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Production of L- methionine by *Bacillus cereus* isolated from different soil ecovars in Owerri, South East Nigeria

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ABSTRACT

A total of 500 bacteria isolated from 20 different soil ecovars were screened for methionine production, three isolates designated as DS-13, AS-9 and RS-16 were recovered. Taxonomic studies revealed that all strains showed good growth and sporulation on most media, utilized various sugars, reduced citrate and was negative for nitrate and urease. Molecular characterization conducted showed that the isolates were different strains of Bacillus cereus. Bacillus cereus RS 16 produced the highest quantity of 1.84mg/ml after 96 hours while Bacillus cereus AS-9 produced the least quantity of 1.21mg/ml after 96 hours.

Key Words: methionine, *Bacillus cereus*, culture medium, soil.

INTRODUCTION

Methionine is an essential amino acid that is required in the diet of humans and livestock. Plant proteins are frequently deficient in methionine and consequently an exclusively vegetable diet may fail to meet nutritional requirements [16].Such deficiencies can only be overcome by an exogenous supply of the essential amino acid.Methionine deficiency has been linked to development of various diseases and physiological conditions including toxemia, childhood rheumatic fever, muscle paralysis, hair loss, depression, schizophrenia, Parkinson's liver deterioration, and impaired growth [17]. Deficiencies can be overcome by supplementing the diet with methionine and, therefore, methionine is of significant interest [7].The L-form of methionine is used extensively in human medicine for a variety of therapeutic purposes, including pH and electrolyte balancing, parental nutrition, pharmaceutical adjuvant, and other applications.

In the recent times, a lot of research efforts have been geared towards the production of amino acids by fermentation methods. Many of these processes seem to be the most economical and practicable means of producing optically active and more readily utilizable amino acids [12].Currently, Nigeria meets all her methionine consumption only through importation. However, methionine can be made available and more economical if produced locally by fermentation.

This research was therefore carried out to isolate and screen bacteria from South east Nigerian soil for methionine production.

MATERIALS AND METHODS

2.1 Organism

Stock cultures of *E .coli* NCCB 1, a methionine requiring auxotroph was purchased from the Netherlands Culture Collection of Bacteria .It was reconstituted on luria broth and maintained on nutrient agar slants at 4^0C

2.2 Isolation of Microorganisms from the soil

Soil samples were collected in sterile polythene bags from soil depth of 5-10 cm of the rhizosphere of different plants found in waste disposal dumps (including soil from an environment in which cassava and maize were grated) in Owerri metropolis. Ten grams of each soil samples were suspended in 100ml of sterile distilled water in a 250ml Erlenmeyer flask. The soil sample was agitated in rotary shaker (Heidoph Unimax 2010) at 150rpm for 20mins. For Heavy soil particles; the suspension was allowed to stand for 30sec before it was serially diluted. Aliquots of 0.1ml of the suspension were then evenly spread on Tryptone Soya Agar (oxoid plates). The plates were incubated at 30^0C for 24h and pure cultures of the isolates stored in Nutrient Agar slants at 4^0C.

2.3 Identification of Bacterial

Cultural characteristics of bacterial isolates on nutrient agar, nutrient broth, tryptone soya agar, mannitol yeast agar and macconkey agar, were examined. Gram staining, spore staining,catalase test, urease test, indole test, motility, growth at 5% NaCl, growth at 6.5% NaCl,methyl red, Voges Proskauer, citrate utilization, ability to reducee nitrate and carbohydrate fermentation were conducted. Taxonomic studies were performed following the methods recommended [3,4, 6,].Bacteria isolate was confirmed by molecular characterization conducted at Macrogen incoporated Europe

2.4 Screening of isolates for methionine production on solid medium:

The isolates were screened for methionine production using a modified medium of [9].Sterilized plates of minimal agar medium containing glucose4.0g; KH_2P0_4, 0.5g; K_2HP0_4, 0.5g; $(NH4)_2S04$, 2.0g; $MgS0_4.7H_20$ 0.001g; $FeS0_4.7H_20$ 0.01g per litre of water with pH adjusted to 7.2, seeded with *Escherichia coli* NCCB 1 were then spread inoculated with each soil isoalate. After 48-72h incubation at 30^0C, the plates were examined for growth of the auxotroph. A total of 500 soil samples were similarly screened, and the Gram reactions of the methionine producing isolates were examined.

2.5 Production of Methionine in Submerged Culture

2.5.1 Seed inoculum:

The medium for seed culture consist of (g/L: peptone, 10.0; yeast extract, 10.0; NaCl, 5.0; water, 1 litre, pH was adjusted to 7.2 with 1N NaOH.The medium was sterilized at 121^0C for 15 minutes. Two loopfuls of each methionine –producing isolates were used to inoculate a 250 ml flask containing 50 ml of the seed medium. The flasks were incubated for 16-18h on a rotary shaker at 120rpm and 300C.

2.5.2 Fermentation

The basal medium used for fermentation composed of KH_2P0_4, 0.5g; K_2HP0_4, 0.5g; $(NH4)_2S04$ 2.0g; $MgS0_4.7H_20$ 0.001g; $FeS0_4.7H_20$ 0.01g; glucose 100.0g; Water I litre; pH was adjusted to 7.2 with 1N Na0H.The medium was sterilized at 115^0C for 10 mins.A 2ml volume (ca 10^4 cells/ml) of each seed culture was used to inoculate triplicate Erlenmeyer flaks containing 20ml of the fermentation medium. After 72h of incubation on the same rotary shaker at 150rpm and 30^0C, growth and methionine produced were determined form the broth culture. Four uninoculated flaks were used as control.

