Metabolism of Pentachlorophenol by an Axenic Bacterial Culture

JOSEPH P. CHU AND EDWIN J. KIRSCH

Environmental Engineering Department, School of Civil Engineering, Purdue University, West Lafayette, Indiana 47907

Received for publication 4 January 1972

A bacterial isolate obtained from a continuous-flow enrichment culture has been shown to metabolize pentachlorophenol as a sole source of organic carbon and energy.

The resistance of chlorophenols to microbial decomposition is said to be dependent on the number and position of chlorine atoms on the phenol ring (1). The widely used pesticide pentachlorophenol (penta) has been considered as one of the most refractory compounds of the chlorophenol family. Penta has been cited as an example of the fallibility of microorganisms in being able to cope with unusual “man-made” chemicals (1). The isolation of a bacterium which both dissimilates and assimilates penta has not been previously reported.

In an attempt to determine the feasibility of biological treatment for industrial wastewater containing penta and other phenolics, heterogeneous populations of microorganisms were shown to liberate radioactive carbon dioxide from 14C-penta (2). Continuous culture technique was used to provide additional enrichment of the penta-degrading microorganisms in the mixed culture. A 1-liter chemostat was inoculated with 100 ml of the mixed culture and aerated with water-saturated air. A mineral salts solution (K2HPO4, 2.0 g; MgSO4·7H2O, 0.2 g; NH4NO3, 0.05 g; tap water, 50 ml; and deionized water, 950 ml) was initially supplemented with Eastman high-purity pentachlorophenol (melting point 189 to 191 C) at 20 mg/liter and supplied to the chemostat at a flow rate of 0.5 liter/day. During 40 days of continuous operation, the penta concentration was increased in gradual increments to 200 mg/liter. The pH of the medium was 7.5, and the culture was sufficiently buffered to maintain penta as a soluble salt. Samples of the chemostat effluent were adjusted to pH 10 ± 0.5 for spectrophotometric scanning between 360 and 260 nm, and the well defined absorbance peak at 318 nm was used in determining concentrations of penta remaining in the culture liquor.

At each incremental increase of penta concentration in the influent, there was a corresponding initial increase in penta concentration in the effluent; however, within 3 days a steady state of 4 mg/liter was established regardless of the influent concentration. The increased rate of penta disappearance indicated that the culture population had been significantly enriched with penta-utilizing microorganisms. The cell density in the effluent was always low, and its turbidity never exceeded 0.05 as measured by absorbance at 600 nm against the un inoculated medium. After 40 days of operation, a portion of the chemostat culture was diluted and plated on Trypticase soy agar (BBL) containing 200 mg of penta per liter. One of the isolates, which was shown to remove penta from solution, was repeatedly reisolated by dilution plating to effect purification. This culture was designated bacterium KC-3. The morphological and physiological characteristics of KC-3 suggest a relationship to the saprophytic coryneform bacteria. A compilation of taxonomic observations is given in Table 1.

The utilization of penta as a carbon and energy source for KC-3 was demonstrated as follows. Cells grown in Trypticase soy agar were transferred to 100 ml of basal salts medium containing 200 mg of penta per liter and were incubated at 30 C on a shaker. A visible increase in turbidity was noted in this flask after 3 days of incubation. Duplicate flasks containing 100 ml of basal salts medium with 26.6 mg of penta per liter were inoculated with 0.5 ml of the above culture. Uninoculated basal salts-penta medium was used as a nongrowth control to check for possible chemical or physical changes of penta in the medium. Basal salts medium without penta was inoculated to check for spurious growth unrelated to the
presence of penta. Concentrations of penta remaining in these flasks were measured spectrometrically in samples clarified by centrifugation, and the number of viable cells was determined by dilution plating. Table 2 shows that in 84 hr the penta concentration was reduced 80% in inoculated flasks and that concurrently the viable cell count increased 50-fold. No penta disappeared in uninoculated flasks, and no increase in viable cell count resulted in cultures without penta.

