SYNERGY OF PATULIN WITH OTHER ANTIBIOTICS

Sir:

Patulin, produced by species of *Aspergillus* and *Penicillium* (see refs 1 and 2) is not difficult to find when screening fermentation broths of these genera for presence of antibiotics. We recently examined a *Penicillium* fermentation broth that appeared to contain patulin among other secondary metabolites. The fermentation broth caught our attention because it contained a possible antibiotic synergist. The antimicrobial spectrum suggested the presence of patulin as did a comparison of the synergy patterns of the broth and authentic patulin (Table 1). This prompted our loss of interest in the antibiotic potential of the fermentation broth. An interest was evoked, however, in the possible site of action of patulin. Thus the putative synergies of patulin with other antibiotics were tested by using patulin and the antibiotics in combination. These results are summarized in Table 2.

Clear synergy is shown with patulin plus rifampin (FICI=0.375) and patulin plus bottromycin (FICI=0.375). Synergy of patulin with efrotomycin is weak (FICI=0.75) and there is no synergy of patulin plus kasugamycin (FICI=1.0). The synergies of patulin with rifampin and with bottromycin are curious since rifampin[3] and bottromycin[4] presumably act at non-vicinal sites. The results are supported however by a synergy test of rifampin with bottromycin that yielded an FICI of 0.45. The previous report[5] of bottromycin plus efrotomycin synergy may be relevant to these data, particularly the weak synergy of patulin with efrotomycin and strong synergy of patulin with bottromycin.

The mechanism of action of patulin has been the subject of several investigations (see refs 6, 7 and 8) with no clear agreement, as yet. MOULÉ and HATEY[6] have shown patulin to inhibit *in vitro* transcription in liver nuclei from rats. The ID_{50} was about 1.3×10^{-3} M[7]. Experiments with rifampin and patulin combinations *in vitro* led to the possibility that both drugs acted on the same step in initiation of transcription. HATEY and GAYE[7] reported inhibition of translation by patulin with lysates from rabbit reticulocytes with an ID_{50} of about $3\sim6\times10^{-4}$ M. With intact reticulocytes the ID_{50} was 2×10^{-4} M. These data indicate patulin is not a strong inhibitor of transcription or translation in these systems. At a concentration of 12 μg/ml, patulin was shown to be a rapid inhibitor of RNA synthesis in *Saccharomyces cerevisiae*[8] lending support to inhibition of transcription as the mechanism of action. This is supported further by the report of TASHIRO *et al.*[9] that patulin is an inhibitor of ribonuclease H, an enzyme that specifically degrades RNA in RNA-DNA hybrids.

The synergy of patulin and rifampin points to transcription as the site of action. However, the synergy of patulin with bottromycin and weak synergy with efrotomycin indicates an inhibition of translation. Thus, our synergy data support two sites of action for patulin. These data are made more interesting by the synergy of rifampin, an inhibitor of transcription[3], and bottromycin, presumably an inhibitor of early translation[4]. TASHIRO *et al.*[9] proposed that patulin acts as an SH blocker. Ribonuclease H is an SH enzyme. The exact enzyme target of bottromycin is not known. If bottromycin does act on an SH enzyme, the synergisms reported here may have

Table 1. Comparative synergy test of patulin and fermentation broths containing unidentified antibiotics.

Test material	Zones of inhibition (mm) on plates containing								
	0	ER	RI	BO	EF	NO	TE	CH	KA
Patulin	15	16	20	22	23	16	15	17	19
Fermentation broth	17	18	32	22	22	19	17	17	20

Nutrient agar plates were seeded, 1%, with a 20-hour shaken culture of *Bordetella bronchiseptica* MB-3551. The plates contained 20% of the MIC of each antibiotic.

0: Control—no antibiotic, ER: erythromycin, RI: rifampin, BO: bottromycin, EF: efrotomycin, NO: novobiocin, TE: tetracycline, CH: chloramphenicol, KA: kanamycin.

Filter discs 3/8″, containing 50 μg of patulin or saturated with the fermentation broth were placed on plates. Diameters of the zone of inhibition were measured after 20 hours incubation at 37°C. We suspect possible synergy if the increase in zone diameter is 5 mm or greater on the plate containing the antibiotic.

Table 2. Synergy of patulin with other antibiotics.

Antibiotic	MIC (μg/ml)	FICI-patulin plus
Patulin	20	
Rifampin	4	0.375
Bottromycin	25	0.375
Efrotomycin	400	0.75
Kasugamycin	10	1.0

Test bacteria were shaken in nutrient broth for 20 hours at 37°C and inoculated (1%) into tubes containing different concentrations of antibiotics. Tube contents were vortexed and poured into petri plates. The MICs were determined visually after 20 hours incubation at 37°C. *Bordetella bronchiseptica* MB-3551 was the test bacterium with one exception. *Pseudomonas stutzeri* MB-1231 was used in experiments with kasugamycin.

FICI is the sum of the fractional inhibitory concentrations. The fractional inhibitory concentration of each antibiotic is the amount in combination that produces the MIC expressed as a fraction of the MIC of the antibiotic alone[10]. If the FICI is <1.0, we accept the combination as synergistic.

some biochemical explanation. However, it has been noted[8] that enzymes without sulfhydryl groups can be inhibited by patulin.

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