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126. Yukio Kameda, Etsuko Toyoura, and Katsuhiko Matsui : Studies on Acylase Activity and Microörganisms. XI.*1 A New Method for resolving DL-Amino Acids by Metabolism of Soil Bacteria.

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In a previous communication[1] it was reported that one strain (KT-231) of soil bacteria metabolized benzoyl derivatives of ε-N-benzoyl-DL-lysine, DL-phenylalanine, and DL-leucine to yield L-amino acids and the corresponding benzoyl-D-amino acids, and that by the metabolism of a strain (KT-233) of soil bacteria, L-threonine and N-benzoyl-D-threonine were obtained from N-benzoyl-DL-threonine. The present paper gives details and further developments.

While engaged in the study of metabolism and hydrolysis by soil bacteria of benzoic acid derivatives, amino acids, and acylated amino acids,[2~9] it was noticed that soil bacteria KT-230, KT-231, KT-232, and KT-233 have metabolic activities shown in Table I.

Table I.
Metabolic Activitiy of Soil Bacteria KT-230, KT-231, KT-232, and KT-233

	KT-230	KT-231	KT-232	KT-233
Benzoic acid (I)	+	+	+	+
Dibenzoyl-DL-lysine (II)	+	+	+	+
Dibenzoyl-DL-lysine (without NH_4Cl)	−	−	−	−
Dibenzoyl-D-lysine (IV)	−	−	−	−
ε-N-Benzoyl-DL-lysine	−	−	−	−
Benzoyl-DL-methionine (V)	+	+	+	+
Benzoyl-DL-methionine (without NH_4Cl)	−	−	−	−
Benzoyl-D-methionine (VII)	−	−	−	−
DL-Methionine	−	−	−	−

\+ 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 observed at 25° in 4 days.

The constituents of the culture medium used in the experiments are as follows : 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; organic substance to be tested, 0.2 g.; distilled water, 100 cc.; pH 7.4~7.6 (adjusted with 10% NaOH).

From Table I, it is to be understood that KT-230, KT-231, KT-232, and KT-233 can utilize benzoic acid, which was produced from dibenzoyl-L-lysine or benzoyl-L-methionine by the action of their acylases, as the sole source of carbon. At the outset the ability of KT-231 to resolve dibenzoyl-DL-lysine was tested as follows : KT-231 was grown at 25° in the culture medium mentioned above containing dibenzoyl-DL-lysine (II). After 3 days, ε-N-benzoyl-L-lysine (III), $[\alpha]_D^{20}$ +19°, and dibenzoyl-D-lysine (IV), $[\alpha]_D^{20}$ +7.5°, were

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obtained in good yield and then a small amount of benzoic acid was obtained. In the same way, it was confirmed that KT-230, KT-232, and KT-233 as well as KT-231 metabolized dibenzoyl-DL-lysine (II) to produce ε-N-benzoyl-L-lysine (III) and dibenzoyl-D-lysine (IV) in good yield.

The ability of KT-230, KT-231, KT-232, and KT-233 was tested to resolve benzoyl-DL-methionine (V) by the same method: KT-230 and KT-231 metabolized benzoyl-DL-methionine (V) to produce L-methionine (VI), $[\alpha]_D^{20}$ +23°, and benzoyl-D-methionine (VII), $[\alpha]_D^{20}$ +19°, but KT-232 and KT-233 produced DL-methionine and benzoyl-D-methionine (VII). It was demonstrated that cell-free extract of KT-232 or bacterial mass of KT-233 could racemize L-methionine to yield DL-methionine. It seems that KT-232 and KT-233 have an enzyme capable of racemizing L-methionine produced from benzoyl-L-methionine.

Subsequently, the metabolic activities of KT-230, KT-231, KT-232, and KT-233 were tested on phenylalanine, leucine, threonine, and their benzoyl derivatives (Table II).

