Microbial Formation of L-Tryptophan from D-Tryptophan

Sirs:

The present paper briefly describes that a large amount of L-tryptophan was produced from D-tryptophan by the intact cells of *Pseudomonas* strain No. 2150. Many investigations have already been reported about the isomerization of D-amino acids, but we have only a few reports concerned with the isomerization of D-tryptophan. Two different mechanisms have been found about the isomeric conversion of D-tryptophan. First of all, Durham\(^1\) reported that the D- to L-isomeric conversion involved the initial oxidation of D-tryptophan to indolepyruvic acid followed by a transamination reaction by a *Flavobacterium* species. On the other hand, Behrman\(^2\) found the presence of a tryptophan racemase in a *Pseudomonas* species. However, an attempt to produce L-tryptophan from D-tryptophan by these isomeric conversions has not yet been reported.

In the course of our investigation on the metabolism of D-tryptophan by bacteria, it was found that a large amount of L-tryptophan was accumulated in the reaction mixture when the intact cells of *Pseudomonas* strain No. 2150 were incubated aerobically in the presence of D-tryptophan and L-amino acids. Results obtained were as follows. *Pseudomonas* strain No. 2150 could grow in an inorganic salt medium containing D-tryptophan as a sole source of nitrogen and produced a reddish brown pigment from D-tryptophan on an organic nutrient medium. The taxonomic properties of *Pseudomonas* strain No. 2150 were examined according to procedure given in Manual of Microbiological Methods.\(^3\) From this examination, it was found that *Pseudomonas* strain No. 2150 was closely resembling *Pseudomonas ovalis*. The microorganisms were grown in the medium consisting of glucose 1.0%, peptone 0.5%, yeast extract 0.2%, KH₂PO₄ 0.1%, K₂HPO₄ 0.1%, MgSO₄·7H₂O 0.05%, n-tryptophan 0.1% ~ 0.2%, and tap water (pH 7.0 ~ 7.4). 100 ml of the medium was introduced into 500 ml shaking flasks and after it was inoculated, the cultivation was performed on a reciprocal shaker for 20 hr at 30°C. The cells were harvested by centrifugation and washed with physiological saline.

The washed cells thus obtained were resuspended to a 1/20M phosphate buffer (pH 7.6), and employed for the reaction. The reaction mixture consisted of D-tryptophan, L-amino acids as amino donor, and the washed cell suspensions. The reaction was proceeded in the test tube under shaking condition at 30°C for 40 hr. L-Tryptophan production was measured by the microbiological assay using an *Aerobacter aerogenes* which was induced by the UV irradiation. Determination of D- or L-tryptophan oxidation activity was carried out by manometric measurement of O₂ uptake. L-Tryptophan was accumulated when the intact cells were incubated with D-tryptophan and L-amino acids. On the other hand, when the same system was incubated in the absence of L-amino acids, a keto acid probably indole pyruvic acid, was accumulated in the reaction mixture, although

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formation of L-tryptophan could not be confirmed.

Furthermore, as shown in Fig. 1, d-tryptophan oxidation activity was stronger than L-tryptophan oxidation activity in the cells grown in the medium supplemented with d-tryptophan. From these results, it was assumed that the mechanism of d- to L-isomeric conversion by the intact cells of Pseudomonas strain 2150 involved an oxidative deamination of d-tryptophan followed by a transamination.

To establish the optimal conditions for the formation of L-tryptophan, various conditions were investigated. Table I shows the change of the isomerization activity during growth. It was found that the cells cultured for 21 hr showed an intensive activity of L-tryptophan formation from d-tryptophan, in contrast, cells cultured for 40 hr was less active.

As shown in Fig. 2, L-tryptophan was produced from d-tryptophan in the presence of L-valine, L-isoleucine, or L-leucine at 80~90% isomerization rate during 40 hr incubation. The effect of d-tryptophan concentration was investigated in the reaction system using L-valine as amino donor. Although the increased amount of d-tryptophan caused to reduce the isomerization rate, the amount of L-tryptophan

<table>
<thead>
<tr>
<th>Culture age of cells (hr)</th>
<th>L-Amino acid added</th>
<th>L-tryptophan formed (µmoles)</th>
<th>Rate of Isomerization (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>L-Glutamic acid</td>
<td>20.8</td>
<td>41.6</td>
</tr>
<tr>
<td>21</td>
<td>L-Leucine</td>
<td>42.9</td>
<td>85.8</td>
</tr>
<tr>
<td>21</td>
<td>L-Phenylalanine</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>21</td>
<td>No addition</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>40</td>
<td>L-Glutamic acid</td>
<td>16.5</td>
<td>32.5</td>
</tr>
<tr>
<td>40</td>
<td>L-Leucine</td>
<td>35.1</td>
<td>70.2</td>
</tr>
<tr>
<td>40</td>
<td>L-Phenylalanine</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>40</td>
<td>No addition</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Reaction mixture: D-Tryptophan 50 µmoles, L-amino acid 100 µmoles, 1/20 M phosphate buffer 1.5 ml, cell suspension 0.5 ml, total volume 2.65 ml. Incubated on shaking at 30°C.

![Fig. 1. Oxidation of D-Tryptophan and L-Tryptophan by Intact Cells.](image1)

**Table I. Changes of Isomerization Activity During Culture Process**

![Fig. 2. Effect of L-Amino Acids on the Conversion of D-Tryptophan to L-Tryptophan.](image2)
accumulation reached to a level of 7.6 mg/ml during 40 hr incubation when 10 mg/ml of D-tryptophan and 15 mg/ml of l-valine were added to the reaction system.

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