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AMINO ACID PRODUCING *NOCARDIA SPP.* FROM SOIL.

BY

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Following the bioautographic technique of Udaka (1959) using a number of different auxotrophic mutants of *E. coli*, two strains of *Nocardia* were isolated, one producing glutamic acid and the other ∝-alanine. The glutamic acid producing strain was identified as *Nocardia ruber* and the ∝-alanine producing strain as *Nocardia citrea*. The two strains were able to grow and produce amino acids in a purely synthetic medium containing glucose, ammonium chloride and mineral salts. The identity of the amino acids in the bacteria free culture filtrate was determined by paper chromatographic analysis using a number of solvent systems as also by chromatography with authentic samples of glutamic acid and ∝-alanine. Further confirmation of the identity of the amino acids was obtained by using 0.2% solution of 1,2 ∝-naphthoquinone 4-sulphonate in 5% sodium carbonate solution as the spraying reagent which gave spots of characteristic colour for glutamic acid and ∝-alanine on the chromatograms.

INTRODUCTION

The excretion of nitrogenous substances by microorganisms was first recognised in the 19th century by Thenard, Pastur, Duclaux and others from their studies on yeasts. In the later half of the 20th century, such studies were greatly accelerated by the development of paper chromatography and other improved analytical techniques. Thus Reindel and Hoppe (1952) in yeasts, Dagley *et al.* (1950) in certain coliform bacteria, Morton and Broadbent (1955) in certain fungi and Perlman and O. Brien (1958) in several species of actinomycetes reported the excretion of amino acids and peptides into the culture media.

Since 1955, Kinoshita *et al.* have made extensive studies on amino acid production by several thousand microorganisms and observed that many mycroorganisms are able to produce amino acids in the culture broth but in amounts so small as to be detected only by paper chromatography. An excellent glutamic acid producing organism *Micrococcus glutamicus* was one of the outsanding strains, isolated by Kinoshita *et al.* (1959), producing glutamic acid at the rate of 30 gm. per litre of the culture filtrate. Following this discovery of Kinoshita, detailed and painstaking screening programmes have been undertaken resulting in the isolation of a good many number of microorganisms producing such large quantities of

amino acids in the medium that they could be industrially utilised for the establishment of large scale fermentations. Among the amino acids obtained by industrial fermentation are glutamic acid and lysine in the U. S. A. and Japan and valine, leucine, isoleucine, tryptophan and certain other essential amino acids in Japan. The fermentative methods for the production of amino acids is preferred because the process is cheaper as compared to other processes and the amino acids so obtained are exclusively in the biologically active L-form and therefore need no resolution before their use. An excellent review on this subject has been made by Dulaney (1967).

During a survey of amino acid producing microorganism present in our soils, several bacteria, fungi and actinomycetes were found to be active, some of them producing appreciable quantities of different amino acids in the culture medium. The present paper reports on a glutamic acid and an ∝-alanine producing organism isolated from soil and contains information on the isolation, characterisation and identification of the producing strains.

Materials And Methods

The bioautographic technique of Udaka (1959) was used for screening of the amino acid producers. The amino acid auxotrophs required for this technique were isolated from *E. coli* K 12 by employing N-methyl-N-nitro:N-nitrosoguanidine as the mutagen, following the method of Ashworth and Kornberg (1969).

Morpological, cultural and biochemical properties of the selected organisms were studied according to the standard methods described in the Manual of Microbiological Methods (1957).

The identity of the amino acids in the bacteria free culture filtrate was confirmed by one and two dimensional paper chromatographic methods using 6 different solvent systems, as also by the characteristic colour of the spots on the chromatograms on spraying them with a 0.2% solution of 1, 2∝-Naphthoquinone 4-sulphonate in 5% sodium carbonate solution.

Results And Discussion

The survey of amino acid producing microorganisms of soil included 245 isolates from 22 soil samples and yieded 26 positive cultures (Chattopadhyay and Banerjee, 1971). Among the positive isolates were two strains of actinomycetes. One strain produced a halo of growth of the glutamic acid auxotroph of *E. coli* and the other produced a similar halo of an ∝-alanine auxotroph around their colonies. The colonies were isolated, brought into pure culture and identified as species of the genus *Nocardia*.

