IX Congreso Internacional de Bacteriología y Micología

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La microbiología y, en especial, la bacteriología y la micología, están iniciando una nueva etapa en su desarrollo producto de la fusión entre la biología molecular y la informática, no más como resultado del acercamiento que se ha experimentando entre estas dos ramas de la ciencia. La bioinformática, de forma general, y en particular los estudios basados en la caracterización del genoma y el proteoma, han abierto las fronteras que limitaban nuestras posibilidades de combatir los agentes infecciosos y de utilizar la biodiversidad a favor del desarrollo humano.

Rita Colwell, de la Fundación Nacional de la Ciencia de los Estados Unidos, dedicó la conferencia de apertura del Congreso al apasionante mundo de la microbiología marina, de donde se conocen apenas 4 000 microorganismos que se estiman en 1% o menos de los que realmente existen.

Es realmente ilimitado este mundo donde la existencia va desde microorganismos que existen a temperaturas inferiores a 0°C hasta otros aislados a temperaturas superiores a 110 °C. El estudio de estos extremófilos que viven y mantienen la actividad enzimática y funcionalidad de sus moléculas en estas condiciones, está ofreciendo información de cómo estabilizar proteínas y conservar sus sitios activos a temperaturas extremas. Entre los extremófilos están también *Deinococcus radiodurans*, que es capaz de reparar el DNA genómico completamente desnaturalizado después de expuesto a enormes dosis radiactivas, y las Halobacterias, capaces de vivir en concentraciones de saturación de sales como las encontradas en las salinas.

El Congreso centró su mensaje en evidenciar que las nuevas tecnologías que permitirán obtener resultados en los próximos años ya se han generalizado, y comienzan a mostrar su potencial en aplicaciones como:

- la modelación e ingeniería de proteínas
- la identificación y caracterización de la biodiversidad
- la aplicación de la biodiversidad en la Bioremediación
- los estudios de patogénesis
- la caracterización y orientación de la respuesta inmune
- el desarrollo de vacunas.

La bioinformática en la microbiología no sólo se ha aplicado en estudios de caracterización molecular, sino también en la obtención de importantes resultados epidemiológicos, donde sobresale el estudio internacional dirigido a relacionar los cambios climáticos con el surgimiento de las epidemias. Así, ha sido posible evidenciar la coincidencia de factores climáticos con epidemias de dengue, malaria y cólera, entre otras. Estos resultados permitirán, en el futuro, prever el surgimiento de epidemias y tomar medidas para su control.

Algunos de los resultados que se presentaron en el Congreso y que reflejan el desarrollo alcanzado con la aplicación de las nuevas tecnologías son:

- *Helicobacter pylori* es el primer microorganismo del que se tiene secuenciado más de un genoma a partir de diferentes aislamientos. De 50 a 60 % de los genes, pudieron ser asociados a alguna función al compararlos a partir de bases de datos de secuencias. Se encontraron 100 genes que varían entre diferentes cepas, lo que pudiera asociarse con las diferencias en su atogenicidad.
- El empleo creciente del control biológico para evitar el daño al medio ambiente y la toxicidad de los agentes químicos, se evidenció con la aplicación en la República Sudafricana de la levadura *Cryptococcus arbidus* para lograr reducir en 60–70% la pudrición de las frutas causada por los hongos *Botrytis cinerea* y *Penicillium expansum*. Las frutas se sumergen antes de envasar en una solución acuosa donde se encuentra suspendida la levadura.
- Rino Rapuoli presentó el trabajo realizado en el Instituto de Investigación IRIS de Chiron, para el desarrollo de una vacuna antimeningocóccica mediante el proyecto de secuenciación del genoma de *Neisseria meningitidis*. Se identificaron más de 600 proteínas de membrana, de las que se lograron expresar, en *E. coli*, unas 350, de las cuales 50 resultaron nuevas y, de estas últimas, 20 mostraron capacidad de generar anticuerpos bactericidas.
- Se desarrolló un método microbiológico para convertir celulosa en etanol utilizando *Klebsiella oxytoca* recombinante en una mezcla con *Saccharomyces pastorianus* y *Kluyveromyces marcianos*, con un rendimiento de 30–40 g/L de etanol en 220 h de fermentación.
- Se evidenció el efecto de los residuos de manano conjugados a la listeriolisina recombinante de *Listeria monocitogenes*, en la inducción de respuesta de mucosa y respuesta de células Th1.
- Se logró expresar las regiones preS2-S del virus de la hepatitis B en el nuevo hospedero *Pichia stipitis* que usa D-xilosa como fuente de carbono.
- Aislamiento de una nueva proteína de la membrana externa de *N. meningitidis* codificada por el gen *nhh*A, el cual presenta 85% de homología entre serogrupos los A, B y C, y fue detectado en 85% de las cepas.
- Se evidenció el efecto antimicrobiano de diferentes péptidos sintéticos, lo que puede tener aplicación en mantener la esterilidad en composiciones farmacéuticas sin necesidad de usar compuestos químicos tóxicos.
- Uso de cepas de *Proteus vulgaris* capaces de crecer en concentraciones de metal entre 0,2 y 0,5 g/L, para la descontaminación de desechos industriales que contienen cromo y que son frecuentes en la industria de curtido de pieles y en la industria de procesamiento del cromo y cromado.

