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Green computing in today's world

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Abstract
This research intends to identify lysine-producing bacteria from rotting banana and pineapple fruits, screen the bacteria for lysine accumulation, and use active isolates to produce lysine in a submerged medium. Despite being a crucial part of both human and animal nutrition, lysine is one of the amino acids that the body does not naturally synthesize. As a result, it is supplemented with food and food items, particularly cereal goods, to improve its protein quality. 'New Market' in Amorji-nike, Enugu State, was where the banana and pineapple fruits were bought; a total of 10 fruits were consumed. Using sterile methods, some degraded fruit tissues from the banana and pineapple were added to the nutritional agar in dishes that were covered and incubated at room temperature. The plates were identified by biochemical tests. The bacteria isolated from the decayed banana and pineapple fruits were *Pseudomonas species*, *Corynebacterium species*, *Staphylococcus aureus*, *Bacillus species*, *Escherichia coli*, *Acetobacter aceti*, *Erwinia herbicola*, *and Gluconobacter oxydans*. Only two of these bacteria—*Bacillus spp*. and *Acetobacter aceti*—were identified as lysine producers.

Keywords: Lysine, supplement, amino acids, banana, pineapple

Introduction
Lysine is an amino acid that is essential in the body but not synthesized biologically by the body. It can be administered by adding it as a food supplement to food and food materials especially cereals to boost the protein quality [1]. Lysine is highly necessary for bone formation of children and growing animals. Lysine has been said to be a deficient amino acid in foods for both man and domestic meat producing animals. Animal feeds for poultry, cattle and other livestock which includes grains and defatted oil seeds only provides little amount of lysine and the amino acid cannot be synthesized biologically necessitating the need for it to be supplemented in their feeds so as to provide the necessary diet [1]. There are other benefits of lysine in man and livestock which includes overcoming angina pectoris, cleaning of the arteries which is an important practice towards cancer prevention, improving bone formation by ensuring there is adequate absorption of calcium hence preventing osteoporosis and production of antibodies which makes a healthy immune system. It is also a very important component of the muscular formation [2]. Lysine can be produced chemically and through biochemical means. The commercially manufactured lysine is 80% produced from biochemical methods and 20% produced using chemical means [2]. In the biochemical production process, the use of fermentation is very economical because the microbial fermentation for production uses substrates from industrial or agricultural wastes or its byproducts [3].
Banana (*Musa acuminate*) as a fruit is very nutritious and delicious and can be eaten raw or as a dessert or can be cooked as a tropical dish. Ripe banana is usually soft and sweet while unripe banana which usually contains starch and fiber is used as a cooking ingredients [4].
Bananas were first planted in South and Southeast Asia and are now grown in a large number of tropical and subtropical nations. They are the second most exported fruit and vegetable in the world. India, the Philippines, China, Ecuador, Brazil, Indonesia, Mexico, Costa Rica, Colombia, and Thailand are the top 10 nations that produce and export significant amounts of bananas [4]. The production of bananas is crucial for developing nations in tropical and subtropical regions since it offers a promising food source for both the domestic market, which can be used to meet local nutritional needs, and for people all over the world.

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Additionally, banana farming offers locals employment opportunities and is crucial to the economies of exporting nations. The majority of bananas used.

There are certain healthy benefits attached to ingestion of banana. In every 118 grams portion of raw edible banana (medium size), contains 105 calories, 27 grams of carbohydrates, and very low fat [4]. Also, Banana has been reported to be good source of vitamin B6 (22%), potassium (12%), vitamin C (17%), magnesium (8%), etc. This nutritional content of banana could be from a variety of factors which includes planting sites, the ripening stage of the fruit, and on the cooking methods. From the aforementioned nutritional facts, it can be seen that bananas are good source of energy and also very rich in carbohydrates and low in fats and hence ere good for weight management. Banana is recommended to people in all areas of life especially sport players and manual laborers that require high energy to work. People living certain sicknesses like high blood pressure and obesity are encouraged to consume banana because the potassium and mineral content aids in lowering the blood pressure.

