NOTES

A NEW PROCESS AND ORGANISM FOR THE FERMENTATION PRODUCTION OF VOLONOMYCIN

V. P. MARSHALL, M. S. LITTLE and L. E. JOHNSON

Research Laboratories, The Upjohn Company, Kalamazoo, Michigan, U.S.A.

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Volonomycin (U-43120, AT-111, NSC-163500) is an antibiotic produced by *Streptomyces paulus*, UC® 5231 (NRRL 8115) which is active *vs.* P-388 leukemia tested in mice[1,2,3]. The physical characteristics and composition of volonomycin indicate that it is a member of a family of antibiotics containing proceomycin and the senfolomycins A and B[1]. Recently, this agent produced by *S. paulus*, UC® 5142 (NRRL 12251) was found to be composed of at least two components designated as volonomycins A and B[4], and to be active as an *in vitro* inhibitor of antibiotic resistant *Staphylococcus aureus* and other Gram-positive bacteria[4,5]. Strain UC® 5142 is a wild type organism isolated independently of strain UC® 5231. Volonomycin is also active *vs. Streptococcus pyogenes* (CD_{50}=2.7 mg/kg) and *S. aureus* (CD_{50}=2.6 mg/kg) when tested subcutaneously using mice[4,5]. In addition, volonomycin is not toxic when tested in this manner at 250 mg/kg[5].

Fermentation Conditions

Streptomyces paulus is stored and maintained on sterile soils in the culture collection of The Upjohn Company. The *S. paulus* containing soils were introduced into a seed medium termed GS-7 which contained Pharmamedia (Traders Oil Mill Co.) and Cerelose (C. P. C. International) each added at 25 g/liter of tap H_2O. The pH of GS-7 was adjusted to 7.2 before sterilization using NH_4OH and the medium was sterilized by autoclaving for 30 minutes. The inoculated 100 ml volumes of GS-7 were shaken at 250 rpm in wide-mouth 500 ml fermentation flasks for 48 hours at 28°C. The mature seed cultures were used as the source of inoculum (5% v/v) for production media designated as 1 and 2. Medium 1 contained Cerelose, 10 g; Pharmamedia, 10 g; dextrin, (C. P. C. International), 20 g; brewer's yeast (Fleischmann Co.), 1 g; and the antifoam agent Ucon (Union Carbide), 10 g added per liter of tap H_2O. After formulation the pH adjustment and sterilization of medium 1 were performed as with GS-7. The composition of medium 2 has been reported[2]. *Streptomyces paulus* was subsequently grown for 4~6 days in these production media in the manner described for GS-7.

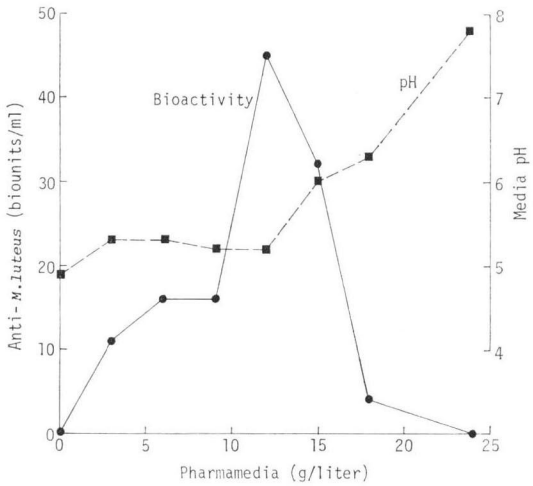
Production of Volonomycin

The biosynthesis of volonomycin was monitored through its inhibition of growth of *Micrococcus luteus*, ATCC 9341 using a disc-agar plate assay. The isolation of the anti-*M. luteus* activity produced by *S. paulus* strains UC® 5142 and UC® 5231, as well as its characterization as volonomycin have been reported[1,4]. The superior production of volonomycin by strain UC® 5142 is shown in Table 1. One biounit of anti-*M. luteus* activity is the amount of volonomycin required to produce a zone of growth inhibition of 20 mm when applied to a 12.7 mm paper disc (Schleicher and Schuell No. 740-E). Using this

Table 1. The synthesis of volonomycin by *S. paulus* strains UC®5142 and UC®5231.

Strain	Medium No.	Anti-*M. luteus* (Biounits/ml)				
		1 day	2 days	3 days	4 days	5 days
UC®5142	1	8	22	77	102	90
UC®5231	1	1	0	6	8	11
UC®5142	2	0	6	28	77	128
UC®5231	2	0	1	4	10	16

Fig. 1. The effect of Pharmamedia addition and pH on the synthesis of volonomycin at 4 days of fermentation.



assay one biounit is equivalent to *ca.* 0.2 μg of volonomycin. On the basis of data derived by chromatography and bioautography *ca.* 85% of the anti-*M. luteus* activity produced using medium 1 appears to be volonomycin.

