SHORT COMMUNICATION

Effect of Growth Rate on the Production of Phytohormone-like Substances by an *Arthrobacter* sp. in Chemostat Culture

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Gibberellins, auxins and cytokinins were produced by an *Arthrobacter* sp. grown in ammonium-limited chemostat culture. Differences in the production of the three substances occurred in response to changes in growth rate.

INTRODUCTION

Many soil micro-organisms (Klämbt *et al.*, 1966; Brown, 1972; Azcón & Barea, 1975; Tien *et al.*, 1979) produce phytohormone-like substances that, like other secondary metabolites, are reported to be synthesized at the end of the exponential phase of growth. Recent studies clearly demonstrated that secondary metabolism is part of the normal metabolism and its extent is controlled by growth rate (Pirt, 1975; Calam, 1979).

Generally little attention has been devoted to the possible relationships between growth rate and phytohormone production by micro-organisms. Holme & Zacharias (1965) reported the production of gibberellic acid by the fungus *Gibberella fujikuroi* grown in continuous culture, but no similar information has been obtained concerning phytohormone synthesis by bacteria.

The purpose of the present work was to investigate the effect of different nutritional and physical factors on phytohormone production by an *Arthrobacter* sp. that has previously been reported to produce auxins and gibberellins (Grappelli *et al.*, 1972) and cytokinins (Grappelli & Pietrosanti, 1975). This paper reports the first results obtained in ammonium-limited chemostat culture of *A. giacomelloi* grown at different dilution rates.

METHODS

Bacterial strain. *Arthrobacter giacomelloi* was isolated and identified in our laboratory (Cacciari *et al.*, 1971).

Culture conditions. Details of the chemostat used, medium (MB₇) for growth, control of culture purity, growth and steady-state conditions were as previously described by Cacciari *et al.* (1979), except that glucose was used instead of mannitol and 0.2 g (NH₄)₂SO₄ l⁻¹ was added to the MB₇ medium. The culture volume was 900 ml and the following dilution rates were used (h⁻¹): 0.015, 0.03, 0.06, 0.10, 0.12, 0.15. The stirring rate of the impeller was 200 rev. min⁻¹; air was supplied at a flow rate of 200 ml min⁻¹ and the temperature was 30 °C.

Phytohormone assay. Steady-state cultures (800 ml) were centrifuged at 16000 g and 4 °C for 15 min to determine biomass concentration and to extract phytohormone-like substances. The supernatant fluid was

acidified with 1 M-HCl to pH 2.8 to 3.0 and shaken with 1 % (w/v) activated charcoal for 2 h. After centrifugation, the charcoal was extracted with 95 % (v/v) aqueous acetone and the acetone was evaporated under vacuum at 30 °C. The moist residue was extracted twice with ethyl acetate and evaporated to dryness. The residue was dissolved in methanol and examined on Whatman no. 1 chromatography paper using two different solvent systems: solvent A was freshly mixed propan-2-ol/ammonia/water (10:1:1, by vol.) to separate auxins and gibberellins; solvent B was butan-1-ol/acetic acid/water (4:1:1, by vol.) to separate cytokinins.

Control chromatograms of authentic indole-3-acetic acid (IAA), gibberellic acid (GA₃) and *N*⁶- Δ^2 -isopentenyladenine (2iP) were developed at the same time. The chromatograms were then cut and assayed using biological tests. The test of Nitsch & Nitsch (1956) was used for auxin activity, with IAA as control. The bioassay for gibberellin-like activity was carried out according to Frankland & Wareing (1960), using GA₃ as control. Cytokinin-like activity was determined by means of the bioassay of Letham (1971), using 2iP as control.

Analyses. Bacterial dry weight was determined by centrifuging and washing 20 ml culture samples and drying the cells to constant weight at 95 °C. Total nitrogen was determined by a semimicro-Kjeldahl method (Bremner, 1965). *Q*_{O₂} *in situ* was determined at 30 °C by a standard manometric method using 2.5 ml culture samples transferred as rapidly as possible to Warburg flasks (Umbreit *et al.*, 1964).