Resúmenes seleccionados de las conferencias dadas en IX Congreso Internacional de Bacteriología y Micología. Agosto 16–20, 1999, Sidney, Australia.

Selected abstracts of lectures given at 9th International Congress on Bacteriology and Mucology. August 16 –20, 1999, Sydney, Australia.

- Identificación de una nueva cepa emergente de *Vibrio cholerae* en la India que difiere de los serogrupos O1 y O139, y es responsable de 5% de los casos de diarrea detectados en Calcuta.
- Se demostró con el modelo de infección por *H. pylori* el papel de las proteínas de shock térmico en la autoinmunidad.

Las aplicaciones de la Bacteriología y la Micología a favor del desarrollo humano han pasado del empirismo al diseño o selección racional basados en métodos bioinformáticos. La ingeniería de proteínas y la manipulación genética de microorganismos han abierto posibilidades ilimitadas de aplicación en la salud, la industria y la preservación del medio ambiente.

Simultaneous saccharification and fermentation of cellulose to ethanol by co-cultures of *Klebsiella oxytoca* P2 and yeast

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Klebsiella oxytoca P2 is a recombinant bacterium containing *pdc* and *adh* genes from *Zymomonas mobilis* which allow it to produce ethanol in high yield (Wood & Ingram, 1992). The organism has been shown to outperform pure cultures and co-cultures of various yeast strains in the simultaneous saccharification and fermentation (SSF) of cellulose to fuel ethanol. This is attributable at least in part to its ability to metabolise cellobiose, which otherwise accumulates during fermentation, inhibiting the action of the cellulase complex thermototerance and low ethanol tolerance: this requires that the SSF be conducted at temperatures well below the optimum for the enzymes as well as limiting the rate of the SSF and the ultimate ethanol concentration achievable. To overcome these limitations, we conducted SSF experiments using co-cultures of *K. oxytoca* and a variety of other thermo- and ethanol-tolerant ethanol-producing microorganisms. The best results were achieved with a SSF incorporating *K. oxytoca* P2 and the yeast, *Saccharomyces pastorianus* 706900 (University of New South Wales) which permitted a 15% increase in ultimate ethanol yield compared to a pure culture of *K. oxytoca* P2, this result is attributable to the ethanol tolerance of *S. pastorianus* which allows it to continue to ferment in the latter stages of batch SSF, thereby raising the ultimate ethanol yield. The higher ethanol productivities able to be attained with the use of *K. oxytoca* P2 in co-culture are expected to yield significant economic benefits in industrial scale ethanol production processes.

Brent E Wood, Ingrain LO. Applied and Environmental Microbiology 1992;July:2103–10.

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Mannan-listeriolysin conjugate induces Th1 and mucosal antibody responses

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Listeria monocytogenes infection is a frequently used model for acquired cellular resistance (ACR). There has been a strong push in recent times to improve vaccines to this class of bacteria, especially the mycobacteria which cause tuberculosis and leprosy. We are using *Listeria* proteins and peptides to investigate the possibility of using a novel adjuvant, namely mannan coupling, to induce ACR. We chose listeriolysin O (LLO), the pore forming haemolysin secreted by *Listeria* as a readily manipulated model. *Listeria* lacking LLO are unable to induce ACR. Resistance is mediated by CD4+ T-cells, CD8+ T-cells and NK cells. IFN-γ plays a key role in activating macrophages for increased bactericidal activity and clearance of infection. The LLO gene was cloned and expressed in *E. coli.* Initial studies have shown that the purified LLO and peptide 215-226 of LLO are able to recall significant amounts of IFN-γ from the spleen cells of mice infected with live *Listeria.* Induction of CTL has also been established. Investigation of the use of mannan as an adjuvant is continuing, with preliminary data indicating that a Manuan-LLO conjugase can induce some IFN-γ, IL-2, IL-12, antigen specific proliferation, as well as significant titres of IgG2a and IgA. If successful this approach could be applied to mycobacterial proteins and peptides.

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Potential application of a *Proteus vulgaris* strain for the bioremediation in industrial effluents contaminated with chromium

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The application of chromium in a variety of industrial activities such as chrome leather tanning, chrome plating, metal cleaning and processing, has lead to release of this metal into the environment in large quantities through industrial effluents. Although several bacterial strains showing chromate-resistance ability have been isolated, there are only a few studies demonstrating their potencial for chromium bioremediation in industrial waste waters. We investigated on a microbial population of an ecosystem polluted from high concentrations of chromium. A selection of the isolated microorganism was effected in presence of the above contaminant metal and some of them were identified using biochemical and morphological analysis. The microorganisms were identified as *Pseudomonas, Proteus, Erwinia,* etc... In particular we performed a study on a strain of *Proteus vulgaris* isolated from these waste waters in increasing concentration of chromium (III) and (VI). An induction of several proteins of different molecular weight able to bound chromium was observed in strains growth at high metal concenttations (0.2–0.5 g/L). The change of oxidation states seems to be the pivotal role of detoxification utilized by these microorganisms. The microorganism showed a good ability to bound chromium. The removal of this metal is also possible with death cells in fact, experiments performed to evaluate the absorption ability on alginate immobilized cells give the same results obtained with viable cells. On the basis of our data we thought that this chromium-reducing bacterium could have potencial for the bioremediation in industrial effluents contaminated with Cr(III) and (VI).

