Fluoroacetate-Metabolizing Pseudomonad Isolated from
Dichapetalum cymosum

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A pseudomonad was isolated from the fluoroacetate-producing plant Dichapetalum cymosum (Hook) Engl. and identified as Pseudomonas cepacia. We established that this isolate was capable of growing in fluoroacetate-enriched solutions without any reduction in growth rate. Our isolate of P. cepacia was capable of defluorinating 2.69 mg of fluoroacetate per 109 cells per h. Fluoroacetate was degraded to CO2 at a rate of 23.53 ng/109 cells per h.

Pseudomonads have been isolated from a variety of materials, including soils, water, spoiled food, and diseased plants and animals (13). Goldman (4) isolated a pseudomonad from soil which is capable of cleaving the fluorine-carbon bond of fluoroacetate. The enzyme which brought about the defluorination was purified and named halodehydrogenase (5). The great stability of the fluorine-carbon bond of fluoroacetate is indicated by its ability to withstand boiling and treatment with concentrated sulfuric acid (15). Sodium fusion at 500°C or refluxing in 30% NaOH is necessary for the complete liberation of fluoride as fluoride from fluoroacetate (12). Kelly (6), Tonomura et al. (18), and Walker and Lien (19) have also reported the ability of soil pseudomonads and other bacteria to metabolize fluoroacetate.

Dichapetalum cymosum causes sudden death to animals and has been recognized as a hazard to livestock in southern Africa (17). Marais (11) isolated the toxic component of the plant and identified it as fluoroacetate. While attempting to establish a callus culture of D. cymosum, it became evident that the plants were invariably infested with bacteria. Although the bacteria appears to infect the pericarp and to a lesser extent the seed, most seeds appear to be aseptic internally before the fruit has become fully ripe (5a). This observation led to the present study.

MATERIALS AND METHODS

Materials. Stems (approximately 2 cm in diameter) and seeds of Dichapetalum cymosum (Hook) Engl. were sampled near Pretoria on three occasions between August and December 1988 and in the Ellisras district (about 250 km northwest of Pretoria) in November 1988. [1,2-14C]fluoroacetate (22 Ci/mol) was obtained from Amersham International plc, Amersham, England.

Isolation of bacteria. Six ripe seeds which had freshly been excised from the fruit and four stems of D. cymosum were thoroughly washed with distilled water and surface sterilized for 20 min in 0.35% sodium hypochlorite [Gillett (Pty) Ltd]. The following procedure was conducted under aseptic conditions: Sodium hypochlorite was removed by washing the seeds and stems with water, and the outer 2 mm of the seeds and stems was removed with a razor blade. The remaining material was twice more sterilized and peeled as described above. The remaining chunks (approximately 3 mm by 3 mm) were incubated in nutrient agar [Biolab (Pty) Ltd] in petri dishes at 29°C.

Identification of bacterial isolates. Cultures isolated from the seeds and stems were tentatively identified by using the API 20 NE system (API System S.A., Lyon, France). This identification was further substantiated by polyacrylamide gel electrophoresis of soluble cellular proteins according to the modification by Van Zyl and Steyn (E. Van Zyl and P. L. Steyn, Syst. Appl. Microbiol., in press) of the method of Kersters and De Ley (7). Pseudomonas cepacia NCPPB 1962, obtained from the National Collection of Plant Pathogenic Bacteria, Ministry of Agriculture, Fisheries and Food, Hatching Green Harpenden, Hertfordshire, England, was used as the reference strain.

Growth of bacteria in fluoroacetate-enriched nutrient solution. The bacteria were grown for 72 h at 29°C in 0.5% liquid yeast extract [Biolab (Pty) Ltd] which was enriched with 1% d-ribose and fluoroacetate in the following concentrations: 10, 25, and 50 mM. The A660 of the cultures was measured, and the density of the bacteria in the culture was determined from a calibration curve of absorbance versus cell number. The bacterial density was 8.5 × 105 cells per ml at the time of inoculation. Each treatment was repeated three times.

Cleaveage of the C—F bond of fluoroacetate. The bacterium was grown in a solution containing 0.5% liquid yeast extract [Biolab (Pty) Ltd], 1% d-ribose, and 50 mM fluoroacetate. The bacterial density at the time of inoculation was 4.3 × 109 cells per ml. Uninoculated medium served as a control. The fluoride concentration of the solutions was measured periodically by means of a fluoride-specific ion electrode (Orion Research Inc). This experiment was repeated three times.

Metabolism of [1,2-14C]fluoroacetate by bacterial isolate. The bacteria were grown in 0.5% liquid yeast extract enriched with 1% d-ribose for 72 h at 29°C, after which the culture was divided into two aliquots, one of which was autoclaved for 15 min at 120°C. Samples (3 ml each) of the autoclaved and unautoclaved cultures were transferred to nylon Conway dishes. Three milliliters of fresh medium was added to a third Conway dish. One microcurie of [1,2-14C]fluoroacetate was added to the solution in each Conway dish, and 0.5 ml of 0.1 M NaOH was pipetted into the center well of each Conway dish before the dishes were sealed with high-vacuum grease (Dow Corning) and Perspex plates. After 48 h of incubation at 29°C, 0.4 ml of each NaOH
solution was mixed with 10 ml of Insta Gel II [Packard (Pty) Ltd] and the radioactivity was determined with a liquid scintillation counter. To verify that the radioactivity trapped in the center wells was in the form of \( ^{14}\text{CO}_2 \), samples of the NaOH solutions were mixed with an equal volume of 1 M Ba(OH)_2 to precipitate the radioactive carbonate and filtered through Whatman no. 42 filter paper with suction. The radioactivity in the filtrates was determined with a liquid scintillation counter.