RESULTS AND DISCUSSION

Arthrobacter giacomelloi was grown in continuous culture on MB₇ medium with ammonium as the growth-limiting nutrient and steady-state cultures were obtained at growth rates between 0.015 and 0.15 h⁻¹. Over this range no washout occurred and the pH value was always between 6.7 and 6.9. Throughout this study constant air flow and stirring rates were maintained. The *Q*_{O₂} value [μ l h⁻¹ (mg dry wt)⁻¹] increased from 17 to 40 with increasing dilution rate, but dissolved oxygen was always detectable and the cultures never became oxygen-limited. The dry weight (1 mg ml⁻¹) and the nitrogen content (40 μ g ml⁻¹) of steady-state cultures were approximately constant and did not vary with growth rate. This organism, unlike other species of the same genus (Dawes & Senior, 1973), did not synthesize large amounts of polysaccharide in response to nitrogen limitation in either batch or continuous cultures. The polysaccharide content of the cells varied from 29 to 35 % of dry weight.

All three phytohormone-like substances were produced in the ammonium-limited continuous cultures of *A. giacomelloi* (Fig. 1). The results obtained showed that the growth rate influenced the synthesis of the three products differently.

The amount of cytokinin-like substance was rather high compared with that obtained in batch cultures in our previous studies on *Arthrobacter*, or in studies reported by others. Phillips & Torrey (1972) in *Rhizobium japonicum* and Coppola *et al.* (1976) in *Escherichia coli* demonstrated that cytokinin synthesis increased during the exponential phase of growth and decreased at the onset of the stationary phase. Coppola *et al.* (1971) related cytokinin production to growth rate in *Saccharomyces cerevisiae*, obtaining different growth rates by altering the nutritional conditions. All the experiments described above were performed in batch cultures grown for several days; therefore it seems difficult to ascribe the effect on cytokinin production only to the growth rates, as the environmental conditions were continuously changing.

The results we obtained in steady-state cultures of *A. giacomelloi* clearly indicated that cytokinin production, under nitrogen limitation, was independent of growth rate over the range 0.03 to 0.12 h⁻¹ while higher and lower growth rates caused a decrease in this biosynthetic activity. The synthesis of gibberellins by *A. giacomelloi* was higher at dilution rates below 0.10 h⁻¹. This behaviour seemed to confirm that gibberellin production was favoured by low growth rates and low availability of a nitrogen source, as reported by Holme & Zacharias (1965) for *Gibberella fujikuroi*. The rather low amount of auxins recovered in the fluid of our steady-state cultures might be accounted for by the presence

Fig. 1. Production of cytokinins (○), gibberellins (●) and auxins (□) as function of growth rate in ammonium-limited steady-state cultures of *Arthrobacter giacomelloi*.

of ammonium-nitrogen, which has been reported to inhibit the biosynthesis of IAA (Lee *et al.*, 1970). Moreover, photodestruction of IAA (Brauner & Brauner, 1954) could have occurred as our growth vessel was not protected from light.

