Note

Microbial Degradation of α -Picolinic Acid, 2-Pyridinecarboxylic AcidMasahiro OHSUGI, Yasuko INOUE,
Keiko TAKAMI and Mayumi NAMIKAWADepartment of Domestic Science,
Mukogawa Women's University,
Nishinomiya 663, Japan

Received October 9, 1980

Pyridine compounds have been found as coenzymes containing nicotinamide and naturally occurring substances such as nicotine. Recently, extensive studies on the quinolinic acid metabolism in the biosynthesis of NAD in bacteria have been reported by Iwai and his co-workers.¹⁻⁵⁾ On the other hand, α -picolinic acid (2-pyridinecarboxylic acid) has been isolated from the culture fluid of *Pyricularia oryzae* as a substance inhibiting the growth of rice plants.^{6,7)} Several reports on the microbial assimilation of α -picolinic acid by *Aerococcus* and *Rhodotorula*,⁸⁾ a Gram-negative rod,⁹⁾ *Arthrobacter*¹⁰⁾ and *Bacillus*¹¹⁾ have been published. Although 6-hydroxypicolinic acid (6-HPA) has been shown as a first oxidation product of α -picolinic acid,⁸⁻¹⁰⁾ little is known of its other metabolites in microorganisms.

In the present study, the isolation of bacteria utilizing α -picolinic acid as sole carbon source and the formation of desthiobiotin from α -picolinic acid are investigated.

We attempted to isolate α -picolinate-assimilating bacteria from soil. The medium contained α -picolinate 0.4%, $(\text{NH}_4)_2\text{SO}_4$ 0.2%, K_2HPO_4 0.1%, KH_2PO_4 0.1%, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.05% and yeast extract 0.01%. Initial pH of the culture fluid was adjusted to 7.0. To a test tube containing 4 ml of the medium was added one loopful of soil. The test tubes were incubated for three to seven days at 28°C with continuous shaking (130 rpm). About twenty strains were isolated from five hundred soil samples by repeating the enrichment culture technique. Of the several pyridine compounds tested, many of the strains isolated showed good growth in a medium containing nicotinate but moderate in α -picolinate. It was remarkable that the strain P-9-1 isolated utilized only α -picolinate among the pyridine compounds tested. This strain could not utilize nicotinate, isonicotinate, quinolinate and dipicolinate. The formation of 6-HPA from α -picolinate in the culture broth during cultivation was checked by estimating the 310 nm absorption substance (6-HPA). Although about 70% of α -picolinate (determined by absorption at 260 nm) added was decomposed during 3 days of cultivation, no formation of 6-HPA was observed. Other degradation products accumulated in the culture fluid were also deter-

mined by gas-liquid chromatography. As shown in Fig. 1, dicarboxylic acids of 2, 5, 6, 7 and 9 carbon chain were formed by the degradation of α -picolinate during 2 days of cultivation. It has been suggested that the formation of glutaric acid (C_5), pimelic acid (C_7) and azelaic acid (C_9)

FIG. 1. Gas-liquid Chromatography of Culture Fluid of Strain P-9-1.

Strain P-9-1 was grown at 28°C for 2 days in 100 ml of the medium described in the text in a 500-ml flask. After the growth, the cultures were centrifuged to remove the cells. The supernatant was concentrated to a small volume *in vacuo* and the organic acids formed were extracted twice with 10 ml of ether. The extract was dehydrated with anhydrous Na_2SO_4 , evaporated to about one tenth volume, and methylated with BF_3 -methanol reagent.¹⁷⁾

The conditions of gas chromatography were as follows: instrument, Shimadzu GC-4BP (FID); column, a glass column (3 mm i.d. $\times$ 2.0 m) packed with 10% DEGS on Shimalite W (60~80 mesh); column temp., initial 30°C, final 200°C; programming rate, 5°C/min; injection and detector temp., 240°C; carrier gas, nitrogen at 60 ml/min; hydrogen, 50 ml/min; air, 800 ml/min; sensitivity $\times$ range, $10^2 \times 64$ (1.6×10^{-9} ampere in full scale). Each peak was identified as shown in the figure: A, α -picolinic acid; C₂, oxalic acid; C₅, glutaric acid; C₆, adipic acid; C₇, pimelic acid and C₉, azelaic acid.