**RESULTS**

*Isolation and identification of bacteria.* Bacterial colonies developed after 3 and 4 days on plates on which stems and seeds, respectively, were incubated. All four stems and three of six seeds used in this study were infested with bacteria. All isolates were identified as *Pseudomonas cepacia* according to the API 20 NE system (99.9%). This preliminary identification was confirmed by polyacrylamide gel electrophoresis of soluble cellular proteins when compared with those of reference strain *P. cepacia* NCPPB 1962.

*Growth of *P. cepacia* in fluoroacetate-enriched nutrient solution.* The addition of fluoroacetate to the nutrient medium had very little effect on the growth of *P. cepacia* even at the relatively high concentration of 50 mM (Fig. 1). The abundance of the bacteria in the different treatments did not differ significantly at the 1% probability level.

*Cleavage of the C—F bond of fluoroacetate.* In both treatments, a small amount of fluoroacetic acid produced during the first 20 h (Fig. 2), after which no additional fluoroacetic acid produced in the control treatment. The *P. cepacia* culture entered its logarithmic growth phase only after 85 h probably because of the small inoculum density. The culture started to produce fluorodecarboxylation and continued to do so for the next 52 h at a constant rate of abut 2.69 mg/10^9 cells per h.

*Metabolism of [1,2-\(^{14}\text{C}\)]fluoroacetate by *P. cepacia.* No radioactivity was found in the filtrates of samples treated with Ba(OH)_2, suggesting that all the radioactivity was in the form of \( ^{14}\text{CO}_2 \). If the \( ^{14}\text{CO}_2 \) that was produced by the live bacteria originated from both carbons of fluoroacetate, then the live *P. cepacia* degraded the fluoroacetate at a rate of 6.423 ng/h. A very small but similar amount of CO_2 equivalent to approximately 0.027 ng of fluoroacetate per h, was produced in the two control treatments. The bacterial density was 1.08 x 10^8 cells per ml when the \([^{14}\text{C}]\)fluoroacetate was added. \( ^{14}\text{CO}_2 \) was produced from \([1,2-^{14}\text{C}]\)fluoroacetate at 336 dpm in nutrient medium alone, 316 dpm in medium with dead *P. cepacia*, and 71, 507 dpm in medium with live *P. cepacia*. (Results are the means of three experiments).

**DISCUSSION**

*P. cepacia* is a soil-inhabiting bacterium which causes root rot of onions (2) and may also be an opportunistic pathogen of humans (3). It seems, however, to have no noticeable effect on the morphology of *D. cymosum*, from which it was isolated. *P. cepacia* is capable of living inside *D. cymosum* probably because it can metabolize fluoroacetate by cleaving the C—F bond. Because a normal tricarboxylic acid cycle operates in all strains of pseudomonads examined (13), it is possible that the carbon of the acetate resulting from the defluorination of fluoroacetate is released as \( ^{14}\text{CO}_2 \) through the bacterial Krebs cycle.

*P. cepacia* is able to utilize a greater number of compounds as sole sources of carbon and energy than any other bacterium investigated in this regard to date (2, 14, 16). The extraordinary nutritional versatility of *P. cepacia* and its ability to colonize both plant and animal tissues represent a degree of adaptability not encountered in most bacteria (9). Such adaptability presumably demands a large number of different biochemical reactions and implies the operation of well-developed, and possibly novel, regulatory mechanisms. Relatively high fluoroacetate concentrations (231.9 mg/kg
of fresh young leaves) are present in *D. cymosum* (11a). The presence of *P. cepacia* in *D. cymosum* may contribute to the degradation of fluorocitrate. *D. cymosum* appears to have a functional Krebs cycle whose aconitase is inhibited by fluorocitrate (1, 10). Because the conversion of fluorocitrate to fluorocitrate apparently is a unique property of certain cell organelles (8), it is conceivable that despite a relatively high overall fluorocitrate concentration in *D. cymosum*, the concentrations in key organelles are kept low, thereby precluding the poisoning of the plant. The possible clustering of *P. cepacia* around such key organelles could considerably assist the plant in preventing fluorocitrate from entering the organelles. On the other hand, it would appear that *D. cymosum* itself also has the ability to degrade fluorocitrate (5a), and this activity could be concentrated in or around the key subcellular organelles. This is, however, speculation and is worthy of further investigation.

With gene manipulation, it might be possible to enable rumen bacteria to break down fluorocitrate. This exciting prospect also needs further investigation.

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LITERATURE CITED


