Communications to the Editor

PRODUCTION OF A NOVEL POLYKETIDE ANTIBIOTIC, JADOMYCIN B, BY *Streptomyces venezuelae* FOLLOWING HEAT SHOCK

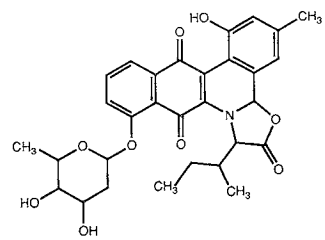
Sir:

The study of the effects of environmental stress on the production of antibiotics by members of the bacterial genus *Streptomyces* has concentrated on the role of nutrient depletion as an initiator of secondary metabolic processes. A general finding is that production of antibiotic compounds rarely occurs during periods of rapid growth in rich media; rather, the onset of their biosynthesis generally coincides with periods of growth-rate reduction following exhaustion of carbon, nitrogen, or phosphate[1]. There is some evidence, moreover, linking genetic control mechanisms which initiate antibiotic production with global regulatory networks which sense fluctuations in nutrient availability and adjust metabolic activities accordingly[2]. Although there appears to be considerable overlap, in terms of induction of common stress proteins, between systems responsive to nutrient limitation and those which react to cellular damage cause by physical agents or toxic chemicals[3], the relationship between non-nutritional stresses, such as the heat shock response, and antibiotic biosynthesis has not been investigated to date. Preliminary work suggests, however, that some heat shock proteins, such as the GroEL chaperonins may be important in antibiotic export and in the assembly of multienzyme complexes involved in polyketide antibiotic production[4]. We report here the discovery of a novel polyketide antibiotic, jadomycin B, the synthesis of which appears to be triggered by heat shock or ethanol treatment.

Streptomyces venezuelae ISP5230† has been studied for its ability to produce the broad spectrum antibiotic, chloramphenicol[5]. Recently we have described the structure of a second antibiotic, jadomycin, the parent compound of a group of novel benzoxazolophenanthridine antibiotics synthesized by *S. venezuelae* during growth at high temperature[6]. Under the fermentation conditions used, the main component in this mixture is a glycosylated form of jadomycin, jadomycin B (Fig. 1). These compounds are unusual in that a nitrogen atom is included in the angled pentacyclic backbone possibly through direct incorporation of an isoleucine molecule. This raises the possibility of directed biosynthesis by providing alternative amino acids in the growth medium. The structure of jadomycin B suggests that it is derived from a polyketide intermediate[7]. This would explain a previous unexpected observation that a polyketide gene probe (*act* I) from *S. coelicolor* hybridized with total genomic DNA from a closely related chloramphenicol-producing strain, *S. venezuelae* UC2374[8].

Because the production of jadomycin B was initially noted during growth at high temperatures, we were interested in examining the effects of a brief heat shock or ethanol treatment on antibiotic biosynthesis (Fig. 2A). Vegetative inocula were prepared by incubating 50 μl of spore suspension (preserved at -20°C in 20% glycerol) in 100 ml of MYM medium[9] for 20~24 hours with shaking (250 rpm) at 27°C. Unwashed mycelium (5 ml) from these cultures was then added to 50 ml of galactose-isoleucine medium (pH 7.3)[6]. Following growth for 6 hours at 27°C with shaking, cultures were: i) shifted to 42°C for 1 hour then shifted back to 27°C; ii) and iii) shifted to 37°C and 42°C, respectively, and maintained at these higher temperatures; iv) supplemented with 6% ethanol (v/v) and maintained at 27°C; v) maintained at 27°C without ethanol supplementation (controls). Biomass was measured as the dry weight of cell material collected from samples by filtration (Fig. 2B). To prepare samples for jadomycin B analysis, 3 ml portions were removed from the cultures at the times indicated

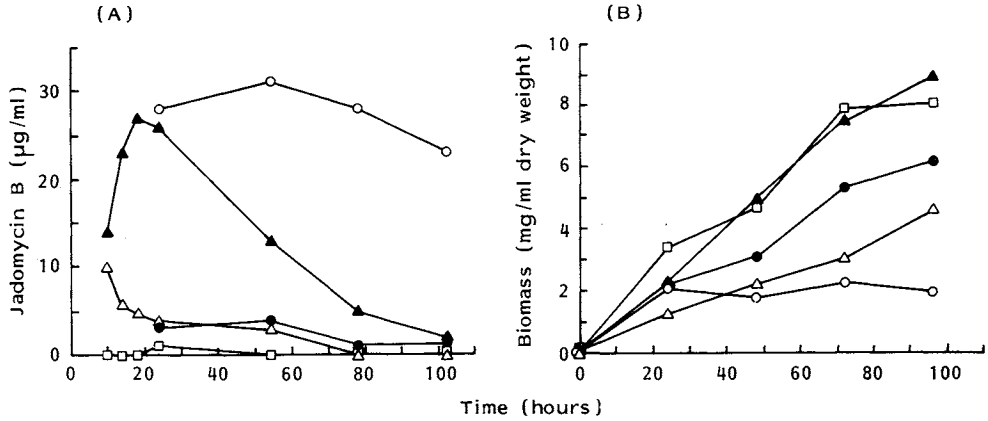
Fig. 1. Structure of jadomycin B.††



† American Type Culture Collection accession number 10712.

