

Studies on Soil Bacteria Capable of Metabolizing Benzoic Acid and Its Related Compounds.

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In this paper we have tried to review the principal results of our research works which have been done in our laboratory for these past five years. For further details, following original papers should be consulted: J. Pharmaceutical Society of Japan, **67**, 1, 41, 76,77; **78**, 118, 119, 120, 127, 171, 172, 213 (1947); **68**, 163 (1948); **69**, 365 (1949) [*cf.* Chemical Abstracts **44**, 1564, 2598 (1950)]

Starting from the investigation on a strain

of soil bacteria which is capable of metabolizing hippuric acid, benzoyl-D, L-alanine, benzoyl-D, L- α -amino-*n*-butyric acid, benzoyl-D, L-valine or benzoyl-D, L-leucine, as the source of carbon and nitrogen, a number of soil bacteria have then been tested in order to determine whether any of them could metabolize benzoic acid and its related compounds as the source of carbon.

General Technical Considerations

1) Basal medium :

The formula for the basal medium :

NH ₄ Cl	0,1g	} After adding 0,2g of the chemical to be tested, the acidity was adjusted to PH 7,0-7,2 with 10% NaOH.
K ₂ HPO ₄	0,1g	
MgSO ₄ · 7H ₂ O	0,05g	
1%CaCl ₂ · 6H ₂ O	2 drops	
1%FeCl ₃ · 6H ₂ O	1 drop	
Distilled water	100 cc.	

The culture medium thus prepared was distributed to sterilized test-tubes, 5-10 cc. for each, and sterilized in a steam sterilizer.

2) Organic compounds added to the basal medium :

- a) Benzoic acid
- b) p-Hydroxybenzoic acid
- c) m-Hydroxybenzoic acid
- d) Salicylic acid
- e) Anisic acid
- f) Piperonylic acid
- g) p-Nitrobenzoic acid
- h) Phenylacetic acid
- i) Cinnamic acid

j) o-Coumaric acid

k) *d,l*-Mandelic acid

3) Method of Cultivation experiments :

0,2g of soil samples taken from different field areas was inoculated into the culture medium and incubated at 25° C for 3-6 days. If luxuriant growth of bacteria has occurred, a roop of the culture fluid was transferred to a new culture medium of the same origine. Such transplantation was repeated at least from three to six times. The bacterial suspension of the last generation was then planted in agar medium, con-

sisted of 1.5% agar and the same culture medium. A series of test-tube experiments was again carried out in order to determine whether the microb isolated

from the agar plate could metabolize the organic compound added to the basal medium.

Results

The data obtained in the experiments carried out, under comparable conditions, with eighteen strains* of soil bacteria from different field areas are presented in Table 1.

Special attention may here be directed to following points:

1) Of 18 strains, 15 were all found seemingly to have a capability of metabolizing benzoic acid as well as p-hydroxybenzoic acid as the source of carbon, and 3 strains, "KT26", "KT31" and "KT33", were capable utilizing p-hydroxybenzoic acid, but not benzoic

Table I.

Comparative Capability for Metabolizing Benzoic Acid and Its Related Compounds by Various Strains of Bacteria from Soil		KT1	KT2	KT3	KT4	KT6	KT7*	KT9	KT13	KT24	KT25*	KT26	KT27	KT28*	KT29	KT30	KT31	KT32*	KT33
Compound added to basal medium	Bacteria isolated from soil																		
	Benzoic Acid	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+
p-Hydroxybenzoic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
m-Hydroxybenzoic	+	-	+	-	-	-	-	-	-	+	-	-	-	-	-	+	+	-	-
Salicylic	-	-	-	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Anisic	-	-	-	-	-	-	-	-	+	+	+	-	+	-	+	-	-	+	-
Piperonylic	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-
p-Nitrobenzoic	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Phenylacetic	+	+	+	+	+	+	+	-	-	+	+	-	-	+	+	+	+	+	+
Cinnamic	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+
o-Coumaric	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-
dl-Mandelic	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-

+ : The bacteria grow very luxuriantly in the culture medium containing the organic compound as a sole source of carbon.

- : No visible growth of bacteria had occurred.

* It was revealed by other experiments that 1) KT 32 is a strong metabolizer of p-methoxycinnamic acid, while KT 25 did not grow in the basal medium containing the same compound, and that 2) KT 7 differs from KT 28 in failing capability of metabolizing either hippuric acid or phenacetic acid as the source of carbon and nitrogen.

* The majority of these bacteria seems to belong to the pseudomonas group.

acid.

2) Attitudes of bacteria against *m*-hydroxybenzoic acid, salicylic acid, anisic acid, piperonylic acid, *p*-nitrobenzoic acid, phenylacetic acid, cinnamic acid, *o*-coumaric acid and *dl*-mandelic acid, are not the same. For example, strain "KT2" differs from strain "KT1" in failing the capability to metabolize *m*-hydroxybenzoic acid. There are only 5 strains, "KT1", "KT3", "KT24", "KT30" and "KT31", which were found to possess a *m*-hydroxybenzoic-acid metabolizing activity. Strain "KT9" contrasted strongly

with any of other strains of soil bacteria in possessing a property to metabolize *p*-nitrobenzoic acid.

Thus, our experiment showed that 1) there occurs in soil bacteria which may luxuriantly grow in a synthetic medium, composed of organic salts only, by utilizing benzoic acid as well as some of its related compounds as the source of carbon, and that 2) there is but a significant difference between bacteria in their capability of metabolizing aromatic acid groups of different structures.

Received Feb. 25, 1951