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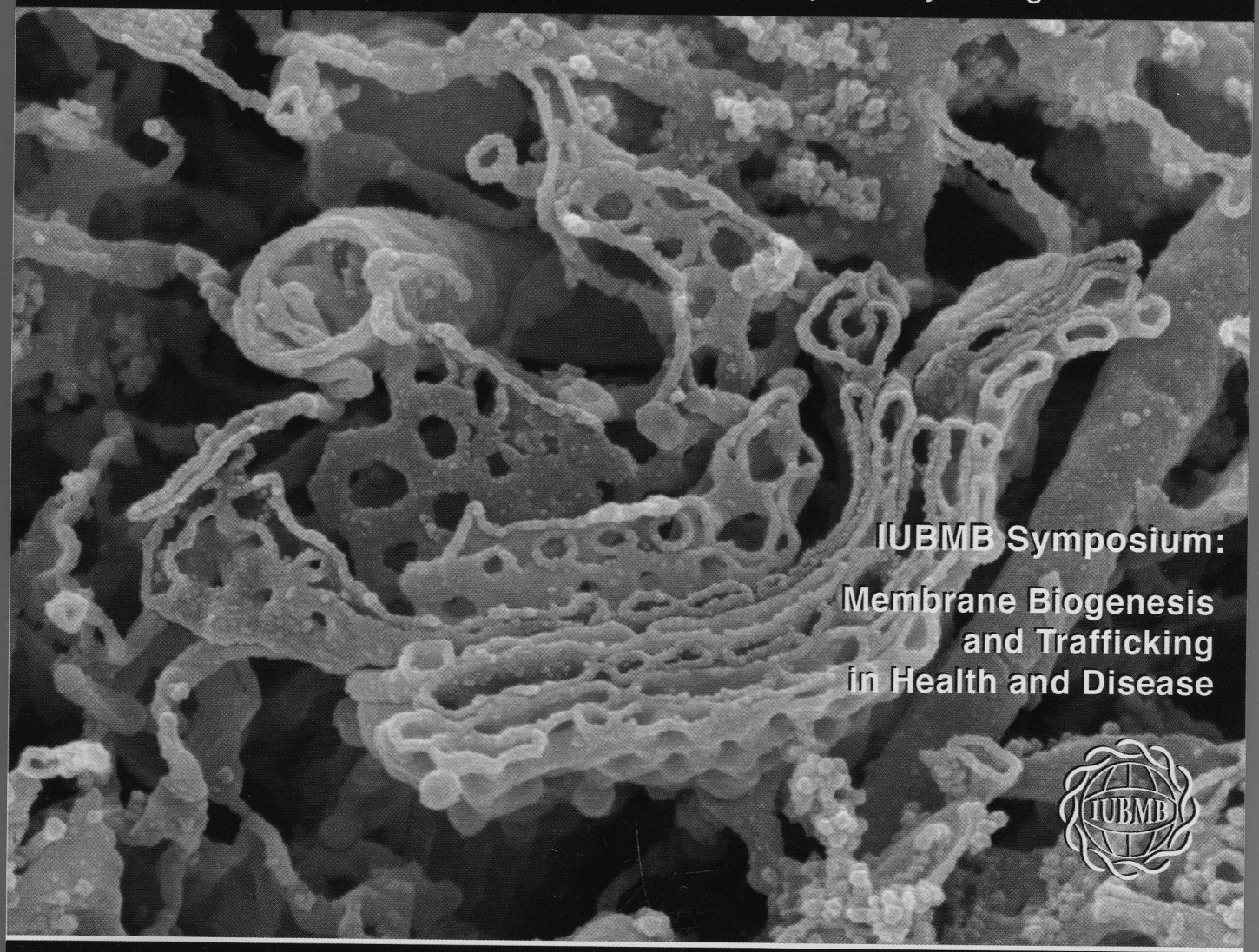

IUBMB Symposium:

Membrane Biogenesis and Trafficking in Health and Disease

10-13 de Noviembre del 2009 San Miguel de Tucumán / Tucumán / Argentina

MI-P61.
COMPARISON OF DEHALOGENASE ACTIVITY BETWEEN PURE AND MIXED CULTURES OF ACTINOMYCETES

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Halogenated compounds are important environmental pollutants. Lindane is a halogenated insecticide prohibited in countries. Its bacterial degradation liberates chlorine by dehalogenases, playing an important role in this process.
Lindane microbial degradation has been studied using pure and mixed microbial cultures. Mixed cultures are suitable for bioremediation because its biodiversity increase the catabolic pathways available for contaminant biodegradation. The aims of this work were to compare dehalogenase activities between pure and mixed cultures of actinomycetes isolated from pesticides contaminated soil, and its identification.
Streptomyces sp. M7, S. coelicolor A3 and four actinomycetes isolates were cultivated alone in minimal medium (MM) with lindane for acclimation. These strains, as pure and mixed cultures, were cultivated in MM with lindane (1.66 mg L-1). Microbial cells were used to obtain cell-free extracts for dechlorinase activity assays. Enzyme activities ranged between 5.14 to 82.39 µmolCl-/h/mg protein. The mixed culture A5-M7 showed the best activity and it was higher than the sum of each activity of pure culture.
The isolates were characterized by 16S rDNA amplifications and sequenced. Actinomycetes were mostly identified as members of *Streptomyces genus*. These native streptomycetes present better ability for lindane bioremediation in mixed cultures.