The similarly well-known fruit pineapple can be considered the second most significant fruit after the banana. More than 20% of the tropical fruit population in the world comes from it [4]. The Amazon basin, where pineapple was cultivated, is believed to have its origins in Brazil and Paraguay. The region between upper Panama and Brazil, Paraguay and Argentina, comprising the northern Amazonian forest and the semi-arid parts of Brazil, Venezuela and Guyanas, has been identified as the zone's most likely area of origin [4]. By 1500, pineapple cultivation had spread around the world after being propagated in tropical regions of Europe. The Cayenalisa (Smooth Cayenne), which was first introduced in Europe, is the most well-liked type. Originally from French Guyana, canned food made its way to Hawaii in the late 19th century. The top pineapple-producing countries worldwide are Thailand, the Philippines, Brazil, and China, which together produce around 50% of the world's pineapples. [6] (India, Nigeria, and Kenya) are a few more significant producers.

Aims and Objectives

To isolate bacteria capable of producing L-Lysine from decayed banana and pineapple.

To screen the bacteria for lysine accumulation.

To produce L-Lysine in submerged medium using active isolates.

Statement of problem

Lysine is actively incorporated in the pharmaceutical industries in the development of balance d composition. The Nigerian industries only meet the lysine requirements only through importation hence involving the spending of huge amount of foreign exchange for the purchase.

Methods and Materials

Materials

Bananas and Pineapples were bought from new market, Enugu state.

Microbial culture used

A typed bacteria culture of *Escherichia coli* NCCB 1841, which is a lysine-requiring auxotroph was obtained from my project supervisor. The culture was inoculated and maintained in nutrient agar slants and stored at 4 ^{o}C. Culture of the lysine auxotroph was maintained on Nutrient agar (Oxoid) slants stored at 4 ^{o}C.

Sterilization of glasswares and media

All glasswares (Petri dishes, conical flasks, test tubes, pipette) used during the research were sterilized using the hot air oven at 180 ^{o}C for 1h. The various growth media used were sterilized with the autoclave at 121 ^{o}C and 15 P.S.I. (pounds per square inch) pressure for 15min; and 115 ^{o}C and 10p.s.i. for 10min.

Sample collection

The fruits samples were purchased from New market Enugu and transported in sterile polythene bags to the Microbiology laboratory of Caritas University Amorji-Nike for microbial analysis.

Bacteria isolation

The fruit samples were washed out in sterile distilled water. 1ml of the resulting effluent was serially diluted tenfold. 1.0g of the pineapple and banana sample was added to 10ml of sterile distilled water in a test tube and placed in a VWRDS0500-2 orbital shaker at 160 rpm for 10min. 1ml of the resulting suspension was serially diluted tenfold. 0.1ml of each dilution was inoculated on properly labeled Nutrient Agar plates. Duplicate plates were prepared for each dilution.

Following incubation of the samples at 30^{0}C for 48h, those plates which contained sufficient number (30 to 50) of discrete colonies were selected, and further isolation (purification) was carried out based on morphological disparity of the colonies. Isolated pure cultures were stored on Nutrient slants at 4 ^{o}C.

Screening of bacteria for lysine production on solid agar medium

The isolates from the fruits were screen to detect if they can produce lysine using a modified medium of Ozulu, *et al*. [6] The minimal agar used has the following composition glucose, 4.0g; $(NH_4)_2SO4$, 2.0; K_2HPO_4, 0.05g; KH_2PO_4, 0.05; $MgSO_4.7H_2O$, 0.1g; $FeSO_4.7H_2O$, 0.01g; $MnSO_4.H_20$, 0.0lg; $CaCO_3$, 0.15g; Agar, 15.0g, water, 1 litre; pH, 5.6. One hundred milliliter (100ml) of the medium was inoculated with 1ml of a 24hrs culture broth of the lysine auxotroph, *Escherichia coli* (NCCB 1841) using the spread plate technique and the plates are incubated for 72h at 30^{0}C and examined for the growth of the auxotroph. The present of halo growth of the *E.coli* shows the ability of lysine production by the isolate. The lysine producers were stored for further studies.

