able for a real industrial expansion. From a purely economic point of view (an important factor for scale-up), it is worth mentioning that on the basis of current distribution prices, microcarriers Nos 1 and 3 or our list, available as dry powder, are cheaper than No. 2 which is supplied, however, ready for use resuspended in the appropriate buffer.

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Ca^{2+}-dependence and metabolic status of an obligate thermophile, *Thermoactinomyces vulgaris*, under shake culture conditions

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Summary. Ca^{2+} stimulates germination of *T. vulgaris* spores. There is a higher mycelial yield as well as higher protein, DNA, RNA and free Pi content in cultures grown in the presence of Ca^{2+} as compared to those grown in the absence of this divalent cation.

The association of Ca^{2+} with thermophilic growth[3,4] as well as with the catalytic activity and stability of thermophilic enzymes[5–8] has been well documented in a variety of microbial systems. However, information concerning the status of germination, growth yield, soluble protein, DNA, RNA and free Pi in response to Ca^{2+} has not been obtained for thermophilic actinomycetes under liquid shake culture conditions. Therefore the present investigation was undertaken using the obligate thermophile *Thermoactinomyces vulgaris*.

A wild-type strain (stock No. 1227) of *T. vulgaris*, which was kindly supplied by Professor D.A. Hopwood, John Innes Institute, Norwich, U.K., was used in the present investigation. The media described by Hopwood & Wright[9] were used with certain modifications[8,10]. Liquid medium was prepared without adding agar and pH was adjusted to 6.8.

Approximately 10^9 spores/30 ml of the medium, in 250-ml conical flasks, were incubated for 6 h (as at this stage the logarithmic phase of growth continues) in a Gyrotory Shaker (New Brunswick, Model G 25) at 50–52 °C at 150 rpm. The mycelium was harvested on Whatman No. 1 filter paper. A homogenate was prepared in glass distilled water with the help of a mortar and pestle, using acid-washed sea sand as an abrasive. The slurry was centrifuged at 20,000 × g for 30 min at 4 °C. The supernatant was retained and stored at 4 °C until used. Germination of spores was examined under a compound microscope and its

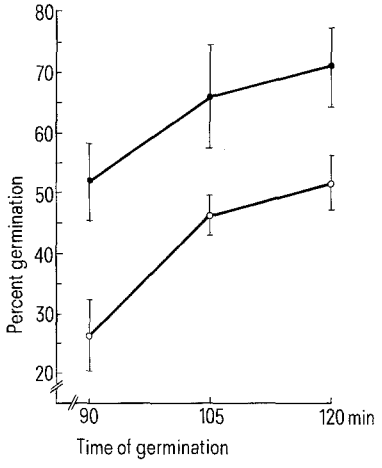

Figure 1. Percentage germination of *Thermoactinomyces vulgaris* spores at different time intervals in the absence (open circles) and presence (closed circles) of Ca^{2+} (0.6 mM) under shake culture conditions. Mean values are based on duplicate readings of 3 independent determinations.

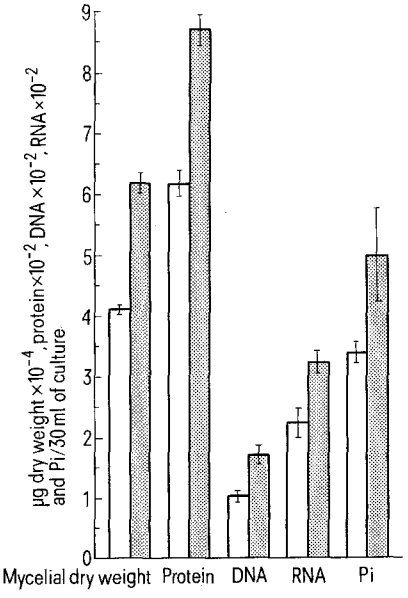

Figure 2. Mycelial dry weight, protein, DNA, RNA and Pi in the liquid shake cultures of *Thermoactinomyces vulgaris* grown in the absence (open bars) or presence (solid bars) of Ca^{2+} (0.6 mM). Mean values are based on duplicate readings of 3 independent determinations.

percentage was calculated on the basis of the ratio of germinated spores to non-germinated ones. Growth yield was measured as µg dry weight/30 ml of the medium. Deoxyribose and ribose nucleic acids were estimated using diphenylamine and orcinol reagents, respectively[11]. Proteins were estimated by using Folin's phenol reagent[12]. Estimation of inorganic phosphate (Pi) was done by forming a complex with ammonium molybdate (phosphomolybdic acid) and its reduction with 1,2,4-amino-naphthol sulphonic acid (ANSA) reagent[13].

