Note

EFFECT OF AZALOMYCIN F ON BACTERIA

SHINICHI SUGAWARA

Central Research Laboratories,
Sankyo Co., Ltd.

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It has previously been reported that azalomycin F could induce permeability changes in *Candida albicans* causing loss of cell constituents and inhibition of substrate uptake which resulted in the death of the organism[1]. The present report is concerned with the effect of the antibiotic on bacteria.

Methods and Results

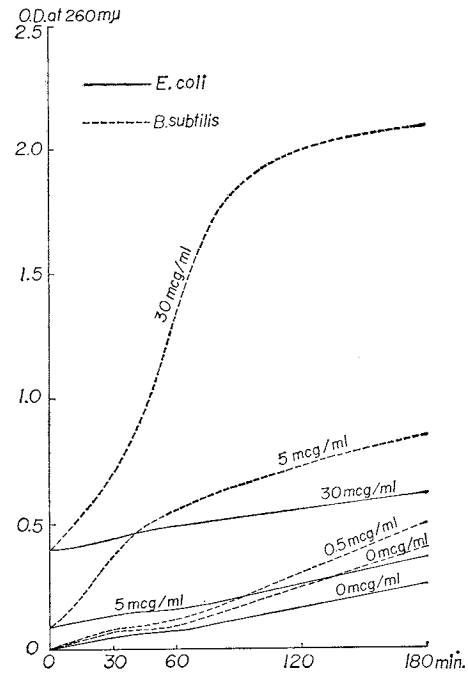
Azalomycin F inhibited the growth of *Bacillus subtilis* PCI 219 at concentrations of 3.0 mcg/ml in STEPHANSON-WHETHAM medium and of 3.0~6.0 mcg/ml in nutrient broth, whereas it failed to inhibit the growth of *Escherichia coli* NIHJ even at concentrations as high as 200 mcg/ml in both media. In the presence of the antibiotic viable cells of *B. subtilis* were rapidly reduced in number during the incubation indicating the drug action is bactericidal (Table 1).

The release of soluble constituents from washed cells by the action of azalomycin F was then examined in the following way[2]. The organism was grown overnight in nutrient broth. Cells were harvested by centrifugation, washed thoroughly in physiological saline, and finally suspended in 1% (w/v) saline to give a suspension containing between 2 and 5 mg dry weight cells per ml. The cell suspension was incubated at 30°C with shaking in the presence of the antibiotic and samples were taken at various times and cooled with ice. Cells were removed by centrifugation at $5,000 \times g$ for 10 minutes, followed by filtration through Millipore filter, and the ultraviolet absorption spectra of the cell-free supernatant fluids measured. The rate of release of 260 mμ absorbing material from *B. subtilis* and *E. coli* is shown in Fig. 1. The leakage of 260 mμ absorbing material from cells of *E. coli* during the 3-hour incubation period was quite low and was attributable to spontaneous lysis of the cells. On the contrary, addition of azalomycin F to cells of *B. subtilis* at concentrations of 5 mcg/ml, which was the minimum inhibitory concentration, or above caused a rapid release of cellular materials into the medium.

Table 1. Activity of azalomycin F against the cell replication of *Bacillus subtilis* in natural and synthetic media

Dose (mcg/ml)	Incubation time (hr)	Viable cell number per ml	
		Nutrient medium	STEPHANSON-WHETHAM medium
0	0	2.3×10^5	4×10^5
0	3	5×10^6	1×10^7
3	3	2.1×10^5	5×10^5
15	3	$<10^3$	1.3×10^3

Fig. 1. Release of bacterial cellular material



Discussion

Among antibiotics there are two major groups in which the primary site of action

involves the cell membrane system They are so-called antifungal polyene macrolide antibiotics, namely nystatin, amphotericin, filipin, trichomycin, *etc.*[3], and antibacterial basic polypeptides, namely polymyxin[4], tyrocidin[5,6], gramicidin[7,8], subtilin[9], *etc.* These antibiotics have characteristics in common such as causing the leakage of cell constituents, their hemolytic action, and their uncoupling activity in the mitochondrial system of rat liver. Streptomycin[10] and novobiocin[11] cause permeability changes only in growing cells. It is quite interesting to note that azalomycin F is neither a polypeptide nor a typical polyenic macrolide, having absorption maxima at 240 and 268 mμ. However, it resembles these antibiotics in its mode of action on both bacteria and fungi. Precise mechanisms and sites of action of these antibiotics may differ in detail and it is therefore necessary to work further on those points as well as the structural elucidation.

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