Lysine production in shake flask fermentation

Innoculum development

A seed medium of the following composition was used to build up the inoculums. [8] Quantities in g/l of the solution used were: peptone, 10; yeast extract, 10; NaCl, 5; pH 5.6 adjusted with orthophosphoric acid. A 100ml Erlenmeyer flask containing 20ml of the seed medium was inoculated with 2 loopfuls of a 48 hour culture of the lysine–producing isolate. The flask was incubated at 30^{0}C for 48hours on a rotary shaken at 160 rpm.

Shake flask fermentation

The composition of the production medium is similar to that of the screening medium with the exception of agar. A cotton plugged Erlenmeyer flask (100ml) containing 20ml of fermentation medium: basal medium (KH_2PO_4, 0.05g; K_2HPO_4, 0.05g; $MgSO_4.7H_2O$, 0.1g; $MnSO_4.H_2O$, 0.01g; $FeSO_4.7HO$, 0.01g; $CaCO_3$, 20g); $(NH_4)_2SO_4$, 10g; glucose, 20g; water, 1 litre; pH 5.6 adjusted with orthophosphoric acid, was used for lysine production. On conclusion of the sterilization, the flask is allowed to cool at room temperature before 1ml of a 48h inoculum of the isolate is inoculated into the fermentation medium. The experiment was performed in duplicate, with uninoculated flask serving as control. The flask was incubated on a rotary shaker (160rpm) at 30^0C for 72h. At the end of the fermentation period, the broth culture was used for lysine determination.

Quantitative determination of lysine

The fermentation broth (5ml) was centrifuged at 5000rpm for 15min and the resultant supernatant was further examined for lysine production using the method described by Chinard. [9] One milliliter of the glacial acetic acid was introduced into the supernatant (1ml) in a test tube and followed by addition of 1 ml of a solution which composed of 0.4ml of 6 M orthophosphoric acid, 0.6 ml of glacial acetic acid and 25 mg of ninhydrin per ml of the acid mixture. The control /blank contains 1ml of glacial acetic acid, 1ml of the acid mixture with the exception of ninhydrin and 1ml of the supernatant. The test tubes were capped its content is properly mixed before it is being heated at 100°C in a water bath for 1 h after which the test tubes were allowed to cool rapidly under tap water and 2ml of the glacial acetic acid was introduced to each tube to make it up to a final volume of 5 ml. Using the spectrophotometer, the optical density of the reacting mixture was read at a wavelength of 515 nm.

Identification of isolates

The isolates were identified using colonial, morphological and biochemical characteristics. The biochemical test performed includes, Gram stain, motility test, catalase test, urease test, indole test, citrate test,

Results

Isolation and screening of isolates for lysine production on solid agar medium

A total of 8 bacteria organisms were screened and two isolates were reported to be active in the lysine production. These isolates produced lysine which resultantly led to the proliferation of the lysine auxotroph, *Escherichia coli*, inoculated into the agar medium. The degree of the halo growth of the isolates is as shown in Table 1, with isolates CHIDERA A3 and CHIDERA C6 indicating high lysine production.

Lysine production in shake flask fermentation

Table 2 shows the result of lysine production by the isolates in submerged medium. The highest lysine yield of 0.36mg/ml was observed in isolate CHIDERA A3, while the lowest yield of 0.15mg/ml was observed in isolate CHIDERA C6. Isolates CHIDERA C6was the most active lysine producer.

Identification of bacteria isolates

CHIDERA A3 was identified as Bacillus Spp and CHIDERA C6 was identified as Acetobacter aceti. The sample was identified by motility test, catalase test, indole test, urease test.

Table 1: Screening for lysine production on solid medium

Bacteria isolate code	Degree of halo growth of *Escherichia coli*
CHIDERA A1	-
CHIDERA A2	-
CHIDERA A3	++++
CHIDERA C4	-
CHIDERA C5	++
CHIDERA C6	-
CHIDERA C7	-
CHIDERA C8	-

Results interpretation:
- No lysine production
++ High lysine producers
++++ Higher lysine producers

Table 2: Production of lysine in submerged medium

Bacteria isolate code	Lysine (mg/ml)
CHIDERA A3	0.36
CHIDERA C5	0.15

Isolation of Bacillus spp AND *Acetobacter aceti*

BANANA and PINEAPPLE sample was collected and processed by serial dilution and pour plate method for the isolation of bacteria. A total of 8 different bacteria species were isolated, and were labeled as follows. CHIDERA A1, CHIDERA A2, CHIDERA A3, CHIDERA C4, CHIDERA C5, CHIDERA C6, CHIDERAC7, CHIDERA C8.

