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ORGANIC ACID PRODUCING MICROORGANISMS IN SOIL OF WEST BENGAL. I BANKURA AND BURDWAN DISTRICT

BY

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Survey of 103 soil samples from two districts of West Bengal shows that more than 38% of the 307 soil isolates have the ability to produce organic acid(s). Soils from Burdwan district are richer in microbial content as well as in the number of potent organic acid producer in comparison to the Bankura district. Most of the potent producers from the two districts are the fungi of genera *Penicillium* and *Aspergillus*, and produce either citric or gluconic acid in the range of 17 to 32 g/l.

INTRODUCTION

Organic acids are widely used in food, plastic, pharmaceuticals and textile industries as well as for numerous other purposes (Prescott and Dunn, 1959 ; Chopra *et al*, 1975). In soil organic acids are produced by various type of organisms (Schueartz *et al*, 1954 ; Sperber, 1958 ; Subramanyam, 1929). Survey of organic acid producing microorganisms in soils of different geographical area has been done by many workers. (Jayaraman and Prasad, 1971 ; Kawatei *et al*, 1969 ; Kawate *et al*, 1976). In the present communication a comparative analysis on the occurrence and distribution of organic acid producing microorganisms in soils of two different districts of West Bengal, observed in course of a brief survey, is reported.

MATERIALS AND METHODS

Isolation of organic acid producing strains : Soil samples were collected from different parts of the district of Bankura and Burdwan and plated on mineral salt agar medium of Doelgar and Prescott (1934) containing 1% calcium

carbonate. After incubation for 3-8 days at 30°C, colonies showing zone of clearance of calcium carbonate around them were picked up on slants. Working cultures were maintained on the mineral salt agar slants of the same medium with 5% glucose as C-source. After good growth the slants were preserved at 4°C and subcultured at intervals.

Cultivation in liquid medium: In order to determine the nature and quantity of organic acid (s) produced, the isolates were cultivated in 50 ml liquid medium in 250 ml Erlenmeyer flasks. The flasks were incubated on a rotary shaker for 3-7 days at 30°C.

Identification of organic acids: The nature of organic acids excreted by the microorganisms was determined by paper chromatography of cell free culture filtrate using following solvent systems : ethanol : methanol : water (9 : 9 : 2, v/v) ; ethanol : methanol : water : formic acid (15 : 15 : 9 : 2, v/v) ; n-butanol : acetic acid : water (4 : 1 : 5, v/v) For detection of spot on the chromatogram 0.05% alcoholic solution of bromophenol blue was used as spraying reagent. Further confirmation about the nature of organic acid was done by using authentic samples of organic acid (s) ran concurrently in the same direction as well as by co-chromatography.

Estimation of organic acids: Organic acids were estimated according to Millis *et al* (1963) by titration of 2 ml portion of culture filtrate against 0·25 N sodium hydroxide solution. That the activity was due to excret organic acid was verified by paper chromatography. Generic identification of the promising strains was done following "A Manual of Soil Fungi" (Gilman, 1967).

RESULT AND DISCUSSION

Screening of 103 soil samples from the two districts involving 307 microoranisms, resulted (Table 1) isolation of 40 microorganisms producing different organic acids. Soils from the district Burdwan appears to be richer in microbial load as well as abundance of organic acid producers. Whereas 27% of microorganisms from Burdwan district produce different organic acid, this value for Bankura district was found to be as low as 9%.

Analysis of the nature of organic acid produced by the positive isolates from the two districts reveals (Table 2) that the isolates produce mostly citric and or gluconic acids. Only 2 isolates from Burdwan district produce itaconic acid together with the citric acid.

