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Neutral lipids and lipase activity for actinorhodin biosynthesis of Streptomyces coelicolor A3(2)

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Neutral lipid accumulation during early growth phase of Streptomyces coelicolor A3(2) was essential for the actinorhodin production during later growth. The activities of lipase and isocitrate dehydrogenase were increasing and decreasing, respectively, suggesting that the degradation products of neutral lipids serve actinorhodin biosynthesis as precursors.

Introduction

There have been reports which described the roles of storage materials in streptomycetes. Glycogen can be used as an energy and carbon source for sporulation (Brana *et al.*, 1986) and trehalose which is accumulated during the developmental cycle, can be used as a protecting agent for membrane integrity from desiccation as well as a carbon or energy source for germination (Martin *et al.*, 1986). Most recently, neutral lipids, mainly triacylglycerols, in the mycelia of streptomycetes were assumed to be used as substrates for the pigmented antibiotic actinorhodin, produced by *Streptomyces coelicolor* A3(2) (Olukoshi and Packter, 1994). In this report, we measured activities of lipase and isocitrate dehydrogenase to show a negative correlation between actinorhodin production and neutral lipid content.

Materials and methods

Strain, media and culture conditions

Streptomyces coelicolor A3(2) was inoculated at about 10^9 spore into 250 ml Erlenmeyer flasks containing 50 ml yeast extract – malt extract broth (YEME, ISP No.2) which was supplemented with 0.2% glucose or with 20 mM $(NH_4)_2SO_4$ when needed. Submerged cultures were grown with shaking at 150 revolution min^{-1}, at 30°C.

Analyses

Actinorhodin was measured on the basis of optical density at 608 nm (Carbo *et al.*, 1995). For lipid extraction, 1 mg of mycelia were harvested, washed twice with distilled water and macerated in the lipid extraction solvent (hexane:isopropylalcohol, 3:2 v/v) (Radin, 1981). Polar and neutral lipids were separated by partition chromatography on silica gel (Christie, 1982) and weighed on an analytical balance. For enzyme assay, mycelia were disrupted in 100 mM Tris-HCl buffer (pH 8.0) by sonication followed by centrifugation for 30 min at 13,000 g, 4°C, and the resulting supernatant was used as a source of enzymes. The protein concentration was measured by Bradford method. Lipase activity was measured titrimetrically by using the commercial kit (Sigma Co.) in which tributyrin was used as a substrate and thymolphthalein as an indicating dye (Dupuis *et al.*, 1993). The amount of enzyme that produced one μmole of free fatty acids per minute was defined as one unit. The activities of isocitrate dehydrogenase and glucose-6-phosphate dehydrogenase were determined by the rate of absorbance change at 340 nm (Passonneau and Lowry, 1993). Units of these two enzymes were determined as μmoles of NADPH produced per minute. Electron microscopy of the mycelia was performed by the method described elsewhere (Brana *et al.*, 1986).

Results and discussion

Neutral lipid contents in mycelia grown in three different media were determined (Fig. 1A). Neutral lipids were accumulated already at two day growth in YEME (about 6.5 mg g $mycelia^{-1}$) and this content was decreasing continuously thereafter, whereas that in YEME+glucose showed relatively little change during growth, maintaining the high initial level. The addition of $(NH_4)_2SO_4$ at the beginning of growth reduced the amount of neutral lipids accumulated and this remained unaltered at low levels throughout the whole incubation period. In contrast, the contents of polar lipids were not dependent on media and growth stages, explaining that the polar lipids carry out their basic