Table II.
Metabolic Activity of Soil Bacteria KT-230, KT-231, KT-232, and KT-233

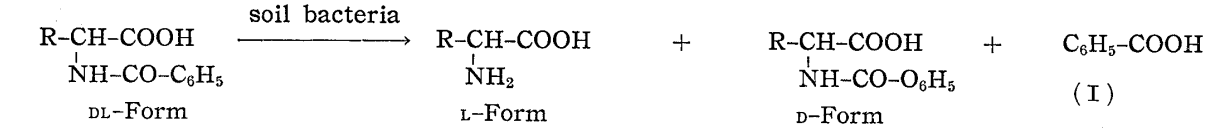
	KT-230	KT-231	KT-232	KT-233
Benzoic Acid (I)	+	+	+	+
Benzoyl-DL-phenylalanine (VIII)	+	+	+	+
Benzoyl-DL-phenylalanine (without NH_4Cl)	−	−	+	+
Benzoyl-D-phenylalanine (X)	−	−	−	−
DL-Phenylalanine	−	−	+	+
Benzoyl-DL-leucine (XI)	+	+	+	+
Benzoyl-DL-leucine (without NH_4Cl)	−	−	+	+
Benzoyl-D-leucine (XIII)	−	−	−	−
DL-Leucine	−	−	+	+
Benzoyl-DL-threonine (XIV)	−	−	−	+
Benzoyl-DL-threonine (without NH_4Cl)	−	−	−	−
Benzoyl-D-threonine (XVI)	−	−	−	−
DL-Threonine	−	−	−	−

+ 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 observed at 25° in 4 days.

From Table II, it is to be understood that benzoyl-DL-phenylalanine (VIII) and benzoyl-DL-leucine (XI) can be resolved by the metabolism of KT-230 and KT-231, but not KT-232 or KT-233, and that benzoyl-DL-threonine (XIV) can be resolved by KT-233, but not by KT-230, KT-231, or KT-232. KT-230 or KT-231 metabolized benzoyl-DL-phenylalanine (VIII) and benzoyl-DL-leucine (XI) to yield the following compounds respectively: L-phenylalanine (IX), $[\alpha]_D^{18}$ −35°, benzoyl-D-phenylalanine (X), $[\alpha]_D^{18}$ −15°, L-leucine (XII), $[\alpha]_D^{18}$ +15°, benzoyl-D-leucine (XIII), $[\alpha]_D^{18}$ +9.5°. By the metabolism of KT-233, L-threonine (XV), $[\alpha]_D^{20}$ −28°, and N-benzoyl-D-threonine (XVI), $[\alpha]_D^{20}$ −25°, were obtained from N-benzoyl-DL-threonine in good yield.

Additionally it has been observed that butyroyl derivatives of ε-N-benzoyl-DL-lysine could be metabolized by KT-232 to produce ε-N-benzoyl-L-lysine (III), $[\alpha]_D^{15}$ +19°, and α-N-butyroyl-ε-N-benzoyl-D-lysine, $[\alpha]_D^{15}$ 0°, which was hydrolyzed by hydrochloric acid to yield D-lysine monohydrochloride, $[\alpha]_D^{15}$ −21°.

KT-230, KT-231, KT-232, and KT-233 were isolated from the soil by using a synthetic culture medium containing dibenzoyl-DL-lysine as the sole source of carbon, and ammonia as the sole source of nitrogen. They seemed to be coccobacillus and have the metabolic activity shown in Tables I and II. KT-230 differs from KT-231 in producing a slimy growth. Morphological studies will be reported elsewhere.



DL-Form	L-Form	D-Form
$R=C_6H_5CONH(CH_2)_4-$ (II)	$R=C_6H_5CONH(CH_2)_4-$ (III)	$R=C_6H_5CONH(CH_2)_4-$ (IV)
$R=CH_3-S-CH_2CH_2-$ (V)	$R=CH_3-S-CH_2CH_2-$ (VI)	$R=CH_3-S-CH_2CH_2-$ (VII)
$R=C_6H_5CH_2-$ (VIII)	$R=C_6H_5CH_2-$ (IX)	$R=C_6H_5CH_2-$ (X)
$R=(CH_3)_2CH-CH_2-$ (XI)	$R=(CH_3)_2CH-CH_2-$ (XII)	$R=(CH_3)_2CH-CH_2-$ (XIII)
$R=CH_3-CH(OH)-$ (XIV)	$R=CH_3-CH(OH)-$ (XV)	$R=CH_3-CH(OH)-$ (XVI)