The morphological characteristics of the two strains are presented below in Table 1.

Table 1. *Morphological characteristics of the isolated strains.*

Characters	∝-alanine producing organism	Glutamic acid producing organism
Vegetative characters	Well defined branched mycelium at the early stages of growth, which later on breaks up into rods and cocci. Mycelium 0.5μ—0.7μ in diameter.	Mycelium branched, eventually splits up into rods and cocci, Mycelium 0.8μ—1.2μ broad.
Spores	Absent	Absent
Motility	Absent	Absent
Gram staining	Gram positive	Gram positive
Acid fast staining	Non-acid fast	Non-acid fast

Cultural characteristics of the two strains are presented in Table 2.

Table 2. *Cultural characteristics of the two strains.*

Characters	∝-alanine producing organism	Glutamic acid producing organism
Colony morphology	Smooth, raised, without ærial mycelium, citron yellow coloured.	At first smooth, colourless later pinkish, with ærial mycelium.
Growth in broth	Thin pellicle and a sediment at the bottom, broth turbid.	With a thick pellicle at the top and sediment at the bottom, broth clear.
Agar stab	Best growth at the top.	Best growth at the top.
Growth on potato	Moderate, white to yellowish.	Vigorous, pinkish.
Growth in synthetic medium	Good growth.	Good growth.
Optimum temperature for growth	Optimum growth at 30°-35°C, but also grows at 45°C.	Optimum growth at 30°-35°C but also grows at 50°C.

Routine physiological and biochemical tests were carried out and the results obtained are presented in Table 3.

Table 3. *Physilogical and biochemical properties of the isolated strains.*

Characters	∝-alanine producing organism	Glutamic acid producing organism
Catalase test	+	+
Nitrate reduction	+	+
Indole Production	—	—
Voges/Proskauer test	—	—
Starch hydrolysis	+	+
Gelatin hydrolysis	+	+
Litmus milk	Coagulated and peptonized	Coagulated and peptonized.
Invertase	+	+
Utilisation of citrate as sole carbon source	+	+
Utilisation of phenol, paraffin or fat as C-source	—	—
Fermentation of carbohydrates		
Arabinose	All the different carbon sources tested were utilized for growth but neither acid nor gas were produced in any case.	All the carbon sources tested, except dulcitol were utilized for growth but neither acid nor gas were produced in any case.
Xylose		
Glucose		
Fructose		
Galactose		
Lactose		
Maltose		
Sucrose		
Starch		
Mannitol		
Inositol		
Dulcitol		
Glycerol		

From the properties studied the organisms were assigned to the genus *Nocardia* of the order Actinomycetales. The characteristic disintegretion of the mycelium into rods and coccal units on aging was the most important diagnostic criterion for the generic identification. The glutamic acid producing strain was found to be related to *Nocardia ruber* (Krasilnikov) as described in Bergy's Manual (1948) but differed from it by its formation of a grey brown diffusable pigment in nutrient broth and gelatin, as also by its capacity to hydrolyze starch and liquefy gelatin. The ∝-alanine producing strain was identified as *Nocardia citrea* (Krasilnikov) on the basis of citron yellow colour of its colonies. Both the isolated strains were able to grow and produce amino acids in a purely synthetic medium containing glucose, ammonium chloride and mineral salts. The nature of the amino acids produced by the organisms was detected by paper chromatographic analysis. The amino acids were identified as glutamic acid produced by *N. ruber* and ∝-alanine by *N. citrea*. While *N. citrea* produced ∝-alanine alone, *N. ruber* was found to produce also small quantity of aspartic acid along with glutamic acid.

Accumulation of amino acids by *Nocardia spp.* has been observed also by others. Nakayama and Hiroshi (1969) reported the production of isoleucine by a strain of *Nocardia golberula* (ATCC 21022). In a survey on lysine producing microorganisms undertaken by the Kyowa Fermentation Industries of Japan, it was reported (1969) that a strain of *Nocardia* sp. (ATCC 21337) was active when ethanol was used as a C-source.

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