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Exploitation of the soil isolates for the production of amino acids

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ABSTRACT

Humans can produce only ten of the twenty amino acids and thus the other amino acids must be supplied in the food. The essential amino acids are arginine (required for the young, but not for adults), histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. Plants are able to make all the amino acids, but humans, on the other hand, do not have all the enzymes required for the biosynthesis of all of the amino acids. L-lysine is one such essential amino acid. It is nutritionally essential, which means that it is essential to human health but cannot be manufactured by the body. For this reason, lysine must be obtained from food that we consume. It may be added to food and feed materials to improve the protein quality. However, fermentative methods seem to be most economical and practicable means of producing lysine. The present work deals with the studies carried out to isolate the amino acid, lysine, producing strains of microorganism from the soil samples collected from different topographic fields. The microbial strains isolated were found to be members of actinomycetes by microscopic and biochemical studies. Finally we tried to explore the microbial strains for the production of the antimicrobial activity and its relation with the amino acid production.

Keywords: actinomycetes ,essential amino acid, fermentation, microbial production, ninhydrin

I.INTRODUCTION

Amino acids play central roles both as building blocks of proteins and as intermediates in metabolism. Humans can produce only ten of the 20 amino acids. The others must be supplied in the food. The essential amino acids are required in the diet for healthy life. Plants, of course, are able to make all the amino acids. Humans, on the other hand, do not have all the enzymes required for the biosynthesis of all of the amino acids. L-lysine is such an essential amino acid. It is nutritionally essential for humans , which means that it is essential to human health but cannot be manufactured by the body. For this reason, lysine must be obtained from food to improve the protein quality. However, fermentative methods seem to be most economical and practicable means of producing lysine and many amino acids. Lysine appears to help the body absorb and conserve calcium and it plays an important role in the formation of collagen, a substance important for bones and connective tissues

including skin, tendon, and cartilage. If there is too little lysine in the diet, kidney stones and other health related problems may develop including fatigue, nausea, dizziness, loss of appetite, agitation, bloodshot eyes, slow growth, anemia, and reproductive disorders. Since it helps with the building of muscle protein, it is useful for patients recovering from injuries and recovery after operations.

Few strains isolated from soil was found to produce large amount of the amino acids. For example the bacterium *Bacillus megaterium* SP 14 accumulated the amino acid, lysine showing the yield of 3.56 mg/ml in a broth culture in 96 hours. Fermentation experiments show that 8.0% (w/v) glucose and 4.0% (w/v) ammonium chloride used as sources of carbon and nitrogen, respectively, in a medium/fermenter volume ratio of 25.0%, influenced accumulation of the amino acid. It is anticipated that the isolation, characterization and the study on actinomycetes can be useful in the discovery of antibiotics along with the amino acid production. The present work deals with the studies carried out to isolate the amino acid producing strains especially lysine from different soil samples and optimization of the process to increase the yields of free amino acid, lysine in a culture broth by the isolated strains. Finally we tried to explore the microbial strains for the production of the antimicrobial activity and its relation with the amino acid production.

II.MATERIALS AND METHODS

2.1 Isolation of microorganisms

In nature microbial populations do not segregate themselves by species but exist in a mixture of many other. In the laboratory, these populations can be separated into pure culture. Therefore the pure culture contains only one type of microorganism which is exploited by the researchers for its characteristic need. With this knowledge our first step was to isolate the microorganism from soils of different topographic locations. Four different soil samples from Barrackpore College ground, Home garden, Corn (*Zea mays*) field and Til (*Linum usitatissimum L.*) field were collected. One gram of each soil sample was mixed in 10ml of sterile distilled water in four different sterile test tubes and subjected to shaker for 15-20 mins. Then these test tubes were kept undisturbed for 15-20 mins. to settle down & different dilutions were prepared for each sample. Discrete colonies were observed after spreading & then they were marked and named accordingly.

2.2 Selection Method for the isolates

From the slants the single colonies were picked and allowed to grow in a medium, which has no amino acid or any compound that may release any free amino group, at 37^0 C for 48 hours. The culture broth was then spotted on a TLC paper and sprayed with ninhydrin reagent. Only four isolates out of 20 isolates produced a ninhydrin-positive spot on the paper. They were isolates namely **CG3, M1, M3 & M4.** So the pure culture of these four isolates were streaked on NA slants and stored at 4^0C.

2.3 Identification of the isolates

After selection of the colonies we prepared the pure culture of the microorganisms. Visualization of the microorganisms in the living state is quite difficult, not only because they are minute but also they are

transparent and practically colorless when suspended in aqueous medium. To study their properties and to characterize into specific group, Simple and Gram staining was done in conjunction with light microscopy.

2.4 Seed Culture and Fermentation

For the fermentative production of amino acids by the four isolated strains of microbes, the first step was preparation of seed culture which will be used as inoculum in the fermentation medium.The medium was sterilized and a 2% seed culture (of four isolates namely CG3, M1, M3 & M4) was used to inoculate four different 500 ml conical flasks containing 50 ml of fermentation medium in each. The flasks were then kept on a shaker at a speed of 170 rpm at 37°C in an incubator. After an interval of every 24 hours assay of this fermentation broth was performed.

2.5 Thin Layer Chromatography

A TLC sheet was taken & marked at four spots for sample loading. Each spot was loaded twice with test samples (24hours old fermentation broth of M1,M3,M4 & CG3) with the help of a capillary tube. After drying TLC sheet was subjected to a saturated Glass chamber containing a solvent system (Butanol: Glacial acetic acid: Distilled water in the ratio of 4: 1: 1) for 4 hours. The solvent front was marked immediately before the solvent dries out. The TLC sheet was kept for drying, overnight. Next day the TLC sheet were sprayed with 0.2% ninhydrin reagent & dried in hot air oven for ease of visualization. The same process was repeated for another solvent system namely Propanol: Pyridine: Glacial acetic acid: water in the ratio of 15:10:3:10.