The observations presented in figure 1 show that in the presence of 0.6 mM of Ca^{2+} (optimal concentration for the growth of *T. vulgaris*), added in the culture medium, there was a higher percentage of germination of spores than in the absence of this divalent cation. The stimulatory effect of Ca^{2+} on germination was more (about 2-fold) at the initial (90 min) stage than at the following stages (105 min and 120 min) of incubation. After about 2 h it was difficult to calculate the percentage of germination because of the formation of a hyphal network and the entanglement of spores in its mesh. Ca^{2+} was found to increase mycelial yield as well as the yield of soluble proteins, DNA, RNA and free Pi by about one and a half fold over the control (fig. 2).

On the basis of these findings it can be suggested that germination is an energy-requiring process, and therefore, it is dependent on Ca^{2+} concentration. This is further supported by our earlier observation that Ca^{2+} is associated with increased hydrolysis of ATP in *T. vulgaris*[14]. Similarly to Mg^{2+} which stabilizes the nonspecific acid and alkaline phosphatases of *T. vulgaris*[15], Ca^{2+} protects its membrane-bound ATPase from thermal inactivation[8] and perhaps the most important function of Ca^{2+} is the stabilization of cell membranes of this obligate thermophile so as to protect the thermolabile components including the precursors of proteins and nucleic acids which are essential for higher mycelial yield at elevated temperature (thermophilic growth) and spore germination.

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Effect of precocene II on acid phosphatase activity in the large milkweed bug, *Oncopeltus fasciatus* (Hemiptera: Lygaeidae)

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Summary. When precocene II was applied to the fourth instar milkweed bug, there was a significant decrease in acid phosphatase activity in the hemolymph at the 40%, 50%, 60%, and 70% time periods in the stadium.

Changes in acid phosphatase synthesis and activity have been shown to be related to juvenile hormone by several workers. Bassi and Feir[1] found that application of a juvenile hormone mimic (Law-Williams, Calbiochem) to 5th instar male milkweed bugs stimulated the synthesis of acid phosphatase near the end of the stadium. They demonstrated that the synthesis was controlled at the transcriptional level[2]. Beel and Feir[3] found an increase in acid phosphatase activity in the hemolymph, salivary glands, and testis of the 5th instar male milkweed bug after treatment with a juvenile hormone mimic. Postlethwait and Gray[4] showed that ovarian acid phosphatase in the adult female *Drosophila melanogaster* was dependent on juvenile hormone levels.

The present study was undertaken to see whether the antiallatotropin, precocene II, would affect acid phosphatase activity in the 4th instar milkweed bug, *Oncopeltus fasciatus,* and thus support the role of juvenile hormone in acid phosphatase activity.

Materials and methods. 4th instar bugs within 1 h of ecdysis were collected from the stock colonies at 1-h intervals between 08.00 h and 12.00 h. The stock colonies were maintained at room temperature of approximately 25 °C and 14L:10D light cycle. After collection 25 bugs were placed in 90 × 50 mm crystallizing dishes with a moistened cotton dental roll and dried milkweed seeds. The day of collection was considered day zero of the stadium and the day when one-half or more of the bugs had undergone ecdysis was the last day of the stadium. For ease of comparing the data with the literature the data are reported in percent of stadium although the measurements were actually made at 24-h intervals.

The 4th instar bugs have endogenous juvenile hormone. Precocene acts on the corpora allata to prevent juvenile hormone from being made[5]. Precocene II was sprayed over the bottom of a petri dish with milkweed seeds at a concentration of 7.0 µg/cm^2 in acetone[5]. Control dishes were sprayed with acetone. The acetone was allowed to evaporate completely before bugs were placed in the dishes. The insects remained in the dishes for the entire stadium. At 4 h after the start of the treatment and then at 24-h