Table 1. *Comparative analysis of organic acid producing mic oorganism in soi of district Bankura and Burdwan, W. B.*

District	No. of soil samples tested	No. of micro-organisms tested	No. of organic acid producing strains			
			Fungi	Bacteria	Actinomycetes	Total
Bankura	49	143	12	1	0	13
Burdwan	54	164	21	6	0	27

Table 2. *Organic acid (s) produced by microorganisms from Bankura and Burdwan district, W. B.*

District	No. of gluconic acid producers	No. of citric acid producers	No. producing citric + gluconic	No. producing citric + Itaconic
Bankura	7	5	1	0
Burdwan	21	4	0	2

Table 3. *Yield of organic acid from the promising cultures from Bankura and Burdwan district.*

Source	Organic acid	Index No.	Generic name	Yield (g/1)
Bankura	Citric	53A	*Aspergillus* sp.	25
		53D	*Aspergillus* sp.	30
	Gluconic	28B	*Penicillium* sp.	20
		62A	*Penicillium* sp.	17
Burdwan	Citric	3A	*Aspergillus* sp.	32
		4D	*Penicillium* sp.	25
	Gluconic	5A	*Penicillium* sp.	25
		72B	*Penicillium* sp.	20
		76A	*Penicillium* sp.	25
		92A	*Penicillium* sp.	20
		93A	*Penicillium* sp.	21

In soil of both the districts gluconic acid producers are greater in number than those producing citric acid.

On the basis of the production of single organic acid in considerable amount 11 isolates producing either citric or gluconic acid have been selected from this survey (Table 3). Whereas in soils of Burdwan district both gluconic and citric acid are produced in good amount by *Penicillium* sp. and *Aspergillus* sp., in soils of Bankura district citric acid in good amount is produced by *Aspergillus* alone. The yield of citric and gluconic acid by fungi obtained from the soils of Burdwan district are considerably higher than those obtained from the Bankura district.

This survey, though in a limited scale, reveals that the organic acid producing microorganisms are fairly well spread in soil. Among the microbes the property of organic acid production is, however, restricted within the fungi and eubacteria. Although some bacteria can produce organic acid in small amounts, organic acids are produced in significant amounts by fungi, more precisely by the members of the genus *Penicillium* and *Aspergillus*.

The distribution of organic acid producing microorganisms in soils of 2 districts is a reflection of their soil conditions. The soils in Bankura district is mostly laterite, and well aerated ; hence harboured by microorganisms most of which could completely oxidise the carbohydrate substrate to CO_2 and H_2O. On the other hand the soils from Burdwan district are clayey-loam, with less aeration. Such soil, as one would expect, harbours microorganisms with incomplete oxidation of carbohydrate substrates, as a result they excrete organic acid (s).

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REFERENCES

Chopra, C. L., Qazi, G. N and Gaind, C. N., (1975), Calcium gluconate by submerged fermentation, *Research and Industry*, **20**(1), 1—3.

Doelger, W. P. and Prescott, S. C, (1934). Citric acid fermentation, *Ind. Eng. Chem.* **26**, 1142–1143.

Jayaraman, K. N. and Prasad, N. N, (1971), Production of organic acid by certain Aspergilli, *Curr. Sci.*, **40**(8), 198–199.

Gilman, J. C. (1967), *A Manual of soil fungi*, Oxford and IBH Publishing Company,

Kawate, S. and Fukuo, T. (1976). Isolation of kojic acid producing fnngi, *Kogaku to gijutsu*, 6(8), 29–35.

Kawate, S., Koike, M., Nakamoto, K. (1969). On the isolation of kojic acid producing microorganisms from peru soil, *Kogaku to gijuisn*, **3**(3), 18–25.

Millis, F. N., Trumpy, B. H. and Palmer. B. M. (1963). The effect of lipids on citric acid production by an *Aspergillus niger* mutant, *J. Gen. Microbiology.*, **30**, 365–379.

Prescott, S. C. and Dunn, C. G. (1959). *Industrial microbiology*, McGraw Hill Book Company Inc., 594.

Schwartz, S. M., Varner, J. E. and Martin, W. P. (I954). Separation of organic acids from several dormant and incubated Ohio soils, *Soil Sci. Ann. Proc.*, **18**, 174–177.

Sperber, J. I. (1958), The incidence of apatite solubilising organisms in the rhizosphere and soil, *Austrian J. Agr, Research.* **9**, 777-781.

Subramanyam, V. (1929). Biochemistry of water logged soils. Part III. Decomposition of carbohydrates with special reference to formation of organic acids, *J. Agr. Sci* **19**, 627–648.