CALCIUM SIGNAL MODULATORS INHIBIT AERIAL MYCELIUM FORMATION IN *Streptomyces alboniger*

Sir:

Aerial mycelium differentiation from the substrate mycelium accompanies a dramatic morphological change in the life cycle of actinomycetes. In our researches on aerial mycelium-inducing substances, we have isolated pamamycin-607 and its four homologues from *Streptomyces alboniger*[1,2], and $Ca(OAc)_2$ from *Streptomyces ambofaciens*[3]. Further investigation showed that Ca^{2+} induced or accelerated aerial mycelium formation in various actinomycete species, including *S. alboniger*. This induction was completely inhibited by ethylene glycol bis(β-aminoethylether)-*N*,*N*,*N*',*N*'-tetraacetic acid (EGTA), a Ca^{2+}-specific chelating agent. Ca^{2+} thus has been shown to play a role in aerial mycelium formation of 21 (58%) out of 36 strains of actinomycetes tested[3].

Ca^{2+} is known as a second messenger in eukaryotes, mediating physiologically important signals in cell responses such as muscle contraction, hormone secretion, and cell division and proliferation[4]. Signal mediations *via* Ca^{2+} are, in most cases, conducted through the action of calmodulin activated as a result of binding with Ca^{2+}. The Ca^{2+}/calmodulin signalling processes are regulated by several chemical modulators that have proved powerful tools for analyzing the role of Ca^{2+}/calmodulin action in cell responses. These modulators therefore could be useful for clarifying the physiological role of Ca^{2+} in aerial mycelium formation by actinomycetes. We here describe the selective inhibititory action of the Ca^{2+}/calmodulin modulators on aerial mycelium formation by *S. alboniger*, confirming that Ca^{2+} functions in aerial mycelium formation.

Three types of Ca^{2+}/calmodulin signal modulators were used: Ca^{2+} channel blockers (verapamil·HCl, diltiazem·HCl and nifedipine), calmodulin inhibitors (ophiobolin A, prenylamine lactate and chlorpromazine), and a Ca^{2+} chelator (EGTA). The paper disc bioassay was used to assess their inhibition of aerial mycelium formation by *S. alboniger* IFO 12738.

All the three types of compounds inhibited aerial mycelium formation by *S. alboniger* (Table 1). Typical inhibition of aerial mycelium formation is shown in Fig. 1. Verapamil, at 0.3 μmol/disc, selectively inhibited aerial mycelium formation, in a dose-dependent manner. Diltiazem also produced selective inhibition at 3.0 μmol/disc. At the same dose, verapamil and diltiazem produced small inhibitory zones of substrate mycelium growth around paper discs. Nifedipine had no effect at 3.0 μmol/disc. Ophiobolin A selectively inhibited aerial mycelium formation at 0.3～3.0 μmol/disc in a dose-dependent manner. In this case, the relatively small increase in the inhibition zone with dose increase is probably due to the low water solubility of this compound. Prenylamine and chloropromazine also produced selective inhibition zone of aerial mycelia, but inhibited substrate mycelial growth more markedly than the other active compounds. EGTA suppressed aerial mycelium formation weakly at 1.0 μmol/disc and clearly at 3.0 μmol/disc, whereas substrate mycelia grew normally within the zone of inhibition.

These experiments showed that aerial mycelium formation of *S. alboniger* is sensitive to all the three types of Ca^{2+}/calmodulin modulators. On the basis of inhibition by Ca^{2+} channel blockers, it seems likely that aerial mycelium formation is induced by exogenous Ca^{2+} that enters the cell through a Ca^{2+} channel. A similar Ca^{2+} channel has been shown in eukaryotic cells. Ca^{2+} uptake by *Bacillus subtilis* is inhibited by Ca^{2+} channel blockers and proteins responsible for the Ca^{2+} uptake have been partial-

Table 1. Selective inhibitory effect of calcium signal modulators on aerial mycelium formation in *Streptomyces alboniger* IFO 12738.

	Diameter of aerial mycelium-inhibition zone (mm) (diameter of substrate mycelium-inhibition zone (mm))		
	Dose (μmol/disc)		
	0.3	1.0	3.0
Ca^{2+} channel blocker			
verapamil	20	30 (+)	35 (14)
diltiazem	0	0	27 (14)
nifedipine	0	0	0
Calmodulin inhibitor			
ophiobolin A	15	18 (10)	20 (11)
prenylamine	26 (20)	26 (20)	38 (30)
cholropromazine	22 (15)	32 (21)	37 (27)
Ca^{2+} chelator			
EGTA	0	14	27

Activity was assayed by the paper disc method on HICKEY and TRESNER's agar medium after 4～6 days of incubation at 28°C.