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Experimental

Preparation of Substrates—Dibenzoyl-DL-lysine, benzoyl-DL-methionine, benzoyl-DL-phenylalanine, benzoyl-DL-leucine, and benzoyl-DL-threonine were prepared according to the procedure described previously.[7] α-N-Butyroyl-ε-N-benzoyl-DL-lysine was prepared as follows: To a solution of ε-N-benzoyl-DL-lysine (10 g.) dissolved in *N* NaOH (100 cc.), butyroyl chloride (4.6 g.) was added under stirring at 5~10° and stirred additionally for 1 hr. at room temperature. The reaction mixture was acidified with HCl and allowed to stand overnight in a refrigerator. The separated crude α-N-butyroyl-ε-N-benzoyl-DL-lysine was collected and recrystallization from AcOEt gave colorless crystals, m.p. 142~143°; yield, 7 g. (54.6%). *Anal.* Calcd. for $C_{17}H_{24}O_4N_2$: C, 63.73; H, 7.55; N, 8.74. Found: C, 63.91; H, 7.52; N, 8.88.

Isolation and Characterization of Soil Bacteria KT-230, KT-231, KT-232, and KT-233—The constituents of the culture medium for isolation of KT-230, KT-231, KT-232, and KT-233 were as follows: Dibenzoyl-DL-lysine, 4.4 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).

0.2~0.5 g. of a soil sample, taken from the herbal garden of this university (KT-230 and KT-231), or a refuse heap of Rokujo village in Takamatsu city (KT-232 and KT-233), was inoculated into 10 cc. of the above culture medium and incubated at 25° for 2~6 days. If luxuriant growth of bacteria occurred and sometimes crystals separated, a loop of the culture fluid was transferred to a new culture medium having the same constituents. Such transplantation was repeated at least twice. The bacterial suspension of the last generation was then planted in an agar medium, containing 1.5% agar in the above culture medium, or 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. KT-230, KT-231, KT-232, and KT-233 seemed to be coccobacillus and could be cultivated successively in various synthetic media containing benzoyl-amino acid (Tables I and II). KT-230 differs from KT-231 in producing slimy growth.

Resolution of α,ε-Di-N-benzoyl-DL-lysine (II) by the Metabolism of KT-230, KT-231, KT-232, or KT-233—i) KT-231 was inoculated into 100 cc. of the culture medium mentioned above containing 1.77 g. of α,ε-di-N-benzoyl-DL-lysine and incubated at 25° for 3 days. The culture medium was adjusted to pH 4.5 with AcOH, evaporated *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 10 cc. of H_2O, neutralized with conc. NH_4OH, and the resulting precipitate was collected by suction. Recrystallization from H_2O gave 0.51 g. (81.7%) of ε-N-benzoyl-L-lysine (III) as colorless leaves, m.p. 270~272°(decomp.); $[\alpha]_D^{20}$ +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, 62.51; H, 7.32; N, 11.25. (III) was characterized as its benzoate, m.p. 146°, $[\alpha]_D^{20}$ −7.5°(c=2, EtOH), obtained in 73.5% yield after recrystallization from Me_2CO and benzene. *Anal.* Calcd. for $C_{20}H_{22}O_4N_2$: C, 67.78; H, 6.26; N, 7.91. Found: C, 67.96; H, 6.56; N, 7.83.

The AcOEt layer was evaporated *in vacuo* to dryness. The residue was washed several times with petr. ether to remove BzOH, and recrystallized from Me_2CO and benzene to 0.70 g. (79.2%) of α,ε-di-N-benzoyl-D-lysine (IV), as colorless plates, m.p. 146~147°; $[\alpha]_D^{20}$ +7.5°(c=2, EtOH). *Anal.* Calcd. for $C_{20}H_{22}O_4N_2$: C, 67.78; H, 6.26; N, 7.91. Found: C, 67.81; H, 6.42; N, 7.97.

