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Bio-recognition capability of *Streptomyces* sp. M7 evaluated in adverse conditions for use as a biological transducer in a Lindane biosensor

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Abstract— **Bio-recognition devices have captured special attention because they combine biological specificity and selectivity with electronics to perform environmental and biomedical analysis. Lindane is a recalcitrant pesticide considered potential carcinogen that has caused serious pollution problems. The purpose of the present study is to evaluate *Streptomyces* sp. M7 ability to dechlorinate lindane in liquid defined media in adverse culture conditions. Bacterial activity was monitored by electrochemical impedance spectroscopy. These results confirm that the microorganism adhered to a solid support is able to grow and to metabolize the organochlorine pesticide as a sole carbon source. Therefore, *Streptomyces* sp. M7 can be applied for a future development of a prototype for lindane detection and quantification.**

I. Introduction

ORGANOCHLORINE insecticides were extensively employed for medical and agricultural purposes [1]. However, indiscriminate use of pesticides has caused serious concern about toxic effects by residues on non-target organisms. Lindane, the gamma isomer of hexachlorocyclohexane (γ-HCH), is a highly chlorinated, recalcitrant pesticide, considered potential carcinogen and listed as priority pollutant by the US EPA [2]. The Salí River (Tucumán, Argentina) is highly contaminated with lindane although this pesticide has been banned in our country several years ago [3]. Pesticides determination requires very expensive methods (e.g. gas chromatography with electron capture detector [4]) and laborious extraction processes. Thus, reliable and cost-effective methods for lindane determination and quantification are of great interest.

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In the last years, electronic devices for bio-recognition are feasible, simple and low-cost alternatives, which can be used in direct environmental measurements. These devices combine specificity and selectivity of biology with electronic miniaturization power. Aerobic degradation of γ-HCH by fungi [5], Gram-negative [6], and Gram-positive [7] bacteria have been reported. Benimeli and co-workers [8], [9] isolated a wild-type streptomycete strain, identified as *Streptomyces* sp. M7 (*SM7*) with high capability of lindane degradation in liquid culture [10]. Therefore, this microorganism may be part of the bio-recognition transducer in a biosensor device for lindane detection and quantification. In previous works we have demonstrated the capability to remove glucose from the liquid culture by the *SM7* grown and adhered to starch-casein agar (SC agar) plates with no shaking and temperature control [11], [12]. Thus, bacterial activity is preserved in hostile conditions which are the possible future work conditions of the final prototype.

Electrochemical impedance spectroscopy has been widely used in the field of microbiology [13]. This non-destructive technique allows detecting, quantifying and even identifying bacteria [14] measuring conductivity changes in the culture medium. Continuing with our previous studies, the aim of this work is to evaluate the capability of adhered *SM7* to a solid matrix to degrade lindane in liquid media by monitoring impedance changes in the culture medium.

II. Materials and Methods

A. Reagents

Lindane (Accustandard Inc., New Haven, USA), solvents and other chemicals were reagent grade and used without further purification. The kit for spectrophotometric determination of glucose was obtained from Wiener lab. All solutions were prepared with ultrapure water (ρ =18 MΩcm) from a Millipore-MilliQ system.

B. Microorganisms and culture media

A previously isolated and characterized strain, *Streptomyces* sp. M7 [8], [9] was used for this work. This strain was stored prior to use on SC agar medium slants containing (in grams per liter): starch, 10.0; casein, 1.0; K_2HPO_4, 0.5; agar, 15.0 [15]. Spores were inoculated in a modified [16] liquid-defined medium (MM) with (in grams

per liter): $(NH_4)SO_4$, 2.0; K_2HPO_4, 0.5; $MgSO_4 .7H_2O$, 0.20; $FeSO_4.7H_2O$, 0.01. Both culture media were adjusted to pH 7.0 prior to sterilization. A stock solution of lindane was sterilized by passing through a 0.22 μm pore size Millipore filter and then added aseptically to the autoclaved medium to a final concentration of 100 μmol L^{-1} (MM+Lin) [17].

C. Batch cultures in synthetic medium

Quantibac cells containing *SM7* spores adhered to SC agar surfaces were inoculated with MM+Lin or MM supplemented with D-glucose (10.0 g L^{-1}), (MM+Glu). Cultivation was carried out for a period of 96 h at 30ºC, without shaking. Control cultures of MM (without lindane or glucose) were also prepared.

