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# 188. Yukio Kameda and Yoshiko Ishii : Studies on Acylase Activity and Microorganisms. XX.*1 Isolation of Soil Bacteria Capable of Resolving Tryptophan by their Acylase on N-Acetyl-DL-tryptophan.

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In the earlier papers[1,2] it was shown that N-acetyl-DL-tryptophan was asymmetrically hydrolyzed to produce L-tryptophan and N-acetyl-D-tryptophan by the acylase of soil bacteria KT 104 (*Pseudomonas* sp.) or KT 241.

In this work two strains (KT 251 and KT 253) of bacteria capable of hydrolyzing asymmetrically N-acetyl-DL-tryptophan (I) were easily isolated from soil samples by using a synthetic medium containing (I) as the sole source of carbon. Both KT 251 and KT 253 seemed to be rod-shaped and exhibited a metabolic activity as shown in Table I.

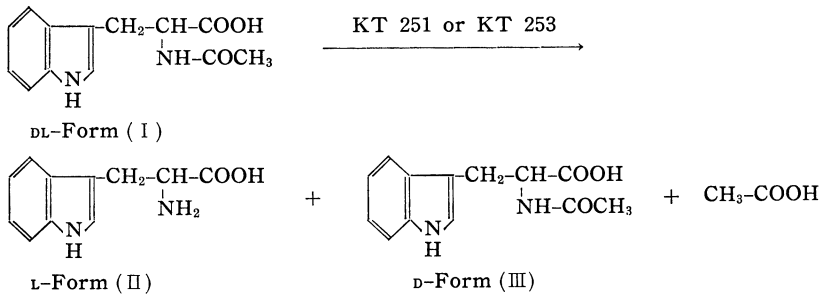
TABLE I. Metabolic Activity of Soil Bacteria KT 251 and KT 253

| | KT 251 | KT 253 |
|---|---|---|
| N-Acetyl-DL-tryptophan | + | + |
| N-Acetyl-D-tryptophan | − | − |
| DL-Tryptophan | + | + |
| Acetic acid | + | + |
| Benzoic acid | − | − |
| *p*-Hydroxybenzoic acid | − | + |
| *m*-Hydroxybenzoic acid | − | + |
| Salicylic acid | − | − |
| Phenylacetic acid | + | + |
| Cinnamic acid | − | − |

\+ Within 4 days at 25°, a luxuriant growth of bacteria was obtained on a culture medium with the particular organic compound as the source of carbon. This cultivation experiment was repeated 3 times in succession.

− Almost no visible growth of bacteria was observed at 25° in 4 days.

Table I shows that both KT 251 and KT 253 can grow in the medium containing N-acetyl-L-tryptophan, but not N-acetyl-D-tryptophan as the sole source of carbon. The ability of KT 251 to resolve N-acetyl-DL-tryptophan was tested in the following manner : To 0.05*M* N-acetyl-DL-tryptophan (I) solution (pH 7.2), the acetone powder of




*1 Part XIX : This Bulletin, **10**, 1146 (1962).

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1) Y. Kameda, E. Toyoura, Y. Kimura, B. Okino : This Bulletin, **6**, 395 (1958). Note that 1-N-acetyl-tryptophan was misprinted for N-acetyl-tryptophan.

2) Y. Kameda, E. Toyoura, Y. Kimura, K. Matsui, M. Kimura : This Bulletin, **10**, 1146 (1962).

KT 251 was added and the mixture was incubated at 37° with a few drops of toluene. After 3 days, L-tryptophan (II), $[\alpha]_D^{15}$ −32°, and N-acetyl-D-tryptophan (III), $[\alpha]_D^{16}$ −28°, m.p. 184~185°, were obtained in good yield. In the same way, it was confirmed that KT 253 also hydrolyzed (I) asymmetrically to produce (II) and (III). (III) was not hydrolyzed by either KT 251 or KT 253.

