

Regulation of the Arginine Dihydrolase Pathway in *Clostridium sporogenes*

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Arginine deiminase activity was induced during the vegetative growth of *Clostridium sporogenes*. The enzyme was sensitive to catabolite repression. The other enzymes of the arginine dihydrolase pathway, namely, ornithine carbamoyl-transferase and carbamate kinase, did not show such variation.

The arginine dihydrolase pathway, which includes the three enzymes arginine deiminase (EC 3.5.3.6), ornithine carbamoyltransferase (EC 2.1.3.3), and carbamate kinase (EC 2.7.2.2), has been recognized as an energy-generating system in some microorganisms (15, 18). These enzymes have been reported in *Pseudomonas fluorescens* (13, 19), *Clostridium botulinum* (9), *Clostridium perfringens* (16), *Streptococcus faecalis* (18), and many *Bacillus* spp. (11). The utilization of arginine through this system has been correlated with growth of *Mycoplasma* (2), motility of a *Pseudomonas* strain (17), and sporulation of *C. botulinum* (12). The present report describes the status of the enzymes of the arginine dihydrolase system during growth and sporulation of *Clostridium sporogenes*.

C. sporogenes (ATCC 19404) was routinely cultured and stored in a cooked-meat medium (Difco Laboratories, Detroit, Mich.). For cultivation of the cells, a medium composed of 3.0% tryptone, 1.0% ammonium sulfate, and 0.1% yeast extract was used (20). A liquid paraffin layer (1 cm) at the top ensured anaerobic conditions during growth and sporulation. The culture was activated by transferring the organism at least twice. A portion (0.1 ml) of the culture containing about 10^8 cells/ml was inoculated into a flask containing sterile medium (150 ml) and was incubated for 16 h at 37°C.

The 16-h culture (8 ml) was inoculated into flasks containing fresh medium (40 ml), and vegetative cells and spores were harvested after 6 and 48 h of incubation, respectively. The cells and spores formed after different periods of incubation were ascertained by staining with 0.1% methylene blue. The total count was determined by plating on Brewer anaerobic agar (Difco) and the extent of sporulation by dipicolinic acid (DPA) formation (5). The cells or spores were harvested at varying intervals of incubation by centrifugation at $3,000 \times g$ for

10 min and washed twice with 50 mM tris-(hydroxymethyl)aminomethane-hydrochloride buffer of the stated pH, and the extracts were prepared by ultrasonication as described earlier (22).

Arginine deiminase activity was determined with L-arginine-HCl as substrate (22), ornithine carbamoyltransferase by the method of Stalon (19), and carbamate kinase by the rate of anhydride formation between adenosine 5'-diphosphate and inorganic phosphate (6), determined by the method of Fiske and Subbarow (3). The protein content in the extract was measured by the procedure of Lowry et al. (7).

The cells of *C. sporogenes* showed normal vegetative growth followed by sporulation (Fig. 1). After an initial lag of 2 h, the exponential phase continued up to between 6 and 7 h, and sporulation commenced after 8 h accompanied by DPA synthesis. Arginine deiminase activity showed enhancement during exponential growth, reaching a maximal value just before the sporulation process commenced, with a subsequent decline (Fig. 2). On the other hand, ornithine carbamoyltransferase and carbamate kinase activities remained unchanged during this period.

Different carbon compounds are known to repress sporulation of some species of bacteria (4, 14). Addition of these to the growth medium also influenced the sporulation process as well as arginine deiminase activity in *C. sporogenes*. The presence of glucose (20 mM), maltose (10 mM), and glycerol (20 mM) caused repression of sporulation to the extent of 100, 90, and 80%, respectively, with concomitant reduction in arginine deiminase activity. Galactose (20 mM) and inositol (20 mM) had no influence on either of these parameters. Ornithine carbamoyltransferase and carbamate kinase did not show variation when any of these compounds was present in the medium. The repression of sporulation as well as of arginine deimi-

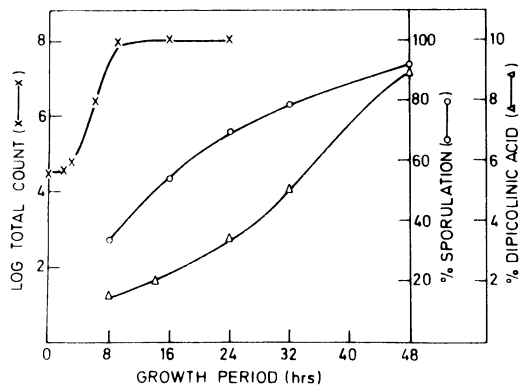


FIG. 1. Profiles of growth and sporulation of *C. sporogenes*. Culture conditions are described in the text. Individual cultures were examined for growth, sporulation, and DPA content at the indicated intervals. DPA was expressed on a percentage of dry weight basis in spores.

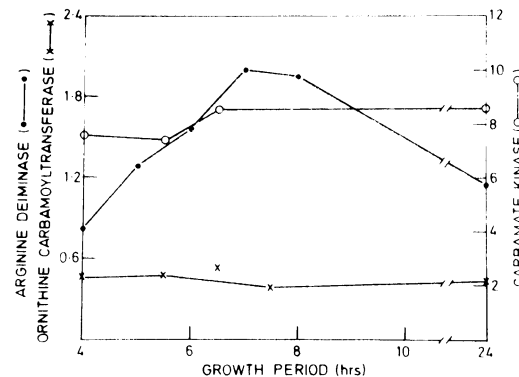


FIG. 2. Activities of arginine dihydrolase pathway enzymes during growth and sporulation of *C. sporogenes*. Activities of arginine deiminase and ornithine carbamoyltransferase have been expressed as micromoles of citrulline formed per milligram of protein per hour and minute, respectively, and that of carbamate kinase as micromoles of inorganic phosphate used per milligram of protein per minute.

nase was dependent upon the concentration of glucose in the medium (Fig. 3). Maximal inhibition of 75% of the enzyme activity was observed beyond 6 mM glucose. The addition of up to 20 mM glucose to the enzyme extract did not cause inhibition of activity. It was observed that an exogenous addition of 1 mg of adenosine 3',5'-monophosphate per ml to the culture did not stop repression of the enzyme, in contrast to earlier reports (1, 8). When glucose-grown cells were suspended in a fresh medium devoid of the hexose, the normal activity of arginine deiminase was restored progressively with the growth.

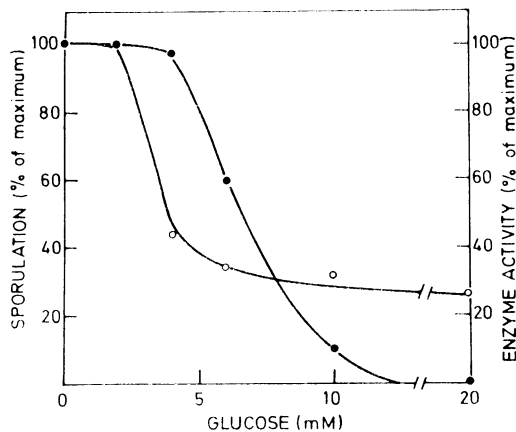


FIG. 3. Effect of varying concentrations of glucose on arginine deiminase (○) and sporulation (●) of *C. sporogenes* cells. Enzyme activity was determined after 6 h of growth, and the extent of sporulation after 48 h.

The foregoing results suggest that *C. sporogenes* utilizes arginine, regulated essentially by arginine deiminase. This enzyme has also been shown to possess regulatory properties as reported earlier (21, 22).

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