D. Apparatus

Impedance measurements were performed with the commercial equipment QUANTIBAC. The instrument is computer controlled and simultaneously measures impedance and turbidity in a culture cell. It is composed by two incubators with variable temperatures independently controlled. The cells are glass bottles of 10 ml capacity with two stainless steel electrodes. The bipolar impedance measured between these two electrodes can be separated into its medium and interface components. The latter is very sensitive to bacterial growth. The equipment uses two frequencies (20 Hz and 20 KHz) in order to separate these two components. It has 30 channels and measures these two electrical and one optical parameters in each channel [18], [19].

A UV-1601 Shimadzu spectrophotometer equipped with PC-interface and analytical software was used for the spectrophotometric determinations.

E. Analytical methods

Each experiment was carried out in duplicate and the results are arithmetic means. Bacterial activity was evaluated by changes in electrical impedance in the liquid medium. In addition, supernatant samples of centrifuged cultures (9,900 x g, 10 min) were used to determine residual either glucose or lindane after 96 h of cultivation.

Glucose determination was carried out by enzymatic assay with a commercial kit based on glucose oxidation by glucose oxidase-peroxidase [20].

For lindane determination by gas chromatograph-electron capture detection, a Hewlett-Packard Model HP 6890 gas chromatograph equipped with an electron capture detector and a HP1 capillary column was used. The chromatographic conditions were: carrier gas (helium) flow rate, 1.5 mL min^{-1}; injector temperature, 200°C; detector temperature, 300°C; and injection volume, 1 μL.

Free inorganic chloride was measured using a modification [21] of the mercury (II) thiocyanate method proposed by Bergmann and Sanik [22].

Quantitative analysis of samples was performed using appropriate calibration standards. All measurements were performed at 25°C.

III. Results and Discussion

Impedance is very sensitive to bacterial content and contains important information about microbiological quality of samples. More specifically, interface impedance, the most sensitive parameter, can present variations up to 70% with bacterial growth, while medium resistance may vary up to 25%. In general, changes in medium conductance recognize the metabolic products generated by microorganism proliferation [13]. With bacteria, contributions to the medium conductance changes are mostly due to proteolytic activity while carbohydrate metabolism is of less importance [23].

Analysis of culture medium resistance showed a considerable difference for cells with *SM7* and MM+Glu or MM+Lin (see Fig. 1). In the first case, liquid medium resistance was found to be near zero during 96 h indicating that metabolic bacterial activity did not change the electrical resistance. On the other hand, cells with *SM7* and MM+Lin showed growth curves, reaching the highest value among 24 h of cultivation. Continuous increments in this electrical value point out that ionic changes in the medium was due to chloride release in the lindane degrading process by the *SM7*. Reference cells (i.e. cells with inoculated MM but with no carbon source) did not present variations on their resistance values during the experiment.

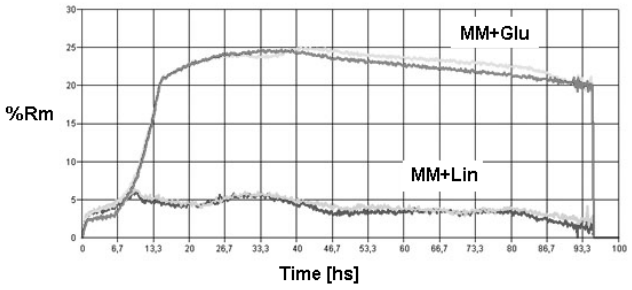

Fig. 1. Medium resistance percent variation curves for *SM7* growing in MM+Glu and MM+Lin.

The interface capacitance is more difficult to interpret because it contains contributions due to the geometry (roughness) of the electrodes and also to the electrochemical characteristics of the interface [24]. In a typical nutrient broth there is a complex mixture of organic and inorganic ions. Bacterial metabolism can modify the double-layer capacitance because of the induced changes in the ionic diameters, in the charges and in the ionic concentrations [14].

A preliminary study of the interface reactance showed a diauxic growth of the *SM7* when asparagine was the nitrogen source in the liquid media (Fig. 2). This problem was solved replacing the amino acid for an inorganic nitrogen source such as $(NH_4)SO_4$ before the experiment was carried out.