FIG. 2. Paper and Column Chromatography of Biotin-vitamins Synthesized during Cultivation of Strain P-9-1.

Paper chromatography: Solvent for development was *n*-butanol-acetic acid- H_2O (12:3:5, v/v).

Column chromatography: Dowex 1 $\times$ 2, formate type, 1.0 $\times$ 43 cm, 30 ml/hr.

A, authentic desthiobiotin; B, authentic biotin; S, sample of the active fraction eluted with formic acid.

might account for the formation of biotin-vitamers.^{12,13)} Next the formation of biotin-vitamers was clarified. The microorganisms were grown at 28°C for 6 days with continuous shaking in a 2-liter flask containing 400 ml of the medium described above. Isolation and identification of the biotin-vitamers formed were carried out by the paper and column chromatographic methods.¹⁴⁾ The biotin-vitamers were quantitatively determined by the biological assay of *Lactobacillus arabinosus* and *Saccharomyces cerevisiae*.^{15,16)} As shown in Fig. 2, the active fraction of biotin-vitamer was obtained by elution with formic acid. From the results of biological activity, bioautographic behavior and elution pattern of the biotin-vitamers on paper and column chromatography, the biotin-vitamer of the eluate was identical with authentic desthiobiotin. Furthermore, the stimulating effect of pimelic acid on the desthiobiotin formation during cultivation of this organism was found. However, the participation of glutaric acid on the biosynthesis of desthiobiotin should be investigated in the future. α -Picolinate assimilating bacteria strain P-9-1 is considered to be a *Alcaligenes* sp. because it: (1) is Gram-negative, coccoid rods, and non-motile; (2) does not produce special pigments, and also does not produce nitrites from nitrates; (3) does not produce acids oxidatively from carbohydrates.

REFERENCES

- 1) K. Iwai, K. Shibata, H. Taguchi and T. Itakura, *Agric. Biol. Chem.*, **42**, 2095 (1978).
- 2) K. Iwai, K. Shibata, H. Taguchi and T. Itakura, *Agric. Biol. Chem.*, **43**, 345 (1979).
- 3) K. Kouno, T. Kumamoto, H. Iwaki and K. Iwai, *J. Gen. Appl. Microbiol.*, **25**, 279 (1979).
- 4) K. Shibata and K. Iwai, *Agric. Biol. Chem.*, **44**, 119 (1980).
- 5) K. Shibata and K. Iwai, *Agric. Biol. Chem.*, **44**, 287 (1980).
- 6) K. Tamari and J. Kaji, *Nippon Nôgeikagaku Kaishi*, **28**, 254 (1954).
- 7) N. Ogasawara and K. Tamari, *Nippon Nôgeikagaku Kaishi*, **31**, 387 (1957).
- 8) S. Dagley and P.A. Johnson, *Biochim. Biophys. Acta*, **78**, 577 (1963).
- 9) M.N. Naik, R.B. Jackson, J. Stokes and R.J. Swaby, *Soil Biol. Biochem.*, **4**, 313 (1972).
- 10) R.L. Tate and J.C. Ensign, *Can. J. Microbiol.*, **20**, 691 (1974).
- 11) O.P. Shukla, *Indian J. Exp. Biol.*, **13**, 80 (1975).
- 12) K. Ogata, Y. Izumi and Y. Tani, *Agric. Biol. Chem.*, **34**, 1870 (1970); *ibid.*, **37**, 1087 (1973).
- 13) K. Ogata, T. Tochikura, M. Ohsugi and S. Iwahara, *Agric. Biol. Chem.*, **30**, 176 (1966).
- 14) K. Ogata, T. Tochikura, S. Iwahara, K. Ikushima, S. Takasawa, M. Kikuchi and A. Nishimura, *Agric. Biol. Chem.*, **29**, 895 (1965).
- 15) L.D. Wright and H.R. Skeggs, *Proc. Soc. Exp. Biol. Med.*, **56**, 95 (1944).
- 16) E.E. Snell, R.E. Eakin and R.J. Williams, *J. Am. Chem. Soc.*, **62**, 175 (1940).
- 17) W.R. Morrison and L.M. Smith, *J. Lipid Research*, **5**, 600 (1964).