipo de suelo o vegetación, insistiendo en el carácter cambiante y fronterizo entre zona terrestre y acuática.

La *Guía* consigue que se conozca y comprenda el funcionamiento y la función de los humedales dentro del conjunto de un ecosistema y su importante papel en la diversidad ecológica. Despierta en el lector no especializado el interés y la curiosidad por estas zonas, al poder apreciar no sólo su belleza aparente, sino las ventajas prácticas de su conservación, a la que individualmente puede contribuir.

Como toda guía, es útil tanto para los expertos, que pueden utilizarla como elemento de consulta, como para los intereresados no especialistas, que pueden extraer de ella un adecuado conocimiento de estas zonas esenciales de nuestra geografía.

Ricard Guerrero
Universidad de Barcelona

MICROBIOLOGÍA SEM **11** (1995), 413

Microbial Biotechnology
Fundamentals of Applied Microbiology

Alexander N. Glazer, Hiroshi Nikaido

W. H. Freeman and Co., New York, 1995.
ISBN 0-7167-2608-4

Tener entre manos un texto que describe muy diversos aspectos aplicados de una ciencia, en este caso la microbiología, desde los temas bioquímicos y genéticos hasta los ecológicos e industriales, exige por parte del lector una selección rigurosa entre la amplia información que ofrece. El objetivo que ha orientado a los autores ha sido conseguir un libro útil y de fácil comprensión para los estudiantes de esas materias, especialmente para los que se mueven en el mundo de la microbiología industrial, y que pueda ser enseñado en un curso académico de microbiología especializada. Por ello, se supone que la intención de los autores no era recopilar y condensar la materia, sino realizar una prospección significativa y digna de profundización.

A lo largo de los quince capítulos del libro, los autores diseccionan los conocimientos más modernos de microbiología para aplicarlos a la industria, tanto en su estado actual como en la previsible orientación futura. Los capítulos están distribuidos en seis partes.

En la primera, resumen de todo el libro, se subraya la importancia y el uso industrial de los microorganismos, en la obtención de nuevos productos o en la mejora de otros, y se indican los riesgos posibles para el medio ambiente. También se describen las diferencias de la taxonomía y filogenia de los diversos grupos de microorganismos. La segunda parte nos transporta al mundo de la genética microbiana, describiendo las diferentes técnicas de introducción de material genético no propio en el genoma bacteriano, y su importancia para la producción de compuestos finales no existentes previamente, o que se encontraban en cantidades ínfimas. La tercera parte se dedica a la mejora genética en microbiología vegetal, destacando el empleo de microorganismos para mejorar las cosechas o hacerlas más resistentes a las plagas o factores adversos del ambiente. La cuarta parte explica la utilización de la biomasa, toda aquella materia orgánica que crece por la conversión fotosintética de la energía solar, para diferentes finalidades, desde fuentes de carbono a fuentes de energía, y explica la degradación de esa biomasa por los microorganismos para obtener los nutrientes esenciales. La quinta parte comenta la producción de diversos aminoácidos y antibióticos por cepas bacterianas mutantes. En la última parte, los autores tratan la utilización de la capacidad natural y artificial de degradación de los microorganismos para eliminar las miles de toneladas de desechos recalcitrantes que se acumulan anualmente, uno de los más graves problemas del desarrollo industrial. Finalmente, otro aspecto que destaca el libro es la expansión de la microbiología, hecho que se deriva de sus múltiples conexiones con las diversas ramas de la ciencia, lo cual puede constituir una gran contribución al desarrollo armónico de la sociedad.

El libro interesa a biólogos y bioquímicos industriales; a ecólogos microbianos que estudian las interacciones con y entre los microorganismos, el último eslabón de la cadena trófica; y a todas las personas con inquietudes por conocer las interrelaciones entre la microbiología y el desarrollo de la industria actual. Pocas veces un libro realizado a partir de una recopilación tan ambiciosa ha resultado tan útil, sobre todo por el interés de la microbiología aplicada en resolver problemas ecológicos, en contraste con otras tecnologías, que pueden provocar cambios irreversibles en el medio ambiente.

Antoni Navarrete
Universidad de Barcelona

MICROBIOLOGÍA SEM **11** (1995), 414

Plastics from Microbes

David P. Mobley (ed.)

Hanser Publishers, Munich, 1994. 270 pp. ISBN 3-446-17367-6.

A principios del siglo pasado, la industria de los productos químicos basados en el carbono obtenía la mayoría de los materiales a partir de organismos vivos. Después de que Wöhler (1828) consiguiera la síntesis de la urea en el laboratorio, los químicos empezaron a desarrollar una gran cantidad de métodos sintéticos para la modificación o la obtención de la mayoría de productos químicos que utilizamos hoy en día, entre los cuales se encuentran prácticamente todos los polímeros.

En los últimos quince años, los avances de la bioquímica y la ingeniería genética en la comprensión y manipulación de las rutas biosintéticas y del material genético, han supuesto el regreso de la química orgánica a sus raíces biológicas.