From the petr. ether, 0.03 g. (9.8%) of BzOH, m.p. 118~120°, was obtained.

ii) KT-230 was grown at 25° for 5 days in 100 cc. of the above medium containing 1.77 g. of dibenzoyl-DL-lysine (II). 0.375 g. (60%) of ε-N-benzoyl-L-lysine (III), 0.78 g. (88%) of α,ε-di-N-benzoyl-D-lysine (IV), and 0.02 g. (6.6%) of BzOH were obtained.

iii) KT-232 was grown at 25° for 5 days in 100 cc. of the above medium containing 1.77 g. of

dibenzoyl-DL-lysine (II). 0.39 g. (62.5%) of ε-N-benzoyl-L-lysine (III), 0.78 g. (88%) of α,ε-di-N-benzoyl-D-lysine (IV), and 0.02 g. (6.6%) of BzOH were obtained.

iv) KT-233 was grown at 25° for 5 days in 100 cc. of the above medium containing 1.77 g-. of dibenzoyl-DL-lysine (II). 0.30 g. (48%) of ε-N-benzoyl-L-lysine (III), 0.79g. (89.3%) of α,ε-Di-N-benzoyl-D-lysine (IV), and 0.02 g. (6.6%) of BzOH were obtained.

Resolution of Benzoyl-DL-methionine (V) by the Metabolism of KT-230, KT-231, KT-232, or KT-233—i) KT-231 was inoculated into 100 cc. of the culture medium mentioned above containing 1.27 g. of benzoyl-DL-methionine (V) and incubated at 25° for 5 days. The culture medium was adjusted to pH 4.5 with AcOH, evaporated *in vacuo* to dryness. The residue was treated with an excess of EtOH. After a few hr. of standing at ca. 5°, the precipitate was filtered by suction, washed several times with hot EtOH, and recrystallized from H_2O and EtOH to 0.315 g. (84.5%) of L-methionine (VI) as colorless plates, m.p. 276~278°(decomp.); $[\alpha]_D^{20}$ +23°(c=2, 5*N* HCl). *Anal.* Calcd. for $C_5H_{11}O_2NS$: C, 40.26; H, 7.43; N, 9.39. Found: C, 40.38; H, 7.61; N, 9.45.

(VI) was characterized as its benzoate, m.p. 95~97°, $[\alpha]_D^{20}$ −19.5°(c=2, EtOH), obtained in 50% yield after recrystallization from H_2O. *Anal.* Calcd. for $C_{12}H_{15}O_3NS$: C, 56.91; H, 5.97; N, 5.53. Found: C, 57.11; H, 6.03; N, 5.19.

The EtOH solution with combined washing from the separation of L-methionine (VI) was evaporated *in vacuo* to dryness. The residue was taken up in a minimum amount of cold water, brought to pH 1.0 by addition of HCl, and extracted with AcOEt. The extract was evaporated *in vacuo* to dryness. The residue was washed several times with petr. ether to remove BzOH and recrystallized from H_2O to 0.43 g. (67.7%) of benzoyl-D-methionine (VII) as white needles, m.p. 95~97°, $[\alpha]_D^{20}$ +19.5°(c=2, EtOH). *Anal.* Calcd. for $C_{12}H_{15}O_3NS$: C, 56.91; H, 5.97; N, 5.53. Found: C, 57.14; H, 5.99; N, 5.23.

From the petr. ether, 0.02 g. (6.6%) of BzOH, m.p. 118~120°, was obtained.

ii) KT-230 was grown at 25° for 6 days in 100 cc. of the above medium containing 1.27 g. of benzoyl-DL-methionine (V). 0.18 g. (48.1%) of L-methionine (VI), 0.30 g. (47.2%) of benzoyl-D-methionine (VII), and 0.01 g. (3.3%) of BzOH were obtained.

iii) KT-232 was grown at 25° for 5 days in 100 cc. of the above medium containing 1.27 g. of benzoyl-DL-methionine (V). 0.31 g. (83%) of DL-methionine, m.p. 270° (decomp.), $[\alpha]_D^{20}$ 0°(c=2, 5*N* HCl), 0.45 g. (71%) of benzoyl-D-methionine (VII), m.p. 95~97°; $[\alpha]_D^{20}$ +19.5°(c=2, EtOH), and 0.01 g. (3.3%) of BzOH were obtained. These results suggested that racemization of L-methionine was due to KT-232.

iv) KT-233 was grown at 25° for 5 days in 100 cc. of the above medium containing 1.27 g. of benzoyl-DL-methionine (V). 0.32 g. (85.8%) of DL-methionine, m.p. 270°(decomp.), $[\alpha]_D^{20}$ 0°(c=2, 5*N* HCl); 0.45 g. (71%) of benzoyl-D-methionine (VII), m.p. 95~97°; $[\alpha]_D^{20}$ +19.5°(c=2, EtOH), and 0.01 g. (3.3%) of BzOH were obtained. These results suggested that racemization of L-methionine was due to KT-233.

