Production of Geosmin in Fermentors and Extraction with an Ion-Exchange Resin

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A method for growing Streptomyces griseus LP-16 in fermentors and extracting and purifying geosmin, using an ion-exchange resin, is described.

Geosmin, trans-1,10-dimethyl-trans-9-decalol (5), is a neutral oil with an earthy odor produced by actinomycetes (4, 7), fungi (9), and blue-green algae (8, 10, 14). Its odor can be detected in water at a concentration of 0.2 µg/liter (3, 13). It has been isolated from garden soil (2), tainted fish (15), Grand Lake, Ohio (13), the Rhine River, and the North Sea (12). Geosmin has been detected in raw (11) and cooked (1) beans and in off-flavored dry white beans (3).

A method for producing geosmin in fermentors is needed to produce analytical standards for environmental and food chemists.

One loopful of a soil culture stock of Streptomyces griseus LP-16 was used to inoculate one flask of soybean meal NZ-amino medium (50 ml of medium in a 250-ml Erlenmeyer flask). (Soybean meal: “Central Flow-Coated—49,” Central Soya, Bellevue, Ohio, 10 g; commercial glucose—Cerelose, 20 g; N-Z Amine A—Humko, Sheffield, Lyndhurst, N.J., 5 g; NaCl, 5 g; tap water, 1 liter; pH before sterilization, 7.5.) After 24 h on a rotary shaker at 28°C, 10 ml of this culture was used to inoculate similar flasks of the same medium. These were incubated in the same manner.

One secondary culture was used to inoculate each 5-liter LabroFerm fermentor (New Brunswick Scientific Co., Edison, N.J.) containing 2.5 liters of the same medium plus 3 ml of Hodag PBG 2000 polyglycol artifoom.

After incubation (28°C; 1 liter of air per min; 300 rpm), whole broths were distilled at atmospheric pressure until 20% of their volume had been collected. The distillates were extracted three times with methylene chloride: 20, 10, and 10% by volume. The combined extracts were concentrated in a stream of warm air to 0.5 ml for assay. Gas chromatography was as previously described (7). A 6-foot (ca. 182.88 cm) column was used, and the column temperature was 120°C, programmed at 5°C per min to 220°C. A 25-mm² peak area equaled 1 µg of geosmin. Amberlite XAD-2 resin (Chemical Dynamics

<table>
<thead>
<tr>
<th>Harvest (day)</th>
<th>Geosmin in half of the distillate (mg)</th>
<th>Eluate fraction (no.)</th>
<th>Geosmin from resin by GC* assay (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>9.8</td>
<td>7</td>
<td>5.2</td>
</tr>
<tr>
<td>2</td>
<td>7.4</td>
<td>8</td>
<td>16.0</td>
</tr>
<tr>
<td>3</td>
<td>4.5</td>
<td>9</td>
<td>5.3</td>
</tr>
<tr>
<td>4</td>
<td>7.9</td>
<td></td>
<td>5.7</td>
</tr>
</tbody>
</table>

* Since half of the distillate is equivalent to 1.25 liters of whole broth, the yields were 7.8, 5.9, 3.6, and 6.3 mg/liter.

** GC, Gas-liquid chromatography.

GC Direct gas-liquid chromatography assay on the methanol solution with a Varian model 28-60 dual-flame instrument with a 4-foot by ½-inch (ca. 121.9 cm by 3.8 mm) column of Apiezon L (10%) on Chromosorb W.

* Geosmin extracted into methylene chloride for the usual gas-liquid chromatography assay.

Co., South Plainfield, N.J.) was swirled five or more times in distilled water, and the fines were decanted and a 100-ml column was prepared. In a typical run, 1.8 liters of distillate was passed through during 45 min, followed by 250 ml of distilled water at the same rate and then methanol. The methanol eluate fractions were collected as soon as the methanol emerged from the column, yielding a geosmin recovery of 89%.

The results are summarized in Table 1. The average level of geosmin production in fermentors was 6 mg/liter, which is better than that ever obtained in flask cultures. Geosmin can be obtained in pure form by preparative gas chromatography (7) or by column chromatography (6). On silica gel, pure geosmin is obtained with approximately 0.5 to 1 g of silica for every 1 mg of geosmin and eluting with methylene chloride.

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