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SHORT COMMUNICATION

IMMUNITY TO MEGACIN A IN PROTOPLASTS OF MEGACINOGENIC *BACILLUS MEGATERIUM*

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Bacillus megaterium 216 MA^+ is sensitive to megacin A when tested with preparations of high concentration (IVANOVICS *et al.*, 1954), while megacin A negative mutants of the strain (MA^-) are about 50 times more sensitive to megacin A than MA^+ (HOLLAND, 1963; OZAKI *et al.*, 1966). The lower sensitivity of MA^+ to megacin A could be interpreted as an expression of partial immunity to megacin, because immunity to colicins is generally incomplete and hence colicinogenic cells are sensitive to high concentrations of a homologous colicin (FREDERICQ, 1958).

Recently, megacin A, an inducible bacteriocin of *Bacillus megaterium* strain 216 was identified as phospholipase A in this laboratory (OZAKI *et al.*, 1966). It was of interest to study the mechanism of immunity to megacin A, which is the first bacteriocin characterized as an enzyme.

To test whether partial immunity is still retained by the protoplasts, protoplast-lysis by megacin A was compared in strains MA^+ and MA^-. The latter strain was isolated in this laboratory by UV irradiation. Bacterial cells were grown in 1.0 per cent casamino acid medium supplemented with NaCl 3 g, K_2HPO_4 0.7 g, KH_2PO_4 0.3 g and Na_2SO_4 0.1 g per liter and the protoplasts were prepared with lysozyme in 15 per cent sucrose medium.

As shown in Fig. 1, protoplasts of MA^- strains were about 30 fold more sensitive to megacin A than those of strain MA^+. These results indicate that immunity to megacin A was still retained at the protoplast-level.

To test the specificity of the immunity, the sensitivities of these protoplasts to phospholipase A of heated " Habu " venom were studied. As seen in Fig. 2, no difference in these sensitivities to the phospholipase A of " Habu " venom was observed. These results showed that partial immunity to megacin A was also found in the protoplasts of strain MA^+ and that the immunity was specific.

Further studies are required on the mechanism of immunity at a molecular level, and studies on the chemical composition, especially the fatty acid composition of phospholipids of both cell membranes are in progress.

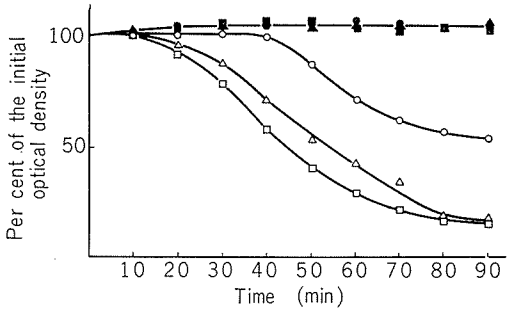

FIGURE 1 The megacinogenic strain (MA^-) and two non-megacinogenic mutants ($MA^- - 1$, $MA^- - 2$) were cultured in casamino acid medium. Cells were washed and suspended in M/15 phosphate buffer containing 15 per cent sucrose and 4×10^{-3}M $MgCl_2$ and treated with lysozyme. 200 units and 2,000 units (final concentration) of megacin A were added, to the protoplasts of strains MA^- and MA^+ respectively. The optical densities were measured at intervals.

□——□ Non-megacinogenic strain 1 ($MA^- - 1$)
△——△ Non-megacinogenic strain 2 ($MA^- - 2$)
○——○ Megacinogenic strain (MA^+)
■——■ $MA^- - 1$ control
▲——▲ $MA^- - 2$ control
●——● MA^+ control

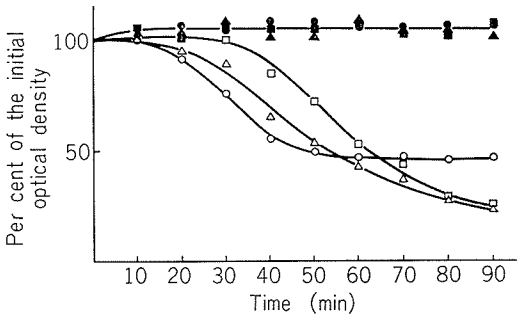

FIGURE 2 Experimental conditions were as in Fig. 1, except that megacin A preparations were replaced by heated " Habu " venom; 10 mg of dry venom were dissolved in M/15 phosphate buffer an dheated at 100°C for 10 minutes (1 : 2,000 dilution in final concentration).

□——□ Non-megacinogenic strain 1 ($MA^- - 1$)
△——△ Non-megacinogenic strain 2 ($MA^- - 1$)
○——○ Megacinogenic strain (MA^+)
■——■ $MA^- - 1$ control
▲——▲ $MA^- - 2$ control
●——● MA^+ control

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