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143. Etsuko Toyoura : Studies on Acylase Activity and Microörganisms. XIV.*2 A Certain Strain of Soil Bacteria capable of resolving Lysine by its Metabolism on α-N-Phenylacetyl-ε-N-benzoyl-DL-lysine.

(*Laboratory of Antibiotics, Faculty of Pharmacy, Kanazawa University**1)

In the previous papers[1~4] it was reported that lysine, methionine, phenylalanine, leucine, threonine, glutamic and aspartic acids, *p*-methoxyphenylalanine, and 3,4-methylenedioxyphenylalanine could be asymmetrically resolved by the metabolism of soil bacteria on benzoyl derivatives of DL-amino acids. The present work is a direct continuation and extension of the same work previously reported.

Four strains (KT-230, KT-231, KT-232, and KT-233) of soil bacteria metabolized dibenzoyl-DL-lysine to produce ε-N-benzoyl-L-lysine and dibenzoyl-D-lysine[2] but it was now revealed that KT-230, KT-231, KT-232, and KT-233 could not metabolize α-N-phenylacetyl-ε-N-benzoyl-DL-lysine (II).

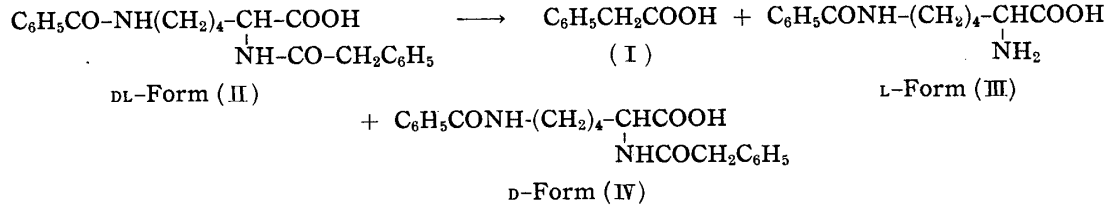
A new strain, which had the metabolic activity shown in Table I was isolated from the soil. It may be seen from Table I that this new bacteria, designated KT-311, can utilize phenylacetic acid (I), which was produced from α-N-phenylacetyl-ε-N-benzoyl-L-lysine by the action of its acylase, and that KT-311 cannot hydrolyse dibenzoyl-L-lysine. The ability of KT-311 to resolve α-N-phenylacetyl-ε-N-benzoyl-DL-lysine was tested. KT-311 metabolizes α-N-phenylacetyl-ε-N-benzoyl-DL-lysine (II) to produce ε-N-benzoyl-L-lysine (III), $[\alpha]_D^{11}$ +19°, and α-N-phenylacetyl-ε-N-benzoyl-D-lysine (IV), $[\alpha]_D^{8}$ −3.3°, in good yield. KT-311 seemingly belongs to the *Pseudomonas* group and has the following characters: Aerobic; rod-shaped; gram negative; yields water-soluble yellowish green pigment that diffuses through the medium.

TABLE. I. Metabolic Activity of KT-311

Phenylacetic acid (I)	+
α-N-Phenylacetyl-ε-N-benzoyl-DL-lysine (II)	+
α-N-Phenylacetyl-ε-N-benzoyl-DL-lysine (II) (without NH_4Cl)	−
α-N-Phenylacetyl-ε-N-benzoyl-D-lysine (IV)	−
ε-N-Benzoyl-DL-lysine	−
Benzoic acid	+
Dibenzoyl-DL-lysine	−

\+ There were luxuriant growths of bacteria within 4 days at 25° on a culture medium (cf. experimental) 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 observed at 25° in 4 days.



*1 Tsuchitoribanaga-machi, Kanazawa (豊浦悦子).

*2 This constitutes a part of a series entitled "Studies on Acylase Activity and Microörganisms" by Y. Kameda. Part XIII: This Bulletin, **7**, 787(1959).

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2) Y. Kameda, E. Toyoura, K. Matsui: This Bulletin, **7**, 702(1959).
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Experimental

Synthesis of α-N-Phenylacetyl-ε-N-benzoyl-DL-lysine (II)—α-N-Phenylacetyl-ε-N-benzoyl-DL-lysine was prepared according to the previously described procedure.[5]

Isolation and Characterization of KT-311—The constituents of the culture medium for isolation of KT-311 were as follows : α-N-Phenylacetyl-ε-N-benzoyl-DL-lysine, 0.2 g.; NH_4Cl, 0.1 g.; K_2HPO_4, 0.1 g.; $MgSO_4 \cdot 7H_2O$, 0.05 g.; 1% $CaCl_2 \cdot 6H_2O$, 2 drops; 1% $FeCl_3 \cdot 6H_2O$, 1 drop; distilled water, 100 cc. pH 7.4~7.6 (adjusted with 10% NaOH).

0.2 g. of a soil sample, taken from a rice field at Takase village in Toyama Prefecture, was inoculated into 10 cc. of the above culture medium and incubated at 25° for 3~6 days. If 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.

This newly isolated soil bacteria was designated KT-311 and its metabolic activity is shown in Table I.

Resolution of α-N-Phenylacetyl-ε-N-benzoyl-DL-lysine (II) by the Metabolism of KT-311—100 cc. of the above sterilized medium containing 1.8 g. of α-N-phenylacetyl-ε-N-benzoyl-DL-lysine in 200-cc. Erlenmeyer flask was seeded with a loop of KT-311 and incubated at 25° for 5 days. The culture thus obtained was heated at 80° for several min. and centrifuged for 10 min. at 8,000*g* to remove the insoluble mass. The supernatant was concentrated *in vacuo* to a small volume, acidified with HCl to pH 1.0, and extracted with AcOEt. The aqueous layer was evaporated *in vacuo* to dryness. The residue was dissolved in ca. 5 cc. of water, neutralized with conc. NH_4OH, and the resulting precipitate was collected by suction. Recrystallization from water gave 0.33 g.(52.8%) of ε-N-benzoyl-L-lysine (III) as colorless leaves, m.p. 270~272°(decomp.); $[\alpha]_D^{11}$ +19°(c=2, 5*N* HCl). *Anal.* Calcd. for $C_{13}H_{18}O_3N_2$: C, 62.38; H, 7.25; N, 11.19. Found: C, 62.25; H, 7.32; N, 11.31.

The AcOEt layer was evaporated *in vacuo* to dryness, the residue was washed several times with petr. ether to remove free phenylacetic acid, and recrystallized from acetone-benzene to 0.75 g. (83.3%) of α-N-phenylacetyl-ε-N-benzoyl-D-lysine (IV) as colorless leaves, m.p. 134~135°; $[\alpha]_D^8$ −3.3° (c=3, EtOH). *Anal.* Calcd. for $C_{21}H_{24}O_4N_2$: C, 68.46; H, 6.57; N, 7.60. Found : C, 68.77; H, 7.09; N, 7.63.

Summary

A certain strain (KT-311) isolated from soil had the metabolic activity shown in Table I and could metabolize α-N-phenylacetyl-ε-N-benzoyl-DL-lysine to produce ε-N-benzoyl-L-lysine and α-N-phenylacetyl-ε-N-benzoyl-D-lysine in good yield. KT-311 could hydrolyse phenylacetyl derivative but not benzoyl derivative of ε-N-benzoyl-L-lysine. KT-311 seemingly belongs to the *Pseudomonas* group.

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