MICRONUCLEUS ASSAY OF RHAMNOLIPIDS BIOSURFACTANT

Sánchez-Noriega Luz María, Valdez-Bastidas Mariel, Romo-Martínez Enrique Jhonatan, García-Magallanes Noemí, Bojórquez-Sánchez Carolina, Amézcua-Vega Claudia*
Universidad Politécnica de Sinaloa. Department of Biotechnology, Mazatlán,Sinaloa, México.
*camezcua@upsin.edu.mx

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Introduction. Rhamnolipids represent an important group of biosurfactants having various industrial, environmental, and agriculture applications. However, little is yet known about their toxic properties to human. The *in vitro* micronucleus assay with cultured human lymphocytes is a mutagenic test system for the detection of chemicals which induce the formation of small membrane bound DNA fragments, micronuclei in the cytoplasm of interphase cells. These micronuclei may originate from acentric fragments or whole chromosomes which are unable to migrate with rest of the chromosomes during the anaphase of cell division (1).

The objective of this preliminary study was to evaluate the incidence of micronuclei/chromosome damage induced by rhamnolipids biosurfactants at different concentrations in cultured human lymphocytes.

Methods. Rhamnolipids biosurfactant was obtained by *P. aeruginosa,* previously it was cultivated on nutrient broth during 48 h. Rhamnolipids-semipurified was obtained according to Amézcua-Vega (2). Blood samples were taken from 5 healthy donors, 20-30 years of age, under sterile conditions with sodium heparine anticoagulant. Semi-purified rhamnolipids in concentrations of 0.25, 0.50, 1 mg/mL were dissolved in RPMI medium. These compounds were added to the cultures just before incubation. The peripheral blood culture with RPMI-1640 medium and phytohemagglutinin (PHA) as mitogen were incubed at 37°C for 24 and 48 h. The micronuclei test was performed by adding cytochalasin-B after 44 h of culture to inhibit cytokinesis (3). At the end of the incubation period, the cells were treated with KCl hypotonic solution (75 nM) and fixed with 1:3 acetic acid:methanol. The fixed cells were put directly on sides and stained with Giemsa solution.

Results. Figure 1 shows, micronuclei (MN) number observed for treated lymphocytes with different concentration of rhamnolipids, having control group as reference. This preliminary study showed that there is not significant difference ($p<0.05$) among the different concentrations of rhamnolipids and the control, were did not observe in both formation of micronuclei and degree of DNA damage.

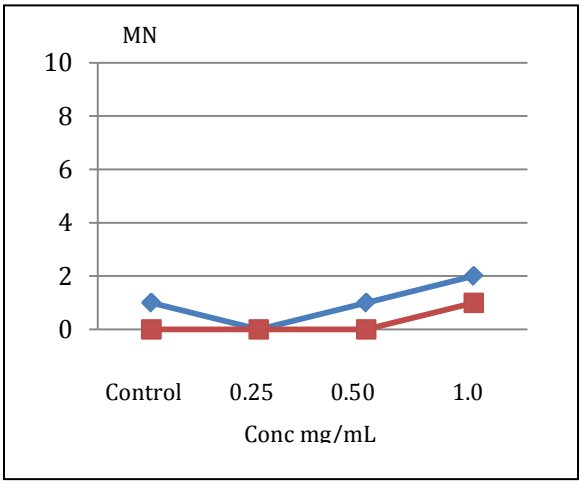

Fig.1. Micronuclei number present in human lymphocytes from healthy donors stimulated with different concentration of rhamnolipids at 24 and 48 h. (■) 24 (♦) 48 h.

Conclusion. Our preliminary results suggested that rhamnolipids biosurfactant have not genotoxic effect.

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