

## Inverse regulation of spore germination and growth by cyclic AMP in *Streptomyces hygroscopicus*

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**Summary.** The cyclic AMP level in germinating spores of *Streptomyces hygroscopicus* rises to a maximum at outgrowth of germ tubes. Exogenous cyclic AMP results in an inverse effect on germination speed and growth.

Bacterial spore germination and outgrowth are considered as an example of differentiation. Yet very little information exists concerning the biochemical and genetic processes necessary for the transition of spores into vegetative cells in the order Actinomycetales<sup>1</sup>. Our previous studies have provided evidence for the occurrence of cyclic nucleotides also in this order, and have additionally indicated that cyclic AMP has a function in the regulation of growth and development in the macrolide antibiotic producer *Streptomyces hygroscopicus*<sup>2</sup>. In this communication we present the results of further investigations that show a fluctuation of cyclic AMP level also during the period of spore germina-

tion and outgrowth, and an opposite effect of this cyclic nucleotide on germination and growth, which may be an interesting mechanism of developmental regulation in this microorganism.

**Materials and methods.** Spores of *Streptomyces hygroscopicus* wild type strain No. JA 6599-7 were harvested from agar medium, by rolling glass beads over the surface, and suspended in 10 ml of a germination medium composed of 1% dextrose, 1% peptone, 0.2% yeast extract, 0.6% sodium chloride, and 1% hydrolyzed casein, passed through a glass filter G3 (Schott and Gen., GDR) and cultivated at 28 °C on a rotary shaker. The assay procedure for cyclic AMP was described earlier<sup>2</sup>. Germination and outgrowth were determined by direct phase-contrast microscopic observation in hourly intervals.

**Results and discussion.** The cyclic AMP content of dormant spores was extremely low (about 2 pmoles per 10<sup>8</sup> spores). But even in the early period of germination, the total level of this cyclic nucleotide was enhanced, as shown in figure 1. A significant maximum was reached at the date of outgrowing germ tubes. These results were confirmed by experiments carried out in another seed culture medium and by the use of a mutant strain (data not shown). Addition of cyclic AMP at inoculation resulted in a reduced speed of germ tube emergence (figure 2, a) until a cyclic AMP concentration was reached which stopped germination almost entirely. But the growth of germ tubes which had already emerged, was stimulated (figure 2, b). It was observed, however, that cyclic AMP at the same concentrations added to the cultures near or during the phase of outgrowth actually stimulated the growth, but without detectable influence on further germination speed. Adenosine or phosphate, respectively, as normal products of hydrolysis did not show any effect.

These results indicate differences in sensitivity to cyclic AMP during the early stage of germination and that of outgrowth, which are phases of important biochemical changes<sup>1,3</sup>. The growth-stimulating effect agrees well with our preliminary experiments<sup>2</sup>, whereas the diverse sensitivity to cyclic AMP may be seen as an interesting biochemical regulator also observed on agar plates. The outgrowing spore is surrounded by a relatively high cyclic AMP concentration – in the case of a solid medium it would be a gradient of cyclic AMP concentration – with a double function: stimulation of its own growth and a simultaneous inhibition of the germination of other spores as possible competitors for the nutrients. The results confirm our suggestion of a regulating function of cyclic AMP in growth and differentiation in *Streptomyces hygroscopicus*.

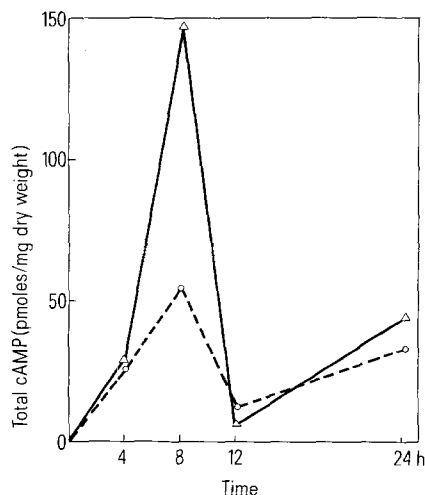


Fig. 1. Cyclic AMP content during spore germination of *Streptomyces hygroscopicus*. Spore concentration: 10<sup>5</sup> ---○---; 10<sup>6</sup> —△—.

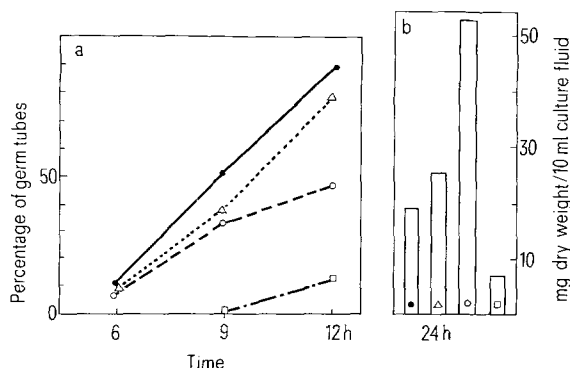


Fig. 2. Germination speed (a) and growth (dry weight) (b) depending on cyclic AMP added at inoculation. The emergence of germ tubes of spores (spore concentration 10<sup>6</sup>) was observed at hourly intervals by phase-contrast microscopy. 24 h after inoculation, the dry weight was determined. Concentrations of added cyclic AMP: none (control), —●—; 4·10<sup>-4</sup> M cyclic AMP, ...△...; 4·10<sup>-3</sup> M cyclic AMP, --○--; 1·10<sup>-2</sup> M cyclic AMP, ---□---.

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