Racemization of L-Methionine by Cell-free Extract of KT-232—KT-232 was grown in 2 L. of bouillon medium (pH 7.2) at 25° for 3 days and the cells were harvested by centrifugation (3,000*g*, 20 min.) and washed with distilled water. The yield of cells, in wet weight, was approximately 20 g. The cells, after having been ground with alumina, were extracted with 50 cc. of distilled water. The alumina and the unruptured cells were removed by centrifugation (3,000*g*, 20 min.) and the cell walls were removed by centrifugation (25,000*g*, 20 min.). Approximately 45 cc. of supernatant solution (cell-free extract of KT-232) was obtained.

A solution of 0.5 g. of L-methionine dissolved in 33 cc. of water was treated with *N* NaOH to pH 7.8. To this aqueous solution, 15 cc. of cell-free extract of KT-232 was added and the mixture was incubated at 37° with a few drops of toluene for 24 hr. The incubation mixture was then adjusted to pH 4.5 with AcOH and the resulting precipitate was removed by filtration. The filtrate was evaporated *in vacuo* to dryness. The residue was recrystallized from H_2O and EtOH to 0.40 g. (80%) of DL-methionine, m.p. 270°(decomp.); $[\alpha]_D^{20}$ 0°(c=2, 5*N* HCl). *Anal.* Calcd. for $C_5H_{11}O_2NS$: C, 40.26; H, 7.43; N, 9.39. Found: C, 40.48; H, 7.56; N, 9.18.

Racemization of L-methionine did not occur with heat-killed, cell-free extract of KT-232.

Racemization of L-Methionine by KT-233 Bacterial Mass with Toluene—In the same manner as described above, KT-233 was grown and the cells were harvested. To 33 cc. of 0.1*M* L-methionine solution (pH 7.8), 5 g. (wet weight) of KT-233 bacterial mass was added and the mixture was incubated at 37° for 24 hr. 0.3 g. (60%) of DL-methionine, m.p. 270°(decomp.); $[\alpha]_D^{20}$ 0°(c=2, 5*N* HCl), was obtained.

Resolution of Benzoyl-DL-phenylalanine (VIII) by the Metabolism of KT-231 or KT-230—i) KT-231 was inoculated into 100 cc. of the culture medium mentioned above containing 1.35 g. of benzoyl-DL-phenylalanine (VIII) and incubated at 25° for 5 days. The culture medium was adjusted to pH 4.5 with AcOH, evaporated *in vacuo* to dryness, and the residue was treated with an excess of EtOH. After a few hr. of standing at ca. 5°, the precipitate was collected by suction, washed with EtOH,

and recrystallized from 70% EtOH. 0.33 g. (80%) of L-phenylalanine (IX) was obtained as colorless plates, m.p. 310~312°(decomp.), $[\alpha]_D^{18}$ −35°(c=2, H_2O). *Anal.* Calcd. for $C_9H_{11}O_2N$: C, 65.44; H, 6.71; N, 8.48. Found : C, 65.65; H, 6.69; N, 8.53.

(IX) was characterized as its benzoate, m.p. 139~140°, $[\alpha]_D^{18}$ +15°(c=2, *N* NaOH), obtained in 69% yield after recrystallization from H_2O. *Anal.* Calcd. for $C_{16}H_{15}O_3N$: C, 71.36; H, 5.61; N, 5.20. Found : C, 71.12; H, 5.78; N, 5.39.