REFERENCES

- AZCÓN, R. & BAREA, J. M. (1975). Synthesis of auxins, gibberellins and cytokinins by *Azotobacter vinelandii* and *Azotobacter beijerinckii* related to effects produced on tomato plants. *Plant and Soil* **43**, 609–619.
- BRAUNER, L. & BRAUNER, M. (1954). Untersuchungen über die Photolyse des Heteroauxins. *Zeitschrift für Botanik* **42**, 83–124.
- BREMNER, J. M. (1965). Semimicro-Kjeldahl method. In *Methods of Soil Analysis*, part 2, pp. 1171–1178. Edited by C. A. Black, D. D. Evans, J. L. White, L. E. Ensminger & F. E. Clark. Madison: American Society of Agronomy.
- BROWN, M. E. (1972). Plant growth substances produced by microorganisms of soil and rhizosphere. *Journal of Applied Bacteriology* **40**, 443–451.
- CACCIARI, I., GIOVANNONZI-SERMANNI, G., GRAPPELLI, A. & LIPPI, D. (1971). Nitrogen fixation by *Arthrobacter* sp. I. Taxonomic study and evidence of nitrogenase activity of two new strains. *Annali di microbiologia ed enzimologia* **21**, 97–105.
- CACCIARI, I., LIPPI, D. & BORDELEAU, L. M. (1979). Effect of oxygen on batch and continuous cultures of a nitrogen-fixing *Arthrobacter* sp. *Canadian Journal of Microbiology* **25**, 746–751.
- CALAM, C. T. (1979). Secondary metabolism as an expression of microbial growth and development. *Folia microbiologica* **24**, 276–285.
- COPPOLA, S., TUCCI, G. & PICCI, G. (1971). Growth rates and cytokinin contents in *Saccharomyces cerevisiae* Hansen. *Giornale di microbiologia* **19**, 20–21, 13–20.
- COPPOLA, S., MARINI, P. & ZOINA, A. (1976). Cytokinin contents and cAMP metabolism during growth of *Escherichia coli*. *Experientia* **32**, 566–567.
- DAWES, E. A. & SENIOR, P. J. (1973). The role and regulation of energy reserve polymers in microorganisms. *Advances in Microbial Physiology* **10**, 135–266.
- FRANKLAND, B. & WAREING, P. F. (1960). Effect of gibberellic acid on hypocotyl growth of lettuce seedlings. *Nature, London* **185**, 255–256.
- GRAPPELLI, A. & PIETROSANTI, W. (1975). Produzione di citochinine da *Arthrobacter* sp. *Atti XVII congresso nazionale di microbiologia, Padova* vol. 1, 299–302.
- GRAPPELLI, A., GIOVANNONZI-SERMANNI, G. & PIETROSANTI, W. (1972). Determinazioni spettrofluorimetriche di sostanze fitormonali prodotte da un *Arthrobacter* sp. *Atti XVI congresso nazionale di microbiologia, Pisa*, vol. II, 269–277.
- HOLME, T. & ZACHARIAS, B. (1965). Gibberellic acid formation in continuous culture. *Biotechnology and Bioengineering* **7**, 405–415.
- KLÄMBT, D., THIES, G. & SKOOG, F. (1966). Isolation of cytokinins from *Corynebacterium fascians*. *Proceedings of the National Academy of Sciences of the United States of America* **56**, 52–59.
- LEE, M., BRECKENRIDGE, C. & KNOWLES, R. (1970). Effect of some culture conditions on the production of indole-3-acetic acid and a gibberellin-like substance by *Azotobacter vinelandii*. *Canadian Journal of Microbiology* **16**, 1325–1330.
- LETHAM, D. S. (1971). Regulators of cell division in plant tissues. XII. A cytokinin bioassay using excised radish cotyledons. *Physiologia plantarum* **25**, 391–396.
- NITSCH, J. P. & NITSCH, C. (1956). Studies on the growth of coleoptile and first internode sections. A new sensitive straight-growth test for auxins. *Plant Physiology* **31**, 94–111.

- PHILLIPS, D. A. & TORREY, J. G. (1972). Studies on cytokinin production by *Rhizobium*. *Plant Physiology* **49**, 11–15.
- PIRT, S. J. (1975). *Principles of Microbe and Cell Cultivation*. Oxford: Blackwell Scientific Publications.
- TIEN, T. M., GASKINS, M. H. & HUBBELL, D. H. (1979). Plant growth substances produced by *Azospirillum brasilense* and their effect on the growth of Pearl Millet (*Pennisetum americanum* L.). *Applied and Environmental Microbiology* **37**, 1016–1024.
- UMBREIT, W. W., BURRIS, R. H. & STAUFFER, J. F. (1964). *Manometric Techniques*, 4th edn. Minneapolis: Burgess Publishing Co.