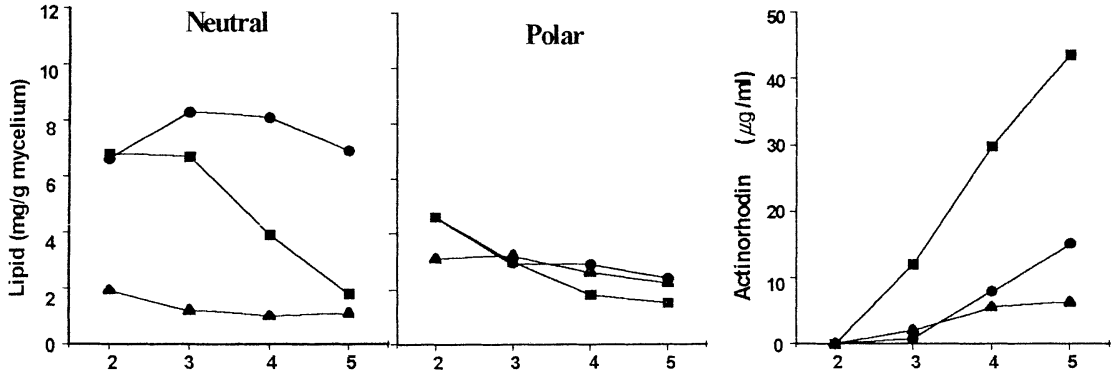

Figure 1 (A) Change of neutral and polar lipid contents and (B) production of actinorhodin during growth of S. coelicolor A3(2) in (■) yeast extract – malt extract without glucose, with (●) 0.2% glucose and with (▲) 20 mM $(NH_4)_2SO_4$.

roles as components of membrane. Meanwhile, the culture grown in YEME showed the most extensive actinorhodin production followed by those in YEME+glucose and YEME+$(NH_4)_2SO_4$ (Fig. 1B). Above result that actinorhodin production was closely related to the neutral lipid decomposition was confirmed by the extensive actinorhodin production of the mycelia initially grown in YEME+glucose when transferred to the half-strength YEME with the concomitant decrease of neutral lipids (data not shown). NH_4^+ showed an inhibitory effect against the neutral lipid accumulation and consquently actinorhodin biosynthesis also at much lower concentration of two mM. The total lipid contents were not NH_4^+ were principally composed of polar lipids. The inhibition of actinorhodin production by NH_4^+ in the earlier paper might be caused by the lack of neutral lipids in mycelia (Hobbs *et al.*, 1990).

In addition to the gravimetrical assay for lipids described above, electron microscopy was carried out to confirm the presence of neutral lipids in *Streptomyces coelicolor* A3(2).

As shown in Fig. 2, neutral lipids appeared as electron transparent droplets in the mycelia from YEME, whereas there were no lipid droplets found in the mycelia from YEME+$(NH_4)_2SO_4$. These results clearly support the previous reports that *Streptomyces coelicolor* A3(2) is one of the examples for the neutral lipid accumulation in bacterial cells and that actinorhodin is derived from the neutral lipids (Olukoshi and Packter, 1994). The lipid accumulation for polyketide biosynthesis was already noticed for leukaemomycin production by *S. grieseus* (Graefe *et al.*, 1984) and the reverse relation between a reserve material and an antibiotic was also known for other streptomycetes (Ranade and Vining, 1993). Our next experiment was to exhibit the change of enzyme activity during growth. The activity of lipase was expected to increase to provide fatty acids for β-oxidation producing acetyl-CoA and