## Experimental

**N-Acetyl-DL-tryptophan (I)**——(I) was prepared according to the procedure previously described.[1]

**Isolation and Characterization of Soil Bacteria KT 251 and KT 253**——The constituents of the culture medium for isolation of KT 251 and KT 253 were as follows: N-acetyl-DL-tryptophan, 0.2 g.; $NH_4Cl$, 0.1 g.; $K_2HPO_4$, 0.1 g.; $MgSO_4 \cdot 7H_2O$, 0.05 g.; 1% soln. of $CaCl_2 \cdot 6H_2O$, 2 drops; 1% soln. of $FeCl_3 \cdot H_2O$, 1 drop; dist. $H_2O$, 100 cc.; pH 7.4~7.6 (adjusted with 10% NaOH). 0.2 g. of a soil sample, obtained from a causeway (KT 251) or an alley (KT 253) in Matsunaga city, was inoculated into 10 cc. of the above culture medium and incubated at 25° for 3~6 days. When a luxuriant growth of bacteria occurred, a loop of the culture fluid was transferred to a new culture medium of the same composition. Such transplantation was repeated at least 3 times. The bacterial suspension of the last generation was then planted in bouillon agar. Culture experiments were carried out in order to determine whether the microbe isolated from the agar plate could grow in the above medium. The newly isolated soil bacteria were named KT 251 and KT 253.

Both KT 251 and KT 253 seemed to be rod-shaped and could be cultivated in various synthetic media (Table I).

**Preparation of Acetone Powder of Soil Bacteria KT 251 and KT 253**——KT 251 was grown in five 1 L. Erlenmeyer flasks, each containing 200 cc. of bouillon (pH 7.4). After keeping at 25° for 4 days, the cells were harvested by centrifugation, and washed with dist. $H_2O$. The cells thus obtained were placed in 200 cc. of $Me_2CO$ cooled to −5°, and the mixture stirred vigorously for 3 min. The solid obtained was collected by suction and washed first with cold $Me_2CO$ and then with cold $Et_2O$. The filter cake of cells was transferred into a sheet of filter paper and worked gently with a spatula until the solvent evaporated, leaving ca. 0.67 g. of a dry powder. In the same way acetone powder (ca. 0.51 g.) of KT 253 was prepared.

**Asymmetric Hydrolysis of N-Acetyl-DL-tryptophan by the Acetone Powder of Soil Bacteria KT 251**——2.46 g. of N-acetyl-DL-tryptophan was suspended in 200 cc. of $H_2O$ and brought into solution at pH 7.8 by the addition of 10% NaOH. To this aqueous solution, 0.4 g. of KT 251 acetone powder was added and the mixture was allowed to digest at 37° with a few drops of toluene. After 3 days the digest was adjusted to pH 4.5 by the addition of AcOH. After heating for several min., the coagulated protein and insoluble mass were removed by centrifugation. The supernatant which came through charcoal was concentrated *in vacuo* until crystallization began. EtOH was then added and the whole was allowed to stand for 12 hr. in a refrigerator. Partially precipitated L-tryptophan was filtered by suction. By concentration of the filtrate and addition of EtOH, some more precipitates were obtained. Recrystallization of the combined precipitates from 70% EtOH yielded 0.4 g. (39%) of L-tryptophan as colorless scaly crystals, $[\alpha]_D^{14}$ −32° (c=1, $H_2O$). *Anal.* Calcd. for $C_{11}H_{12}O_2N_2$: N, 13.72. Found: N, 13.93.

The filtrate from L-tryptophan was concentrated to dryness and the residue was dissolved in ca. 3 cc. of $H_2O$. The solution was acidified with HCl and the resulting precipitate was collected by suction. Recrystallization from 50% MeOH gave 0.7 g. (57%) of N-acetyl-D-tryptophan, m.p. 184~185°, $[\alpha]_D^{15}$ −28° (c=2, EtOH). *Anal.* Calcd. for $C_{13}H_{14}O_3N_2$: N, 11.38. Found: N, 11.47.

**Asymmetric Hydrolysis of N-Acetyl-DL-tryptophan by the Acetone Powder of Soil Bacteria KT 253**——To 200 cc. of 0.05*M* N-acetyl-DL-tryptophan solution, 0.4 g. of KT 253 acetone powder was added and the mixture was incubated at 37° for 5 days. 0.3 g. (29%) of L-tryptophan and 0.75 g. (61%) of N-acetyl-D-tryptophan were obtained.

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## Summary

Two strains (KT 251 and KT 253) of bacteria capable of resolving tryptophan by their acylase on N-acetyl-DL-tryptophan were isolated from soil samples by using a synthetic medium containing N-acetyl-DL-tryptophan as the sole source of carbon. Both KT 251 and KT 253 could be cultivated in various synthetic media.

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