Utilization of Phenol and Cresols by *Bacillus stearothermophilus*, Strain pH24

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**INTRODUCTION**

The degradation of aromatic compounds by mesophilic micro-organisms (reviewed by Dagley, 1971) and the oxidative breakdown of phenolic compounds by several genera of aerobic bacteria and yeasts is well documented (Bayly, Dagley & Gibson, 1966; Feist & Hegeman, 1969; Neujahr & Varga, 1970; Neujahr, Lindsjo & Varga, 1974). However, there is little information concerning the utilization of aromatics by thermophiles. Experiments with enrichment cultures suggest that thermophiles can degrade aromatic rings and phenol-oxidizing cultures have been reported (Egorova, 1942, 1946). More recently, the oxidation of benzoic acid by an unidentified thermophilic bacterium isolated from soil was described (Buswell & Twomey, 1974). We report the growth of *Bacillus stearothermophilus*, strain pH24, on phenol and the isomeric cresols, and the ability of intact bacteria to oxidize a variety of aromatic compounds.

**METHODS**

*Bacillus stearothermophilus* (strain pH24), was isolated from industrial sediment by elective culture at 55 °C in a semi-defined basal medium which contained phenol as the major carbon source. The strain was maintained on slopes of basal medium solidified with 2% (w/v) Difco agar.

Cultures were grown in a mineral salts medium containing 1 ml trace elements solution (Barnett & Ingram, 1955)/l, plus (g/l): K₂HPO₄, 0.5; NH₄Cl, 1.0; MgSO₄·7H₂O, 0.02; yeast extract (Oxoid), 0.2; Casamino acids (Difco), 0.1; aromatic carbon source, 0.5; the pH was adjusted to 7.2. Succinate or sodium gluconate (2 g/l) replaced the aromatic substrate for the growth of non-induced bacteria.

*Bacillus stearothermophilus*, strain pH24, was grown routinely in 2 l baffled Erlenmeyer flasks each containing 1 l of the above medium. The flasks were incubated at 55 °C in an orbital incubator shaker (Gallenkamp) operated at 150 rev./min. Cultures were harvested in late exponential phase (12 to 14 h) by centrifugation (20000 g, 10 min, 25 °C), and washed once with phosphate buffer (0.1 M-KH₂PO₄ adjusted with 2 M-NaOH). When required for manometric studies the pellet was resuspended in the same buffer to give a bacterial density of 15 to 20 mg dry wt/ml.

The extinction values of bacterial suspensions were measured with an EEL nephelometer (blue filter) and a standard curve prepared relating bacterial dry weight (dried at 120 °C to constant weight) to extinction. Oxygen uptake was measured at 55 °C by conventional Warburg manometry. Reaction mixtures contained 250 μmol phosphate buffer pH 7.0 (0.1 M-KH₂PO₄ adjusted with 2 M-NaOH), substrate (3 μmol) and bacterial suspension
**Short communication**

**Table 1. Oxidation of substrates by B. stearothermophilus, strain PH24**

<table>
<thead>
<tr>
<th>Test substrate</th>
<th>Phenol</th>
<th>o-Cresol</th>
<th>m-Cresol</th>
<th>p-Cresol</th>
<th>Sodium gluconate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenol</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>o-Cresol</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>m-Cresol</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>p-Cresol</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Benzoate</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Catechol</td>
<td>+</td>
<td>NE</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>3-Methylcatechol</td>
<td>+</td>
<td>NE</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>4-Methylcatechol</td>
<td>+</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>+</td>
</tr>
<tr>
<td>Glucose</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>+</td>
</tr>
</tbody>
</table>

+, Substrate oxidized without a lag period; −, substrate not oxidized after 3 h; NE, not evaluated.

(3 to 5 mg dry wt) in 3·0 ml H₂O. All chemicals were obtained from commercial sources and purified by recrystallization or sublimation where necessary.

**RESULTS AND DISCUSSION**

**Organism identification**

Although the taxonomy of the aerobic, thermophilic, spore-bearing bacilli is still far from satisfactory, considerable advances are apparent from several recent taxonomic studies of this group (Molyneaux, 1952; Smith, Gordon & Clark, 1952; Allen, 1953; Walker & Wolf, 1971). In accordance with the generally accepted principle of ‘species patterns’ the organism used in this study has been typed as *Bacillus stearothermophilus*, strain PH24.

Characteristically, the bacteria were Gram-positive rods with swollen terminal and sub-terminal thick-walled oval endospores. The minimum temperature for growth was 40 °C and the maximum 72 °C; no growth occurred under anaerobic conditions. Tests on cultures grown at 55 °C revealed that acetylmethylcarbinol was not produced, citrate was not utilized, no acid was produced from mannitol, and nitrate was strongly reduced to nitrite. No growth occurred in 5% (w/v) NaCl broth, or below pH 6·0 in a Lab-Lemco beef extract-peptone medium. Casein and gelatin were strongly hydrolysed but not starch. Only in this latter respect did our strain differ from the ‘species pattern’ for *B. stearothermophilus* as defined by Smith *et al.* (1952).

**Utilization of aromatic compounds as carbon sources for growth.** Of a wide range of aromatic compounds tested, only phenol and the isomeric cresols were utilized as growth substrates. Growth rates and final yields were highest on phenol and p-cresol, with mean generation times at 55 °C of 50 and 70 min, respectively. The organism had a low tolerance to phenol; concentrations above 0·05% were inhibitory.

**Oxidation of aromatic compounds by washed cell suspensions**

Phenol-grown bacteria metabolized phenol and o-, m- and p-cresol without a lag (Table 1) but oxidation of the isomeric nitrophenols, fluorophenols, chlorophenols, hydroxybenzoic acids and benzoate was not observed. When any of the four aromatic substances (phenol or o-, m- or p-cresol) was the growth substrate, all these phenolic compounds were oxidized without a lag period. Bacteria harvested from gluconate media, however, did not metabolize the aromatic substrates even after 3 h, indicating the inducible nature of the enzymes.
involved. Bacterial suspensions induced by growth on phenol, m-cresol and p-cresol rapidly oxidized catechol, 3-methylcatechol and 4-methylcatechol. In reaction mixtures containing catechol and 4-methylcatechol, yellow intermediates appeared which showed identical spectral characteristics in alkaline, acidic and neutral conditions with those of the respective meta-cleavage products, namely, 2-hydroxyxymuconic semialdehyde and 2-hydroxy-5-methylmuconic semialdehyde. Further, extracts of phenol-induced bacteria of this strain oxidized catechol and several catechol derivatives via the meta-cleavage route (Buswell, 1974; Buswell, Roberts & Twomey, 1974).

Mesophilic bacteria have been widely reported to catabolize phenol and the isomeric cresols by converting them before aromatic-ring fission into catechol and methylcatechols, respectively. As far as we are aware, little work on aromatic metabolism has been done using thermophilic micro-organisms, nor for that matter is much information available on the degradation of aromatics by Bacillus species in general. The present results indicate that conversion of benezienoid compounds operates in both groups, and further investigation of the enzymes which catalyse the breakdown pathways and comparison with the corresponding functional enzymes in mesophiles are in progress.

REFERENCES