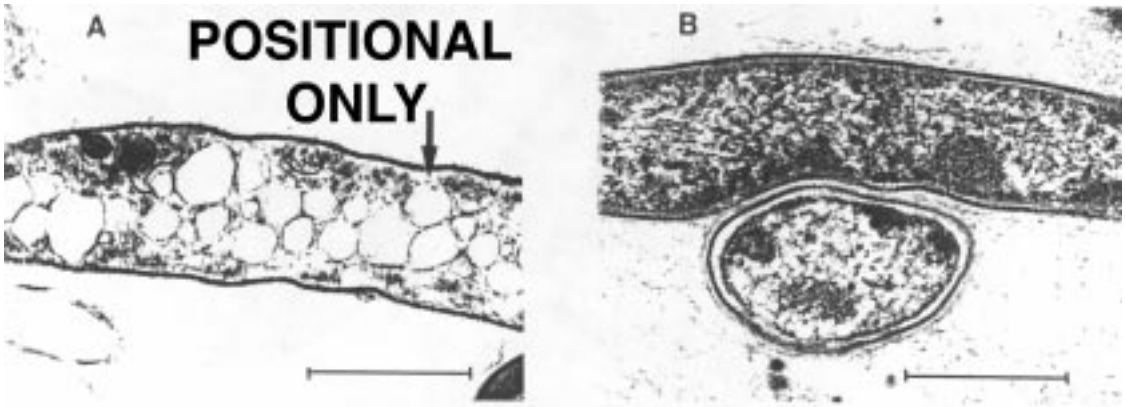

Figure 2 Transmission electron micrographs of S. coelicolor A3(2) grown in (A) yeast extract – malt extract and in (B) yeast extract – malt extract + 20 mM $(NH_4)_2SO_4$. A Bar equals 0.5 μm, arrow indicates lipid droplets.

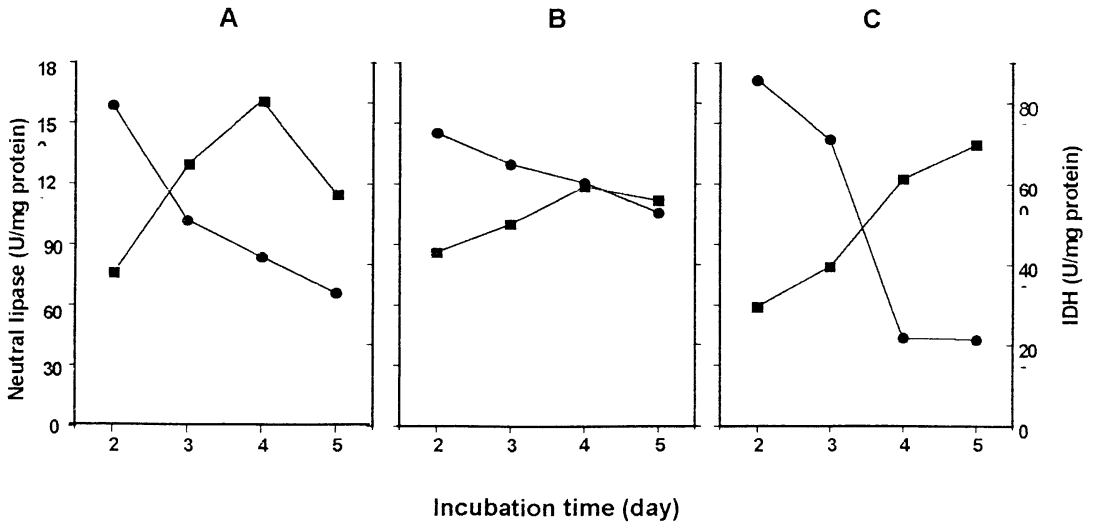

Figure 3 Changes in (■) neutral lipase and (●) isocitrate dehydrogenase (IDH) activities during growth of S. coelicolor A3(2) in yeast extract malt extract without (A) glucose, with (B) 0.2% glucose and with (C) 20 mM $(NH_4)_2SO_4$.

malonyl-CoA, the starting and extending unit, respectively, for actinorhodin biosynthesis. At the same time, the tricarboxylic acid cycle should be interrupted to lead these precursors exclusively to the biosynthesis of actinorhodin, for which we measured isocitrate dehydrogenase as a representative enzyme of tricarboxylic acid cycle. As shown in Figure 3, in YEME a two fold increase of lipase activity was accompanied by a two fold decrease of isocitrate dehydrogenase activity, whereas in YEME+glucose relatively less significant changes in both enzyme activities were observed. In YEME+$(NH_4)_2SO_4$, although there was few neutral lipids accumulated in mycelia, the activity changes were similar to those in YEME. Meanwhile, the activity of glucose-6-phosphate dehydrogenase in the three different liquid media was decreasing throughout the whole incubation time (data not shown), indicating that the increase of lipase was necessary to provide a carbon and energy source for the later growth phase.

Therefore, under these culture conditions, the accumulation of neutral lipids during the early growth phase was prerequisite for actinorhodin production, and the metabolic flow was important in connection with the pigment production, so that fatty acids of neutral lipids were destined for actinorhodin.

Acknowledgement

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