Table 3: Cultural characteristics of the isolates

Isolation properties	Chidera a1	Chidera a2	Chidera a3	Chidera c4	Chidera c5	Chidera c6	Chidera c7	Chidera c8
Morphological observation	light green and Flat colony	Brown colony And occurred in Pairs	Round and smooth Surface arranged in Clusters	Milky white colony	Very large greenish Wide spreading surface	White colonies and Straight rods	Mucoid colonies which Have entire margins	White colony with edges And glistering appearance
Microscopic observation	Gram-ve rod	Gram-ve rod	Gram-ve rod	Gram+ve rod	Gram+ve rod	Gram –ve rod	Gram + rod	Gram + rod
Gram's reaction	-	-	-	+	+	-	+	+

Table 4: Confirmatory test of bacteria isolates

Isolates	Motility Test	Catalase Test	Indole Test	Ureas Test	Citrate Test	Oxidase Test	Probable Organisms
Chidera A3	-	+	-	-	+	-	*Bacillus spp*
Chidera C6	-	+	-	-	+	-	*Acetobacter aceti*
Key: Positive (+) Negative (-)							

Discussion

Out of a total of eight isolates only two microorganisms were isolated from Pineapple and Banana from New market Enugu were found to be lysine producers Table 1. The isolates CHIDERA A3 and CHIDERA C6 were used for more experiments on lysine accumulation in submerged medium.

From the results gotten from this study, the production of lysine producing microorganisms can be isolated from Pineapple and Banana, CHIDERA A3 and CHIDERA C6. From the result in Table 2, the lysine produced by the isolates as indicated by the halo growth of *Escherichia coli* Lysine auxotroph inoculated on the agar medium. This result is in accordance with that of Ezemba *et al*. [8], in their work: *Microbacterium lacticum*; a lysine producing Bacterium isolated from Oil contaminated soil in South East Nigeria, where it was seen that a lysine producing bacteria could be isolated from the environment.

After the isolates were screened further for lysine production in submerged fermentation as presented in Table 2 Isolate CHIDERA A3 gave the higher lysine yield of 0.36 mg/ml concentration and CHIDERA C6 gave the least lysine yield 0.15 mg/ml.

Table 3 shows the morphological and biochemical identification of isolate in which *Bacillus spp* indicated gram positive, catalase positive, indole negative, motility positive, urease negative, while *Acetobacter aceti* indicates gram negative, catalase positive, indole negative, motility negative, urease negative.

The other bacteria isolates are: *Staphylococcus aureus, Escherichia coli, Corynebacterium spp, Pseudomonas spp, Erwinia herbicola, Gluconobacter oxydans*. Some of the bacteria isolate are gram + ve, while some are gram – ve.

Lysine in the growth medium was to examine the growth of auxotroph and halo growth of the *E. coli* which was described by Ezemba *et al*. [3], in their work: Optimization of Fermentation Conditions of *Bacillus thuringiensis* EC1 for Enhanced Methionine Production. The Quantitative determination of lysine which indicated that CHIDERA A3 produced more lysine than CHIDERA C6.

Conclusion

From this study it can be deducted that lysine producers like *Acetobacter and Bacillus* species can be isolated from banana and pineapple. It also showed a better approach in screening for lysine producers using lysine auxotroph. The approach not only saves time but also prevents laborious method that are frequently practiced. This method if well-developed could secure steady availability of lysine and reduce its importation into the country.

Recommendation

Lysine and its products are used in many industrial process, further studies will be needed to determine whether the lysine from banana and pineapple posses properties which can be used in industrial processes.

Conflict of Interest

Not available

Financial Support

Not available

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