MI-P62.
KEY ROLE OF Spo0A OF *B. subtilis* PROBIOTIC ON THE INNATE IMMUNE RESPONSE & PATHOGEN EXCLUSION

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Antibiotic resistance has become a major clinical problem. One of the causes has been attributed to their use as growth promoters in animal feed. Probiotics could replace them by generating a protective barrier against harmful microorganisms after colonization of the intestinal mucosa and by stimulating the innate immune response. We studied the effect of probiotic *B subtilis* spores on weight gain, immune response, and pathogens exclusion on chickens. Weight gain resulted to be 5% higher than control group in chickens fed daily with supplements of spores. We also found that Spo0A the master regulator of sporulation is involved in the activation of complement system via classical, alternative and lectin pathways. In contrast, a mutant strain lacking active Spo0A was unable to activate complement system. Spo0A-dependent activation of complement systems by B subtilis reached C5 convertase and binding of C9 was not detected. This could indicate that bacteria are opsonized and then phagosytated by macrophages. Macrophages would then serve as APC rising a specific immune response and thus protection of the host against bacterial pathogens. Moreover, Spo0A was also found to be responsible of exclusion of binding to extracellular matrix proteins of several bacterial pathogens such as *Staph. aureus, Salm. enterica, Vibrio cholerae, Pseud. aeruginosa* and *Clostriidum perfringens*.

MI-P63.
PRESENCE OF REACTIVE PROTEINS AGAINST ANTI-UBIQUITIN ANTIBODY IN HALOPHILIC MICROORGANISMS

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Ubiquitin is a highly conserved protein that takes part in important processes within eukaryotes, but is not present in prokaryotes. However, both eukaryotic and prokaryotic cells contain ubiquitin like proteins (Ubls), which share similar folding and role with ubiquitin.
We previously found proteins recognized by anti-ubiquitin antibodies plus a PCR-product codifying for a peptide with ubiquitin-like structure in haloalkaliphilic archaea. Based on these results, a search for proteins that react with the antibody against ubiquitin was performed in several halophilic archaea and bacteria. For this, microorganisms growth was followed by estimation of OD_{600}, and DNA, RNA, and protein contents. Protein extracts were separated by 1D SDS-PAGE and subjected to Western blot test using a polyclonal anti-ubiquitin antibody. All samples displayed immuno-reactive bands and these results were confirmed by 2D gel electrophoresis. Four most characteristic reactive spots (Mr 50-55 kDa and Ip 3-4) were excised from the gel and subjected to protein sequence determination. The analysis of a 53 kDa spot from *Halobacterium halobium* showed that it corresponds to the elongation factor EF-1α, which is not an Ubl but takes part in misfolded protein degradation mediated by ubiquitin. Its relationship with Ubls is being analyzed.
Supported by CONICET and UNMdP.

MI-P64.
DETECTION OF δ-PCCH AND 1,4-TCDN IN Streptomyces CELL-FREE EXTRACTS BY THE DECHLORINASE ON δ-HCH

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The synthesis of dechlorinase in Streptomyces sp. M7 was induced when the microorganism was grown in the presence of lindane (δ-HCH) as the only carbon source. It was grown in MM medium containing 100 µg ml-1 δ-HCH and incubated at 30 °C for 48 and 96 h. After incubation, pellets were aseptically harvested by centrifugation.
The cell-free extract was used for extraction of δ-HCH, δ-pentachlorocyclohexene (δ-PCCH) and 1,3,4,6-tetrachloro-1,4-cyclohexadiene (1,4-TCDN). They were extracted by solid phase extraction using C18 columns and the residue was resuspended in hexane. Routine quantitative determinations of lindane catabolism were carried out with gas chromatograph- electron capture detection-mass spectrometry analysis.
The δ-PCCH and 1,4-TCDN were detected, both being products of the dechlorinase activity from the cell-free extract at 48 and 96 h of growth of *Streptomyces sp.* M7 in MM with lindane. The appearance of δ-PCCH (Rt 6.26 min) and 1,4-TCDN, (Rt 5.29 min), the first and second product of the lindane catabolism by a specific dechlorinase was proposed by other researchers in *Sphingomonas*. The relative abundance of both increased one and half times, at 96 h compared to 48 h of growth. Were not found in MM with lindane. This is the first time that an enzyme with dechlorinase activity has been demonstrated in an actinomycete strain isolated in Tucumán, Argentina.