Short Paper

Effect of Josamycin on Form and Number of Cells Forming One Chain of *Streptococcus* sp.*¹

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Bacteria under the effect of antibiotics change their form due to inhibition of production and synthesis of materials for growth. *In vitro*, josamycin (JM) shows a strong antibacterial activity to *Streptococcus* sp., isolated from diseased fish.¹⁾ In this paper, the morphological changes of *Streptococcus* sp. exposed to JM was observed under light-microscope for the purpose of investigating antibacterial effect.

Streptococcus sp. YT-3 was cultured in brain heart infusion (BHI) broth at 37°C for 24 h. One ml of 100 fold diluted broth culture was added into 4 flasks with 98 ml of BHI broth. Immediately, one ml of various concentrations of JM solution were added and incubated at 37°C without shaking. The final concentrations of JM in each flask were 0.39, 0.20, 0.10 and 0 µg/ml. A sample of each culture broth was taken for analysis the number of colony forming unit (CFU), by agar dilution method, and staining of cells with methylene-blue after 0, 3, 6, 12, 18, 24, 48 and 72 h. The bacterial form and number of cells forming one chain were observed under light-microscope.

The initial concentration of bacteria in each broth was 4.1×10^4 CFU/ml. Bacteria could grow in the presence of each JM concentration except 0.39 µg/ml. In 0.39 µg/ml JM concentration, the number of CFU gradually decreased and a change of cell form could not be observed. In JM concentrations allowing growth, it was revealed that the bacterial cell form become larger than non-treated cells and the number of cells forming one chain increased. As well as the cells were well stained by methylene-blue (Fig. 1). The time-lapse effect of various JM concentrations on *Streptococcus* sp. CFU and the number of cells forming one chain are shown in Fig. 2. Enlargement and increase of cell chains were observed at late log phase and these phenomena ceased slowly at stationary phase.

As for the morphological affect of protein synthesis inhibitory agents on bacteria, Shockman²⁾ reported that *Streptococcus faecalis* exposed to chloramphenicol developed cell wall thickness. Nakao *et al.*³⁾ reported that *Staphylococcus aureus* exposed to erythromycin (EM), spiramycin (SPM), lincomycin and clindamycin developed extreme thickness and multilayer formation of cell walls and also increased the incorporation of L-lysine in the cell wall fraction but decreased the protein fraction. The phenomena observed in this study might be a cause of thickening of cell walls because JM is macrolide as EM and SPM, and their antibacterial actions are similar. It is suggested that thickening of the division wall followed by an increase in number of cells per chain, especially at log phase growth,

Fig. 1. Light-microscopic observation of *Streptococcus* sp. YT-3 on BHI broth at various concentrations of josamycin. A: 0 µg/ml. B, C: 0.20 µg/ml. Bar indicate 10 µm.

Fig. 2. Influence of josamycin at different doses on the viable cell count (CUF) and number of cells forming one chain of *Streptococcus* sp. YT-3. A, x: 0.39 µg/ml; B, □: 0.20 µg/ml; C, △: 0.10 µg/ml; D, ○: 0 µg/ml; a: 1-5; b: 6-10; c: 11-20; d: 21-50; e: 51-100; f: 100<; ND: not detected.

showed the ability of bacteria to produce protein was at its lowest point in comparison to cell wall synthesis.

Reference

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*¹ Chemotherapeutic study of josamycin against streptococcal infection in cultured yellowtail-V.

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