The EtOH solution with combined washing from the separation of L-phenylalanine was evaporated *in vacuo* to dryness. The residue was taken up in the minimum amount of cold water, brought to pH 1.0 by addition of HCl, and extracted with AcOEt. The extract was evaporated *in vacuo* to dryness, the residue was washed several times with petr. ether to remove BzOH, and recystallized from H_2O to 0.30 g. (44.5%) of benzoyl-D-phenylalanine as colorless needles, m.p. 139~140°, $[\alpha]_D^{18}$ −15° (c=2, *N* NaOH). *Anal.* Calcd. for $C_{16}H_{15}O_3N$: C, 71.36; H, 5.61; N, 5.20. Found : C, 71.16; H, 5.67; N, 5.45.

ii) KT-230 was grown at 25° for 5 days in 100 cc. of the above medium containing 1.35 g. of benzoyl-DL-phenylalanine (VIII). 0.28 g. (68%) of L-phenylalanine (IX), 0.20 g. (29.6%) of benzoyl-D-phenylalanine (X), and 0.02 g. (6.6%) of BzOH were obtained.

Resolution of Benzoyl-DL-leucine (XI) by the Metabolism of KT-231 or KT-230—i) KT-231 was inoculated into 100 cc. of the culture medium mentioned above containing 1.18 g. of benzoyl-DL-leucine (XI) and incubated at 25° for 5 days. The culture medium was treated according to the resolution procedure of benzoyl-DL-phenylalanine. 0.24 g. (72.8%) of L-leucine (XII) (recrystallized from H_2O and EtOH) was obtained as colorless plates, m.p. 293~295°(decomp.), $[\alpha]_D^{18}$ +15°(c=2, 5*N* HCl). *Anal.* Calcd. for $C_6H_{13}O_2N$: C, 54,94; H, 9.99; N, 10.68. Found : C, 54.75; H, 10.25; N, 10.41.

(XII) was characterized as its benzoate, m.p. 103°, $[\alpha]_D^{15}$ −9.5°(c=2, EtOH), obtained in 68% yield after recrystallization from benzene. *Anal.* Calcd. for $C_{13}H_{17}O_3N$: C, 66.36; H, 7.28; N, 5.95. Found: C, 66.64; H, 7.37; N, 5.84.

0.35 g. (59.3%) of benzoyl-D-leucine (XIII) (recrystallized from benzene) was obtained as colorless prism, m.p. 103°, $[\alpha]_D^{18}$ +9.5°(c=2, EtOH). *Anal.* Calcd. for $C_{13}H_{17}O_3N$: C, 66.36; H, 7.28; N, 5.95. Found : C, 66.49; H, 7.42; N, 5.67.

0.01 g. (3.3%) of BzOH was obtained from petr. ether as colorless plates, m.p. 118~120°.

ii) KT-230 was grown at 25° for 5 days in 100 cc. of the above medium containing 1.18 g. of benzoyl-DL-leucine (XI). 0.235 g. (71.8%) of L-leucine, 0.24 g. (40.7%) of benzoyl-D-leucine, and 0.01 g. (3.3%) of BzOH were obtained.

Resolution of Benzoyl-DL-threonine (XIV) by the Metabolism of KT-233—KT-233 was inoculated into 100 cc. of the culture medium mentioned above containing 1.12 g. of benzoyl-DL-threonine (XIV) and incubated at 25° for 6 days. The culture medium was adjusted to pH 4.5 with AcOH and evaporated *in vacuo* to dryness. The residue was treated with an excess of EtOH. After a few hr. of standing at ca. 5°, the precipitate was collected by suction, washed several times with hot EtOH, and recrystallized from H_2O and EtOH to 0.19 g. (63.4%) of L-threonine (XV) as colorless plates, m.p. 260°(decomp.); $[\alpha]_D^{20}$ −28°(c=2, H_2O). *Anal.* Calcd. for $C_4H_9O_3N$: C, 40.33; H, 7.62; N, 11.76. Found : C, 40.15; H, 7.73; N, 11.50.

(XV) was characterized as its benzoate, m.p. 146°, $[\alpha]_D^{20}$ +25°(c=2, H_2O), obtained in 45% yield after recrystallization from AcOEt and benzene. *Anal.* Calcd. for $C_{11}H_{13}O_4N$: C, 59.18; H, 5.87; N, 6.28. Found : C, 59.30; H, 5.99; N, 6.24.