III.RESULTS

1.1 Determination of Gram character

Following the Gram staining procedure it was noted that the M1 & CG3 cultures resemble Actinomycetes group, which takes crystal violet color showing branching and sub-branching of hyphae. It was also seen that M3 is a gram negative rod , which are mainly scattered in arrangement and M4 is Gram positive coccus and they appears in clumps (Table1). During fermentation, growth was observed in the fermentation medium containing M1, M3, M4 and CG3 cultures. In CG3 marked conical flask; yellowish white, ball like growth of the isolates were observed (Fig.1).

1.2 Analysis of Amino acids produced

3.2.1 Preliminary Test - Paper chromatography was employed for the detection of amino acids in the fermentation broth of four different isolates.1ml of each fermentation broth of four different isolates were transferred to four different eppendorfs & they were centrifuged at 3000 rpm for 10 mins. The supernatant different microbial cultures were collected. The pH of the supernatant was noted. This broth was applied to the center of the activated short TLC strip with the help of a fine capillary tube. After drying, each TLC strips were sprayed with 0.2% ninhydrin solution and kept in hot air drier for few minutes. Pinkish purple coloration of the spots was observed in each TLC strip, indicating that the microorganisms were able produce free amino acids (Fig.2).

3.2.2 Thin Layer Chromatography

Thin layer chromatography of some standard amino acids along with the test samples were performed in two different solvent systems namely Propanol: Pyridine: Glacial acetic acid: water in the ratio of 15:10:3:10, and with the solvent system (Butanol: Glacial acetic acid: Distilled water in the ratio of 4: 1: 1) in separate glass chambers.

The four isolates were found to produce more than one amino acid extracellularly in the medium as seen in TLC plates (Fig. 3). The Rf values of all the three spots were calculated (Table 2) and compared with the Rf values of standard amino acids. Among the 3 amino acids produced extracellular by the test organisms (CG3 & M1) grown in various carbon source in the fermentation medium, two spots were found to be similar to known amino acids, lysine and aspartic acid. The 3rd spot remained unidentified as its Rf value did not match with the Rf values of standard amino acids used. The fermentation medium was a non amino acid containing medium or in other words it does not contain any compound which will release free amino group to give ninhydrin positive test. So, it can be easily concluded that the test organisms (M1, M3, M4 & CG3) were able to produce three extracellular amino acids. Two of which may be lysine and aspartic acid along with one unidentified amino acid.

3.3 Assay of antimicrobial activity

The fermentation broth of the isolates CG3 and M1, which were found to be actinomycetes were subjected to antimicrobial assay against a Gram +ve test microorganism. CG3 showed a good antimicrobial activity (Fig.4) while the M1did not exhibit any such activity.

IV.FIGURES AND TABLES

Table1. Determination of Gram character

Isolate Number	Gram character	Morphology
M1	Actinomycetes	Filamentous
M3	Gram -ve	Rods
M4	Gram +ve	Coccus in clumps
CG3	Actinomycetes	filamentous

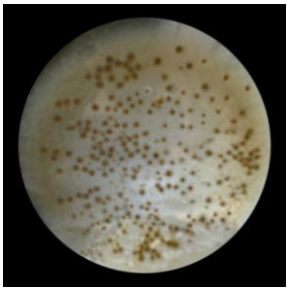

Fig.1 Yellowish white, ball like appearance CG3 at base of conical flask

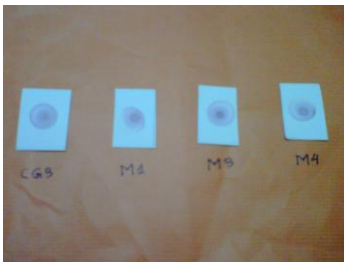

Fig.2 TLC strips with purple spots

Table 2. Rf values of the three spots obtained from Four microbial isolates

Name of the isolate	**Distance travelled by Solvent Front** (in cm)	**No: of spots produced**	**Distance travelled by test sample** (in cm)	**Rf value**
M3	6.6	3	1.3 2.2 3.5	0.196 0.333 0.507
CG3	6.8	3	1.1 2.1 3.7	0.161 0.308 0.544
M4	7	3	1.2 2.3 3.3	0.171 0.328 0.471
M1	6.9	3	1.4 2.2 3.5	0.202 0.318 0.507

Fig.3 TLC sheet of test samples (M1, M3, M4 & CG3)

Fig.4 Zone of inhibition exhibited by CG3 against Gram +ve microorganism.

V.CONCLUSION

Of all the 20 isolates, four isolates were found to give the purple spot with the ninhydrin in their broth, though they were cultured in a medium which was devoid of any amino group. It was also found that it is not a single spot but a mixture of three spots in all the isolates which was confirmed by thin layer chromatography. The two positive spots were confirmed to be lysine and aspartic acid when TLC with standard amino acids was done. However a third spot remained unidentified as it did not match with the available standard amino acids. Two strains namely M1 and CG3 were found to the members of Actinomycetes, while M3 was found to be a Gram -ve and M4 was found to be Gram +ve bacteria. Since actinomycetes are known for the production of antimicrobial substances we explored these two strains further and found that only strain CG3 showed the antimicrobial activity in the fermentation broth, while the strain M1 showed no such property. The biochemical characteristics of the strain CG3 showed that it is catalase positive, urease –ve, can hydrolyse starch and can also reduce nitrate. Starch & Peptone was found to be the best for optimal production of antimicrobial substance. Hence, the actinomycetes CG3 was the most desirable strain which could be exploited for the production of amino acids as well antibiotic, which may aid in human health.

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