The ability of KC-3 to dissipate penta was further demonstrated by analyzing the CO\(_2\) liberated when randomly labeled \(^{14}\)C-penta was respired. Cells of KC-3 grown in basal salts medium containing 200 mg of penta per liter were harvested by centrifugation at 20,000 \(\times g\), washed twice in 0.02 m phosphate buffer at pH 7.8, and resuspended in buffer to a density of 0.4-mg cell dry weight per ml. A 2.5-ml amount of the cell suspension and 0.5 ml of \(^{14}\)C-penta solution, containing 72 \(\mu\)g of penta or 34,560 counts/min, were placed in a Warburg flask and incubated at 25 C. The radiochemical purity of the penta was in excess of 99.5% (3). A hyamine hydroxide-impregnated filter paper strip was placed in the center well to trap CO\(_2\) evolved. The radioactivity on the strip was determined by liquid scintillation counting in a scintillator containing 0.4% 2,5-diphenyloxazole (PPO) in equal volumes of toluene and 2-ethoxyethanol. The evolution of \(^{14}\)CO\(_2\) is shown in Fig. 1. The maximum rate of \(^{14}\)CO\(_2\) evolution was 4,900 counts per min per hr. Expressed as oxidation of penta carbon to CO\(_2\), this represents conversion rate of 10 \(\mu\)g of penta per hr per mg of cell dry weight. At the end of 24 hr, 73% of the \(^{14}\)C-penta

![Graph showing CO\(_2\) evolution](image)

**Fig. 1.** \(^{14}\)CO\(_2\) evolved from biooxidation of \(^{14}\)C-pentachlorophenol.

| Table 1. Taxonomic characteristics of bacterium KC-3 |
|-------------------------------|---------------------------------|
| **Criteria**                  | **Description of organism**     |
| Cell morphology               | Slender bacilli, 2 to 4 \(\mu\)m long, nonmotile, non-spor-forming, frequent V-shapes, occasional palisade arrangements and Chinese character aggregates |
| Staining                      | Gram-variable but predominantly gram-negative with metachromatic granules, non-acid-fast, granules stain with Sudan Black B |
| Colonial morphology           | Circular, convex, entire, lemon-yellow colonies on complex organic media including Trypticase soy agar and nutrient agar. Small, pale-yellow colonies on cellobiose-mineral salts agar and tiny opaque colonies on pentachlorophenol-mineral salts agar. Gelatin not liquified, nitrate not reduced, glucose oxidized aerobically but not fermented, catalase-positive, peroxidase-positive, cytochrome oxidase-negative, no growth in lauryl tryptose broth, no growth in brilliant green bile broth, cellobiose, glucose, acetate, and selected chlorinated phenols utilized as sole organic carbon sources. Sucrose, lactose, citrate, glutamic acid, xylene, pyruvate, malate, and cellulose do not support growth in a mineral salts medium. |
| Physiological and cultural    |                                  |

| Table 2. Disappearance of pentachlorophenol and growth of bacterium KC-3 in basal salts media |
|-----------------------------------------------|-----------------------------------------------|
| **Growth medium**                            | **Penta (mg/liter)** | **No. of viable cells (10^5/ml)** | **Incubation time (hr)** | **Incubation time (hr)** |
| Basal salts + penta, inoculated              | 26.6               | 25.5               | 21.2               | 5.3               | 7               | 40               | 440               |
| Basal salts + penta, uninoculated            | 26.6               | 25.2               | 20.2               | 4.8               | 7               | 43               | 370               |
| Basal salts, inoculated                       | 26.6               | 26.6               | 26.6               | 26.6               | 0               | 0                | 0                 |
| Basal salts, inoculated                       | 0                  | 0                  | 0                  | 0                  | 7               | 5                | 0.2               |
added appeared in $^{14}$CO$_2$ collected. The culture centrate showed no detectable residual penta when examined by spectrophotometry or scintillation counting.

**LITERATURE CITED**

1. Alexander, M. 1965. Persistence and biological reactions of pesticides in soils. Soil Sci. Soc. Amer. Proc. 29:1–7.
2. Kirsch, E. J., and J. E. Etzel. 1972. Microbial decomposition of pentachlorophenol. J. Water Pollut. Contr. Fed., in press.
3. Rogers, R. R., J. E. Christian, J. E. Etzel, and G. S. Born. 1971. The synthesis and purification of uniformly labeled carbon-14 pentachlorophenol. J. Label. Compounds 12:149–153.