The EtOH solution was combined with washing from the separation of L-threonine and evaporated *in vacuo* to dryness. The residue was taken up in the minimum amount of cold water and brought to pH 1.0 by addition of HCl. The aqueous mixture was extracted with AcOEt and the extract was evaporated *in vacuo* to dryness. The residue was washed several times with petr. ether to remove BzOH and recrystallized from AcOEt and benzene to 0.39 g. (69.6%) of benzoyl-D-threonine (XVI) as colorless plates, m.p. 146°; $[\alpha]_D^{20}$ −25°(c=2, H_2O). *Anal.* Calcd. for $C_{11}H_{13}O_4N$: C, 59.18; H, 5.87; N, 6.28. Found : C, 58.93; H, 5.59; N, 6.20.

0.01 g. (3.3%) of BzOH was obtained from petr. ether as colorless plates, m.p. 118~120°.

Resolution of α-N-butyroyl-ε-N-benzoyl-DL-lysine by the Metabolism of KT-232—KT-232 was inoculated into 100 cc. of the culture medium mentioned above containing 3.20 g. of α-N-butyroyl-ε-N-benzoyl-DL-lysine and incubated at 25° for 10 days. The culture medium was treated according to the resolution procedure of dibenzoyl-DL-lysine and 0.84 g. (67.2%) of ε-N-benzoyl-L-lysine was obtained and recrystallized from H_2O as colorless leaves, m.p. 270~272°(decomp.); $[\alpha]_D^{15}$ +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, 62.49; H, 7.38; N, 11.23.

0.90 g. (56.2%) of α-N-butyroyl-ε-N-benzoyl-D-lysine (recrystallized from AcOEt) was obtained as colorless crystals, m.p. 110~112°; $[\alpha]_D^{15}$ 0°(c=2, EtOH). *Anal.* Calcd. for $C_{17}H_{24}O_4N_2$: C, 63.73; H, 7.55; N, 8.74. Found : C, 63.61; H, 7.78; N, 8.87.

α-N-butyroyl-ε-N-benzoyl-D-lysine (0.5 g.) was refluxed with 6*N* HCl (10 cc.) for 6 hr. After being chilled, the separated benzoic and butyric acids were extracted with ether. The aqueous layer

was evaporated *in vacuo*, the residue was taken up in EtOH, and the EtOH solution was neutralized with pyridine. The resulting precipitate was collected by suction and recrystallized from H_2O and EtOH to 0.15 g. (52.8%) of D-lysine monohydrochloride as colorless crystals, m.p. 256°(decomp.); $[\alpha]_D^{15}$ −21°(c=2, 5*N* HCl).

α-N-Butyroyl-ε-N-benzoyl-L-lysine was prepared from ε-N-benzoyl-L-lysine and butyroyl chloride in 50% yield as colorless crystals, m.p. 110~112°, $[\alpha]_D^{15}$ 0°(c=2, EtOH). *Anal.* Calcd. for $C_{17}H_{24}O_4N_2$: C, 63.73; H, 7.55; N, 8.74. Found : C, 63.93; H, 7.81; N, 8.51.

Summary

Four strains (KT-230, KT-231, KT-232, and KT-233) of soil bacteria were isolated and their metabolic activities were tested on ε-N-benzoyl-lysine, methionine, phenylalanine, leucine, threonine, and their benzoyl derivatives (Tables I and II). KT-230, KT-231, KT-232, and KT-233 metabolized dibenzoyl-DL-lysine to produce ε-N-benzoyl-L-lysine and dibenzoyl-D-lysine respectively in good yield. KT-230 and KT-231 metabolized benzoyl-DL-methionine to produce L-methionine and benzoyl-D-methionine, but KT-232 and KT-233 produced DL-methionine instead of L-methionine. It was demonstrated that cell-free extract of KT-232 or bacterial mass of KT-233 could racemize L-methionine to yield -DL-methionine. KT-230 and KT-231 metabolized benzoyl-DL-phenylalanine and benzoyl-DL-leucine to yield L-phenylalanine, benzoyl-D-phenylalanine, L-leucine, and benzoyl-D-leucine. By the metabolism of KT-233 L-threonine and N-benzoyl-D-threonine were obtained from N-benzoyl-DL-threonine in a good yield. α-N-Butyroyl-ε-N-benzoyl-DL-lysine was metabolized by KT-232 to yield ε-N-benzoyl-L-lysine and α-N-butyroyl-ε-N-benzoyl-D-lysine.

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