†† The isolation and structure elucidation of jadomycin B will be detailed elsewhere (S. W. AYER *et al.*, manuscript in preparation).

Fig. 2. Jadomycin B production (A) and growth (B) of *Streptomyces venezuelae* cultures which were: i) heat shocked for one hour at 42°C (▲); ii) maintained at 37°C (●); iii) maintained at 42°C (△); iv) supplemented with ethanol (6%, v/v) (○); v) maintained at 27°C (control) (□).



and filtered (Whatman No. 1 paper) in order to remove mycelium. The samples were then re-filtered using 0.22 μm filtration units (Millex-GV, Millipore) and 1 ml portions were evaporated *in vacuo*. The residues were resuspended in 200 ml of methanol-water (1 : 1), sonicated for three 30 second bursts (Bronson 3200 sonicator water bath), and finally filtered again using 0.45 μm filtration units (Ultrafree-MC, Millipore). The filtered samples were then analyzed using HPLC (Hewlett Packard 1090). Samples (20 μl) were applied to a reversed-phase column (2 × 250 mm, Vydac ODS) and jadomycin B was eluted at a flow rate of 0.2 ml/minute with a linear gradient of 50 : 50 acetonitrile (0.1% trifluoroacetic acid)-water (0.1% trifluoroacetic acid) to 100% acetonitrile (0.1% trifluoroacetic acid) over 20 minutes. Detection was 313 nm. A purified sample of jadomycin B was used as an instrument calibration standard.

Of the heat treated fermentations, the heat shocked cultures accumulated the highest jadomycin B titers, which peaked 12 hours following heat shock, then began to decline throughout the following 96 hours (Fig. 2A). This production pattern is highly unusual for secondary metabolites as most antibiotics tend to accumulate following the end of the growth period. Control cultures maintained at 27°C without heat shock accumulated negligible amounts of jadomycin B, while cultures maintained at 37°C and 42°C produced moderately higher concentrations (Fig. 2A). The fate of the jadomycin B as it disappears from the cultures is unknown, but we have noted the appearance of a new peak in the HPLC traces coinciding with its depletion. Cultures grown at higher temperatures showed decreasing biomass accumulation compared with 27°C controls (Fig. 2B), while the heat shocked cultures, following a brief lag, resumed growth at the control rate.

Of the many alternative inducers of the heat shock response in bacteria, ethanol treatment comes closest to mimicking the effects of heat in terms of induction of heat shock proteins[10]. We were, therefore, interested in examining the effects of ethanol treatment on the production of jadomycin B. We found that high titers could be obtained during growth at 27°C when cultures were supplemented with ethanol (6% v/v, final concentration), six hours after inoculation (Fig. 2A). In the ethanol-supplemented cultures the jadomycin B production phase was more prolonged compared with the heat-treated cultures and maximum titers were obtained at 48 hours. Growth of the cultures, however, was arrested soon after the addition of ethanol (Fig. 2B). A one-hour exposure of cells to ethanol followed by replacement into fresh medium resulted in a brief burst of jadomycin B production but had little effect on growth (data not shown).

In the past, the study of the heat shock response in *Streptomyces* was largely neglected, but recent work has suggested that the response may be linked to morphological and possibly also physiological differentiation in this bacterium[11]. The mechanism of ethanol induction of jadomycin B production is unknown and is currently under investigation, but we suggest that it relates to the ability of ethanol treatment to trigger a stress response. Alternatively, ethanol could be supplying polyketide precursors,

although when a similar concentration of sodium acetate was added to cultures no jadomycin B production was observed. In support of the hypothesis that production of jadomycin B can be initiated by a variety of agents which trigger a heat-stress response in *S. venezuelae*, we have noted the appearance of this antibiotic following infection with bacteriophage SV1 (J. L. DOULL *et al.*, manuscript in preparation). The present report represents the first description of a heat shock inducible antibiotic, and as such, provides some insights into the role of this stress response in the morphologically and physiologically complex *Streptomyces*.

Acknowledgments

We thank Professor L. C. VINING for helpful discussions and the use of laboratory facilities. This work was supported, in part, by grants from the Natural Sciences and Engineering Research Council of Canada and the Mount Saint Vincent University Committee on Research and Publications. This is NRCC publication number 34849.

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(Received December 28, 1992)

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