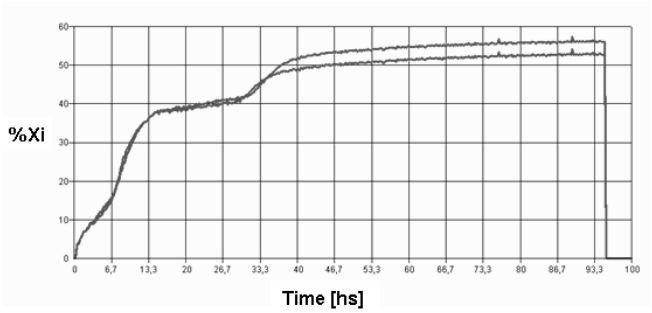

Fig. 2. Diauxic growth of *SM7* with asparagine as nitrogen source.

Analysis of interface reactance also showed growth curves for cells with *SM7* and MM+Lin suggesting changes in the electrodes interfaces. In this case, maximum values were achieved within 48 h. When glucose or glucose and lindane were present in the liquid medium, morphology of the curves differed noticeable from the curves of culture conditions previously analyzed as can be seen in Fig. 3.

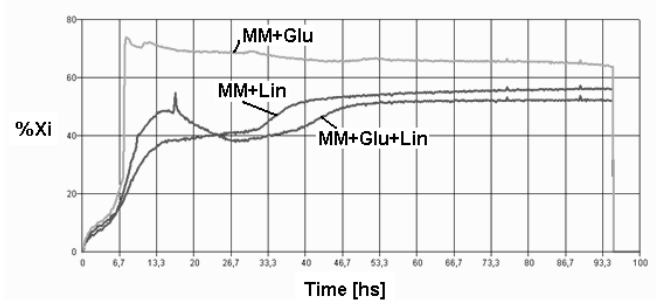

Fig. 3. %Xi growth curves for *SM7* growing in MM+Glu, MM+Lin and MM+Glu+Lin.

In addition, bacterial activity was evaluated at the end of the experiment by a model carbon source, i.e. glucose, and by the halogenated insecticide as a sole carbon source by means of residual glucose and lindane determinations in the liquid media. After 96 h initial glucose concentrations significantly diminished indicating *SM7* activity. Lindane degradation was slower indicating a prolonged adaptation of the bacterial metabolism to the culture conditions. Free inorganic chloride present in the supernatant samples was according with lindane dechlorination whereas no chloride release was found in samples with MM+Glu.

When both carbon sources were present at the same time in the liquid medium, the *SM7* did not consume glucose and lindane at equal rates according to previous work [8]. Instead, lindane degradation began after an adaptation period of time.

IV. Conclusion

SM7 capability of degrading organochlorine pesticides remains unalterable under unfavorable culture conditions, i.e. without shaking and adhered to a solid matrix. Therefore, *SM7* may be used as the biological transducer to detect and quantify lindane in bio-recognition dispositives for environmental studies. Impedance measurements appear to be useful in determining the *SM7* metabolism and to distinguish between glucose and lindane as carbon source. This is a promising transduction method to use in the biosensor.

References

[1] J. C. Quintero, M. T. Moreira, G. Feijoo and J. M. Lema. "Anaerobic degradation of hexachlorocyclohexane isomers in liquid and soil slurry systems". Chemosphere, vol. 61 (2005), pp. 528-536.

[2] K. Walker , D. A. Vallero and R. G. Lewis. "Factors influencing the distribution of lindane and other hexachlorocyclohexanes in the environment". Env. Sc. Tech., vol. 33 (1999), pp. 4373-4378.

[3] A. P. Chaile, N. Romero, M. J. Amoroso, M. Del V. Hidalgo and M. C. Apella. "Organochlorine pesticides in Sali River. Tucumán". Rev. Bol. Ecol. Vol., vol. 6 (1999), pp. 203-209 (in Spanish).

[4] A. J. Hedley, L.L. Hui, K. Kypke, R. Malisch, F. X. R. van Leeuwen, G. Moyd, T.W. Wong and E. A. S. Nelson. "Residues of persistent organic pollutants (POPs) in human milk in Hong Kong". Chemosphere, vol. 79 (2010), pp. 259–265.

[5] B. K. Singh and R. C. Kuhad. "Biodegradation of lindane (γ-hexachlorocyclohexane) by the white-rot fungus Trametes hirsutus". Letters in App. Microbiol.., vol. 28 (1999), pp. 238-241.

[6] B. K. Singh and R. C. Kuhad. "Degradation of the insecticide lindane (γ-HCH) by the white-rot fungi Cyathus bulleri and Phanerochaete sordida". Pest Manag. Sc., vol. 56 (2000), pp. 142-146.