The production of volonomycin by *S. paulus*, UC® 5142 using medium 1 with Cerelose added at 10 g/liter responded positively to the addition of Pharmamedia up to the level of 12 g/liter (Fig. 1). At levels of Pharmamedia >12 g/liter there was a reduction in the amount of volonomycin produced. This reduction correlates with the elevation of medium pH to a level at which volonomycin is unstable[4] (Fig. 1). In order to realize the maximum advantage of Pharmamedia addition to medium 1, the level of Cerelose was increased (Fig. 2). The maximal production of volonomycin using medium 1 type media was obtained with Pharmamedia at 19 g/liter and Cerelose at 22 g/liter. This improved medium was designated as medium 1A.

The growth of *S. paulus*, UC® 5142 is sensitive to volonomycin incorporated into agar media at concentrations $\geq$10 μg/ml. The role of Ucon at 10 g/liter of fermentation medium 1 appears to be at least partially related to the partitioning of volonomycin into the Ucon phase during fermentation. Such sequestering of volonomycin was systematically investigated through the incorporation of XAD-2 (Amberlite) into a modified medium 2 before sterilization at levels between 1 and 12 g/liter (Fig. 3). At 3 days of fermentation uniform aliquots of these experimental flasks were taken for determination of their anti-*M. luteus* activities. Each aliquot was divided equally with half receiving acetone treatment. Using this elution technique a volume of acetone equal to the sample volume was mixed with the sample and was held stationary at room temperature for 15 minutes. The mixture was then agitated for one minute. Both the eluted and noneluted samples were centrifuged at 5,000$\times g$ for five minutes. The supernatant fluids of each sample were then assayed for anti-*M. luteus* activity. The data presented in Fig. 3

Fig. 2. The effect of Cerelose and Pharmamedia on the synthesis of volonomycin at 4 days of fermentation.

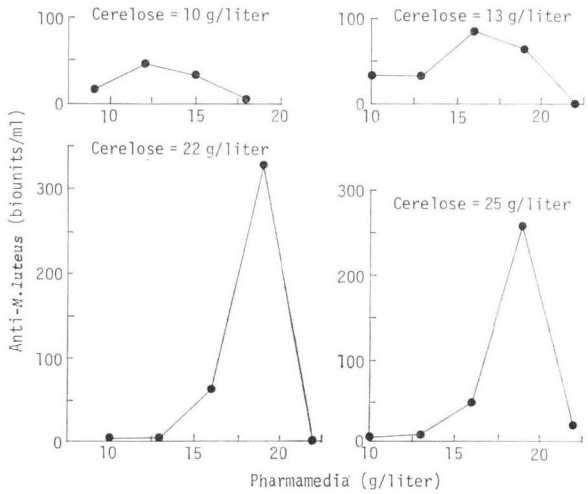
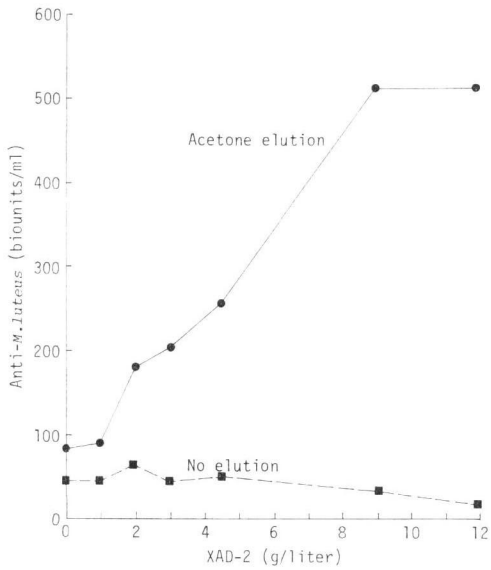


Fig. 3. The effect of XAD-2 on the synthesis of volonomycin at 3 days of fermentation using a modification of medium 2.

This medium designated as 2A contained Cerelose, 10 g; malt extract, 30 g; soybean meal, 12 g; and corn steep liquor, 5 g added per liter of tap H_2O.



show that the volonomycin removed from the XAD-2 by acetone treatment resulted in anti-*M. luteus* titers at least 5-fold greater than those obtained in fermentations lacking XAD-2. The binding and sequestering of volonomycin by XAD-2 during fermentation protects *S. paulus* from its antibacterial effect and allows its synthesis to occur at higher levels.

Acknowledgments

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