Fig. 1. Selective inhibition by calcium signal modulators of aerial mycelium formation in *Streptomyces alboniger* IFO 12738 (Dose: μmol/disc).

(A) Verapamil, (B) diltiazem, (C) ophiobolin A, (D) EGTA.

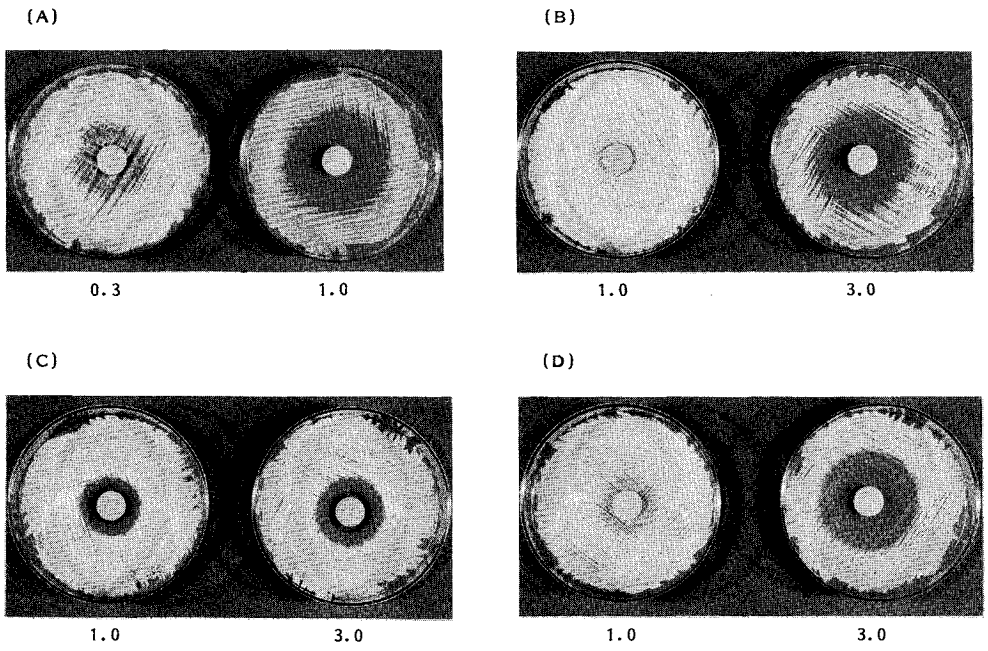
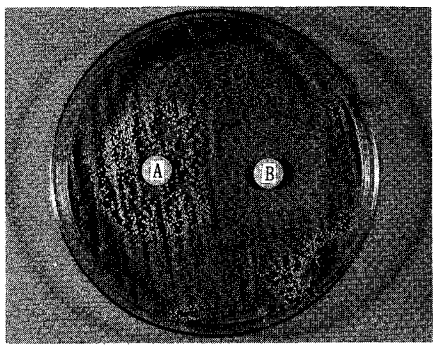


Fig. 2. Inhibition by EGTA of aerial mycelium-inducing activity of pamamycin-607.



Activity was assayed with an aerial mycelium-negative strains of *S. alboniger*. A: Pamamycin-607 (3 μg/disc) B: EGTA (3 μmol/disc). The other conditions were the same as footnoted in the Table 1.

ly purified[5]. Ca^{2+} channel blockers also inhibit the chemotaxis of *Bacillus brevis* to L-alanine at concentrations comparable to those needed for the inhibition of Ca^{2+} uptake[6]. The fact that Ca^{2+} channels function in some bacteria support that a similar Ca^{2+} channel system functions in aerial mycelium formation by actinomycetes. The three calmodulin inhibitors tested also inhibit aerial mycelium formation in *S. alboniger*. A calmodulin-homologous protein with Ca^{2+}-binding ability has been found in *Saccharopolyspora erythraea* (formerly *Streptomyces erythraeus*), although its physiological function is not yet known[7]. Our findings that calmodulin inhibitors act on aerial mycelium formation in *S. alboniger* suggest that the calmodulin-like protein functions in aerial mycelium differentiation in *S. alboniger*.

Our additional experiment showed that in the presence of EGTA which traps free Ca^{2+} in the medium, pamamycin-607 did not show aerial mycelium-inducing activity (Fig. 2). This may suggest that pamamycin-607 activity is expressed through the Ca^{2+} signaling system.

For further characterization of the regulatory role of Ca^{2+} on aerial mycelium formation by *S. alboniger*, we are now investigating the relation between the intracellular Ca^{2+} concentration and pamamycin-607.

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(Received February 3, 1992)

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