Así, el interés por la síntesis microbiana de polímeros ha dejado de ser una curiosidad científica para convertirse en una posibilidad tecnológica de gran alcance con múltiples aplicaciones de interés para numerosos campos de la industria. Gran parte de este interés está motivado por consideraciones medioambientales: obtención de polímeros biodegradables o bien de polímeros a partir de substratos más baratos (fuentes de carbono no dependientes del petróleo y renovables, como los residuos agrícolas o el dióxido de carbono).

También es posible la obtención de polímeros mediante procesos a temperatura y presión ambientales (más baratos energéticamente), o con reacciones que de otra manera serían muy difíciles o muy costosas. Otro objetivo son las nuevas aplicaciones tecnológicas (piezas biocompatibles para uso médico, nuevos materiales, compuestos de alto valor añadido.).

Por todo ello, han empezado a aparacer libros sobre este tema apasionante, y al mismo tiempo de gran valor económico, entre los cuales se encuentra esta obra dirigida por David P. Mobley. Una prueba de todo lo expuesto es que la mayor parte de las aplicaciones industriales descritas has sido aportadas por los propios responsables en compañías privadas o en centros de investigación estatales. Este hecho supone, por una parte, la oportunidad de poder contemplar los procesos anteriores tanto desde el punto de vista académico como industrial y comercial, y, por otra, la posibilidad de establecer contacto directamente con los científicos y técnicos que están en la primera línea de este campo, gracias a las referencias que aparecen en los distintos apartados.

El libro está dividido en diferentes capítulos, en cada uno de los cuales se da toda la información sobre un proceso en concreto. En el libro se describen ampliamente los procesos de obtención de estos polímeros o prepolímeros —bien a partir de materiales básicos, bien mediante la realización de alguna reacción química imposible desde el punto de vista de la síntesis clásica— de origen bacteriano, entre los que se encuentran policarbonatos, polisulfonas, polifenilenos, proteínas estructurales o los prometedores y fácilmente biodegradables poli-ß-hidroxialcanoatos.

Se trata de una obra básica para todos aquellos interesados por los polímeros y por los nuevos materiales con base orgánica, pero también para todos los que se dedican a la bioquímica, la microbiología, la ecología microbiana y la ingeniería genética aplicadas.

Jaume Mir
La Seda de Barcelona

Normas para los autores

Microbiología SEM (la revista científica de la Sociedad Española de Microbiología, SEM) acepta artículos y notas de investigación originales dentro del campo de la microbiología y, ocasionalmente, artículos de revisión. Textos en inglés (preferentemente) o español. La aceptación corresponde al Consejo Editorial. Sólo se admitirán trabajos inéditos que no estén pendientes de publicación en cualquier otra revista. Los originales publicados en *Microbiología SEM* podrán ser reproducidos siempre que se indique su origen.

PRESENTACIÓN DE LOS ORIGINALES. Los artículos estarán escritos a máquina, a doble espacio, en hojas UNE A-4 por una sola cara, numeradas correlativamente y con un amplio margen en la parte izquierda. No deberán exceder de 16 páginas impresas, incluyendo tablas y figuras (lo que corresponde aproximadamente a 25 hojas mecanografiadas). Los artículos incluirán una primera página en la que se indicará por este orden: Título del artículo, nombre y apellido del autor o autores, centro en el que se ha realizado el trabajo y dirección completa del mismo, así como de tres a cinco "palabras clave". En los artículos en español se deberá incluir una versión inglesa del título. Los artículos constarán de: Resúmenes en inglés y en español (de no más de 250 palabras cada uno), Introducción, Materiales y métodos, Resultados, Discusión, Agradecimientos y Bibliografía. Las secciones de Resultados y Discusión se podrán juntar en una sola.

Las abreviaturas, símbolos y siglas deberán seguir las recomendaciones de la Comisión IUPAC-IUB sobre nomenclatura bioquímica. Deberá emplearse siempre el Sistema Internacional de Unidades (SI).

La bibliografía será citada en el texto mediante números y se dispondrá numerada y en orden alfabético de acuerdo con los ejemplos que se ofrecen a continuación:

Miller, J. H. (1972). Experiments in Molecular Genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Seeberg, E., Nissez-Meyer, J., Strike, P. (1976). *den*V gene of bacteriophage T4 determines a DNA glycosilate specific for pyrimidine dimers in DNA. J. Virol. **35**, 790–797.

Tomasz, A. (1984). Building and breaking in the cell wall of bacteria. The role for autolysins. *In* Nombela, C. (ed.), Microbial Cell Wall Synthesis and Autolysis, pp. 3–12. Elsevier Science Pub., Amsterdam.

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Microbiología SEM (the official journal of the Spanish Society for Microbiology, SEM) publishes original research articles, research notes and reviews covering all aspects of microbiology. All submissions should be written in English (preferably) or Spanish. The decision to accept manuscripts is made by the Editorial Board. Submission of an article to this journal is understood to imply that it has not previously been published and that it is not being considered for publication elsewhere. Consent will be given for reproduction of papers published in this journal if the source is credited.

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PREMIO

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Se convoca un premio al mejor trabajo biográfico sobre la vida del Profesor Vicente Callao Fabregat, destacando sobre todo sus actividades científicas y valores humanos, con motivo del 25 aniversario de su fallecimiento.

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El plazo de recepción termina el día 31 de octubre de 1996.

El Presidente
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