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Cytoxicity of Bacterial Chitinases

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Chitinases are enzymes that hydrolyze the C1 and C4 link of chitin, a B -1,4 polymer of N-acetylglucosamine. Since fungal cell walls are rich in chitin, the use of chitinases as antifungal agents in foods and feeds is promising. Several studies reported the potential of bacterial chitinases as antifungal agents. These studies showed the ability of the enzymes to lyze cell wall and inhibit growth of fungi (Horikoshi and Iida, 1958;1959, Horikoshi and Sakaguchi, 1958, Llyod et al., 1965, Morrissey et al., 1976, Barrows-Broaddus and Kerr, 1981, Ordentlich et al., 1988, Inbar and Chet, 1991, Gay et al., 1992, and Gupta et al., 1995).

Crude chitinases from the following bacteria namely; *Bacillus* circulans B1037, Arthrobacter luteus B1077, *B. circulans* B1045, *Streptomyces griseus* B1562, *Serratia marcescens* LPC19 B1748 and *S. marcescens* LPM42 B1749 were produced and assayed. Their antifungal activity against *Aspergillus flavus* and *A. parasiticus* was demonstrated (Doyungan, 1996). The crude chitinases were able to lyze mycelia and inhibit spore germination of the test molds.

Cytotoxicity of the pure and crude bacterial chitinases were analyzed following the Cytotoxicity-MEM Elution-MTO23 technique developed by the North American Science Associates, Incorporated (NAmSA), Northwood, Ohio, USA. The analysis was conducted by the Microbiological Service Unit of the National Institute of Molecular Biology and Biotechnology (*BIOTECH), UPLB.*

Results show that the pure chitinase from S. marcescens and the six crude chitinases were toxic to mouse fibroblast cells at different dilutions (Table 1). All the fibroblast cells were lyzed (100% lysis) at different dilutions.

However, cytotoxicity analysis of the corn kernels immersed for 24 hours in the pure and crude chitinases showed that only chitinases obtained from *A. luteus* and *S. griseus* were toxic and caused lysis (up to 90%) of the fibroblast at 1:8 and 1:4 dilution respectively (Table 2).

The observed lysis of the mouse fibroblast cells can be attributed to the alteration/s in their cell membrane. The cell membrane contains carbohydrates as structural component, usually there are oligosaccharides side chains linked covalently to proteins to form glyco-

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Table 1. *Cytotoxic activity of the crude chitinases in mouse fibroblasts*.*

ENZYME	PARAMETERS				
	Confluent monolayer	Intracellular granulation	Swelling	Lysis CTE	Score
Control (-)	+	-	-	-	N
Control (+)	+	-	-	100% up to 1:4	T
5Bc	+	-	-	100% up to 1:4	T
6Al	+	-	-	100% up to 1:64	T
7Bc	+	-	-	100% up to 1:32	T
9Sg	+	-	-	100% up to 1:32	T
11Sm	+	-	-	100% up to 1:32	T
14Sm	+	-	-	100% up to 1:4	T

*Based on the report of cytotoxicity test conducted by the Microbiological Service Unit of the National Institute of Molecular Biology and Biotechnology (BIOTECH), UPLB (Appendix R)

Legend:

Control (-) - minimum essential medium (MEM)

*Table 2. Cytotoxic activity of the crude chitinase-treated corn kernels in mouse fibroblasts.**

ENZYME	PARAMETERS				
	Confluent	Intracellular monolayer	Swelling granulation	Lysis CTE	Score
Control (-)	+	-	-	-	N
Control (+)	+	-	-	-	N
5Bc	+	-	-	-	N
6Al	+	-	-	90% up to 1:8	T
7Bc	+	-	-	-	N
9Sg	+	-	-	90% up to 1:4	T
11Sm	+	-	-	-	N
14Sm	+	-	-	-	N

*Based on the report of cytotoxicity test conducted by the Microbiological Service Unit of the National Institute of Molecular Biology and Biotechnology, UPLB (Appendix S).

proteins. Oligosaccharides containing N-acetylglucosamine monomers in B-1,4 linkages are likely to be present in the membrane. Exposure of these oligomers to the enzyme could have trigerred changes in the membrane permeability resulting to the lysis of the fibroblasts. On the other hand, corn kernels treated with the chitinases showed to be non toxic to the fibroblasts except those treated with *A. luteus* and *S. griseus* crude chitinases. It is probable that changes in the enzyme following treatment to corn kernels had occurred causing loss of the capacity of the enzyme to lyze fibroblast cells. Probably, some compounds in the corn changes the inhibitory effect of chitinase. However, the cytotoxicity of A. luteus and S. griseus crude chitinases can be attributed to the presence of other substances in the culture filtrate.

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