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Identification of a novel gene encoding an outer membrane protein of *Neisseria meningitidis*

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Neisseria meningitidis is a Gram-negative bacterial pathogen of humans which commonly colonises the nasopharynx and may also cause invasive disease such as meningitis. In previous studies we identifíed homologues of the adhesin AIDA-1 of *Escherichia coli* which contain repetitivo DNA motifs which have been previously associated with phase variable virulence genes. In this study we have identified a further homologue of AIDA-1. This gene has been designated *hiaNm* as analysis of the complete coding sequence revealed that it is more closely related to the adhesins Hia and Hsf of *Haemophilus influenzae.* Using rabbit polyclonal antisera raised against a partially purified fusion protein consisting of maltose binding protein and the *hiaNm* gene product, HiaNm, we were able to demonstrate expression of HiaNm in wild-type *N. meningitidis,* but not in a *hiaNm* knockout mutant strain. Western immunoblot analysis of total cell proteins and outer membrane complex preparations confirmed that HiaNm was localised to the outer membrane. HiaNm-specific bacten'cidal activity was also shown in the anti-HiaNm polyclonal sera. We showed that *hiaNm* is present in 71/71 strains of *N. meningitidis* representative of all the major disease-associated serogroups, based on Southem blot analysis. We propose that HiaNm represents an interesting new outer membrane protein of *N. meningitidis* which may prove to be a useful target for future vaccines.

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Immunological role of heat shock protein 60 in *Helicobacter pylori* infection: study on malt lymphoma with *H. pylori* infection

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Gastric MALT lymphoma is closely associated with *Helicobacter pylori* infection. Ulcerative lesions were induced by oral challenge with *H. pylori* into gastric mucosa in severe combined immunodeficient (SCID) mice that have been transplanted with lymphocytes from MALT lymphoma patients. Gastric mucosa from patients with MALT lymphoma was analyzed by immunohistochemistry with anti-*H. pylori* and anti-heat shock protein (Hsp) antibodies. Foilicular dendritic cells of germinal centres in the stomachs affected by MALT lymphoma were immunostained with anti-*H. pylori* polyclonal antibodies and with anti-human HSP60 mAb. Furthermore, existence of autoantigen shared by *H. pylori* and human gastric epithelial cells was confirmed. Antibody titers to HGC-27 cells were significantly elevated in *H.* pylori-positive MALT lymphoma patients when compared with titers in patients with other gastroduodenal diseases and in healthy subjects. The sera from MALT lymphoma patients often reacted with a protein considered to be Hsp 60 on immunoblotting, and showed elevated antibody titers to the recombinant human Hsp6O by both ELISA and immunoblotting. Antigenic similarity between Hsp 60 and *H. pylori* HspB was documented by immunoblotting using anti-Hsp 60 mab and computer analysis of amino acid sequence. Whole HspB and two special regions of HspB (a domain containing T cell epitope cluster and a domain existing both T cell epitope cluster and similarity region of amino acid sequence) prepared as GST-fusion proteins. We are now investigating immunological role of Hsp with these recombinant proteins.

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The xylose-fermenting-yeast *Pichia stipitis* as host for the heterologous production of the hepatitis B niiddle protein

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The yeast *Pichia stipitis* and the related *Candida shehatae* are the best xylose-fermenting yeasts thus far described. D-xylose is the predominant pentose sugar in hemicellulose, the second most abundant renewable carbon source in nature. Therefore, with the aid of a suitable host-vector system, *P. stipitis* has the potential of producing recombinant proteins on D-xylose as abundant substrate. The envelope of the hepatitis B virus is made up of three proteins, namely the major protein (encoded by the S gene), the middle protein (encoded by the *preS2-S* gene) and the large protein (encoded by the *preS1-preS2-S* gene). These viral genes have already been successfully expressed in *Saccharomyces cerevisiae.* In this study the expression of the *PreS2-S* gene in *P. stipitis* is undertaken. Tbe hepatitis B middle protein gene, *preS2-S,* was cloned under control of the *S. cerevisiae* PGKI promoter into a *P. stipitis* episomal vector carrying the *P. stipitis URA3* gene as a selectable marker. The vector was introduced into a *P. stipitis ura3* auxotrophic strain by means of electroporation. The presence of the recombinant plasmid in the transformants, was established through Southern blotting analysis and the transcription of the *preS2-S* gene in *P. stipitis* transformants was verified by Northern blotting analysis. The production of the recombinant viral protein in *p. stipitis* was established by protein dot blot and western analysis.

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