2.6 Determination of growth of the isolate:

Growth of each isolate was determined turbidimetrically using the culture broth in spectronic 21 spectrophotometre(Camspec).

2.7 Analytical methods

Quantitative determination of L-methionine in the culture broth without purification was carried out by the modified calorimetric method of [8].A 5ml volume of the culture broth was centrifuged at 5,000xg for 20 minutes and the cell free supernatant was assayed for L-methionine.1 ml of 5N NaOH was added to each tube followed by the addition of 0.1 ml of 10% sodium nitroprusside solution with through mixing. The mixture was allowed to stand for 10 min. Then two milliliters of 3% aqueous solution of glycine was added to the reaction mixture with frequent shaking over

a period of 10 min. After an additional 10 min interval, 2ml of concentrated *ortho*-phosphoric acid was added drop wise to the mixture with shaking. Colour development was allowed to proceed for 5 min and the colour intensity measured at 540 nm in a spectrometer. A blank containing distilled water and all other reagent served as the 100% transference standard. Results obtained with the test samples were interpolated on a standard methionine curve.

RESULTS AND DISCUSSION

A total of 500 soil samples from different soil ecovars were screened for methionine production on solid medium, 3 organisms designated as DS-13.AS-9 and RS-16 were recoverd. All isolates showed good growth on Nutrient Agar, Tryptone Soya Agar and Mannitol Yeast Agar. No growth was observed on Macconkey Agar. The isolates were Gram positive spore formers with spores positioned centrally. Growth was observed at a temperature range of 26-45^{0}C with the optimal range of 30-37^{0}C.Nitrate was not reduced by the isolates. All organisms reduced citrate but were all negative to urease. DS -13 and AS -9 were able to grow in 6.5% NaCl while RS-16 did not grow. Methyl red, catalase, starch hydrolysis were positives for all isolates.RS-16 fermented mannitol, maltose and glucose. AS- 9 fermented glucose, lactose, xylose. RS- 16 fermented mannitol and glucose with gas production. All isolates were motile.

All isolates were confirmed by molecular characterization conducted at Macrogen Incorporated Europe as *Bacillus cereus* DS13, *Bacillus cereus* AS 9 and *Bacillus cereus* RS16.The results shows that most bacterial species are capable of producing methionine and that methionine producing organisms may be fairly well distributed in nature. Several researchers have shown that microbial excretion of methionine is not restricted to a particular group of bacteria. [5, 10, 13, 14, 18, 19,] reported amino acid excretion in bacteria.

Bacillus cereus DS 13 and *Bacillus cereus* RS 16 produced 1.46mg/ml and 1.84mg/ml after 96 hours fermentation period respectively while *Bacillus cereus* AS 9 produced 1.21 mg/ml after 96 hours. This report agrees with the works of [1] that produced 3.48mg/ml and 1.35mg/ml methionine levels using *Lactobacillus plantarum* and *Bacillus* spp after 96 and 72 hour incubation period respectively. [2] also reported 4.5 g/l methionine yield after 76 hours of incubation

This preliminary study has shown that methionine producing bacteria can be isolated from Nigerian soil. This fermentative process of methionine production when developed will reduce importation of methionine in Nigerian and make it more readily available.

Table 1: Production of methionine in submerged culture

Isolate code	Growth(O.D)	Methionine(mg/ml)
DS13	1.728	1.46
AS9	1.826	1.21
RS16	1.864	1.84

DS: isolate from dump site
AS: isolate from abattoir soil
RS: isolate from the rhizosphere

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REFERENCES

[1]Anike N, Okafor N, Afr.J. *Food Agr Nutr Dev* **2008** 8, 77-90
[2] Banik A, Majumdar S, Indian J Exp Bio **1974**.12, 363-365
[3] Barrow G, Felthan R, *Cowan and Steel's Manual for the Identification of Medical Bacteria.* 3rd edn. Cambridge University Press, Great Britian **1993**
[4] Buchanan RE, Gibbon NE, *Bergey's Manual of Determinative Bacteriology*.8th edn. Williams and Wilkins Co. Baltimore **1974**
[5] Dagley S, Dawes EA, Morrison GA, *Nature* London **1950**.165, 437-438

[6] Finegold SM, Baron EJ, *Barley and Scott Diagnostic Microbiology*. 8th edn.Th CV Mosby Company, U.S.A **1990**
[7] Gomes J, Kumar D, *Biotechnol Adv* **2005** 23, 41–61.
[8]Greenstein JP, Wintz M, Methionine. *Chemistry of the amino acid*, vol. 3.John Wiley & Sons.**1961** pp 2125–55.
[9]Kase H, Nakayama K, *Agri Biol Chem* **1975** 39,161–8.
[10]Kimura E, Kikuchi Y, Kawahara Y, Goto S, Kurahashi O, Nakamatsu T, US patent **2002** 6,338956.
[12]Kinoshita S, Udaka S, Shimono M, *J. Gen. Appl. Microbiol.* **1957** 3,193–205.
[13]Kumagai H, *Adv Bioch Eng* **2000** 69, 71-85
[14] Leuchtenberger W, *Biotechnol* **1996** 6,492
[15] Pfefferle W, Lotter H, Friedrich, Degener W,*European patent* **1993** 532,867.
[16]Pham CB, Galvez FCF, Padolina WG, *ASEAN Food J* **1992** 7, 34-37
[17] Rose WC, Coon MJ, Lockhart HB, Lambert GF, *J Biol Chem* **1955** 215,101-110
[18]Roy SK, Biswas SR, Mishra AK, Nanda G, *J Microbiol Biotechnol* **1989** 4, 35–41.
[19]Sharma S, Gomes J, *Eng Life Sci* **2001** 69–73.