[7] A. De Schrijver and R. De Mot. "Degradation of pesticides by actinomycetes". Critical Reviews in Microbiol. vol 25 (1999), pp. 85-119.

[8] C. S. Benimeli, M. J. Amoroso, A. P. Chaile and R. G. Castro. "Isolation of four aquatic streptomycetes strains capable of growth on organochlorine pesticides". Biores. Tech. vol. 89 (2003), pp. 133-138.

[9] C. S. Benimeli, R. G. Castro, A. P. Chaile and M. J. Amoroso. "Lindane removal induction by Streptomyces sp. M7". J. Basic. Microbiol. vol. 46, pp. 348-357.

[10] C. S. Benimeli, M S. Fuentes, C. M. Abate and M. J. Amoroso. "Bioremedation of lindane-contaminated soil by Streptomyces sp. M7 and its effects on Zea mays growth". Intern. Biodet. and Biodegrad. vol. 59 (2007), pp. 148-155.

[11] M. L. López Rodriguez, C. E. Giacomelli; R. E. Madrid and C. J. Felice. "Optimización de la capacidad de bio-reconocimiento superficial de Streptomyces sp. M7" in XVI Congreso Argentino de Fisicoquímica y Química Inorgánica, Salta, Argentina, 2009.

[12] M. L. López Rodriguez, C. E. Giacomelli; C. J. Felice and R. E. Madrid. "Evaluación de actividad de Streptomyces sp. M7 adheridas a un soporte sólido" in 6to Congreso Iberoamericano sobre Sensores, IBERSENSOR 2008, Sao Paulo, Brasil, 2008.

[13] N. Ramirez, A. Regueiro, O. Arias and R. Contreras. "Electrochemical impedance spectroscopy: an effective tool for a fast microbiological diagnosis". Biotecn. Aplicada, vol. 26 (2009), pp. 72-78.

[14] C. J. Felice, R. E. Madrid, J. M. Olivera, V. I. Rotger and M. E. Valentinuzzi. "Impedance microbiology: quantification of bacterial content in milk by means of capacitance growth curves". J. Microbiol. Meth. vol. 35 (1999), pp. 37-42.

[15] D. A. Hopwood. "Genetic análisis and genome structure in Streptomyces coelicolor". Bacter. Reviews, vol. 31 (1985), pp. 373-403.

[16] M. L. López Rodriguez, G. L. Luque, R. E. Madrid, C. J. Felice, N. F. Ferreyra, C. E. Giacomelli and G. A. Rivas. "Cuantificación de asparagina en cultivos celulares con electrodos compósito de canotubos de carbono codificados con cobre" in V Congreso Argentino de Química Analítica, Bahía Blanca, Argentina, 2009.

[17] M. J. Amoroso, R. G. Castro, A. Duran, G. Oliver and R. T. Hill. "Chromium cation uptake by aquatic actinomycetes". J. Ind. Microbiol. Biotechnol. vol. 26 (2001), pp. 210-215.

[18] R. E. Madrid, J. C. Felice, M. E. Valentinuzzi. "Automatic on-line analyzer of microbial growth using simultaneous measurements of impedance and turbidity". Med. Biol. Eng. Comp., vol. 37 (1999), pp.789-793.

[19] R. E. Madrid, C. J. Felice, Assignee: UNT-CONICET, “Equipment for analyzing microbial contamination by impedance and turbidity”, Argentine Patent AR014143B3 (Dic, 2006).
[20] P. Trinder. “Determination of blood glucose using 4-amino phenazone as oxygen acceptor”. J. Clin. Pathol. vol. 22 (1969), pp.246.
[21] S. F. Pesce and D. A. Wunderlin. “Biodegradation of lindane by a native bacterial consortium isolated from contaminated river sediment”. Intern. Biodet. and Biodeg. vol. 54 (2004), pp. 255-260.
[22] J. G. Bergmann and J. Sanik. “Determination of trace amounts of chlorine in naphtha”. Analytical Chem. vol. 29 (1957), pp. 241-243.
[23] G. Suhren and W. Heeschen. “Impendance assays and the bacteriological testing of milk and milk products”. Milchwisenschaft vol. 42 (1987), pp. 619-627.
[24] C. J. Felice. “Monitor digital de microorganismos: aspectos teóricos y prácticos”. PhD dissertation, INSIBIO-UNT, Tucumán, Argentina, 1995.