

# Lysine Producers from Decayed Banana and Pineapple Fruits

<sup>a</sup>Ezemba, C.C\*, <sup>b</sup>Okpata, C.C., <sup>a</sup>Etikudike, V. O. <sup>c</sup>Osuala, O.J\*, <sup>b</sup>Ilozor, J. C., <sup>b</sup>Okoye, S. C., <sup>b</sup>Akagu, S., <sup>b</sup>Ezejiolor, C. C.,  
<sup>a</sup>Mmaduekwe, C. J., <sup>a</sup>Udoye, I.W., <sup>d</sup>Ezemba, A.S., <sup>a</sup>Ezeokolie, C.M., <sup>a</sup>Okoye, P.C., <sup>a</sup>Chikelue, M.S. <sup>d</sup>Anakwenze, V.N. <sup>a</sup>  
Ezeigwe, C. and <sup>c</sup>Okoye A.C.

<sup>a</sup> Department of Microbiology, Chukwuemeka Odumegwu Ojukwu University, Uli, Anambra State, Nigeria.

<sup>b</sup> Department of Microbiology and Biotechnology, Caritas University, Amorji-Nike, Enugu State, Nigeria.

<sup>c</sup> Department of Pharmaceutical Microbiology and Biotechnology Madonna University Elele, Rivers state, Nigeria.

<sup>d</sup> Department of Microbiology, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

<sup>e</sup> Department of Applied Microbiology and Brewery, Enugu State University of Science and Technology, Enugu State, Nigeria.

Corresponding Authors: Ezemba C.C/ [Constancechinnvere790@gmail.com](mailto:Constancechinnvere790@gmail.com) (+234803744096) and Victor O.E/  
[victordike0703@gmail.com](mailto:victordike0703@gmail.com) (+23470 373 66997)

**Abstract:** This study is aimed at isolating bacteria capable of producing lysine from decayed banana and pineapple fruits, screening the bacteria for lysine accumulation, and producing lysine in a submerged medium using active isolates. Lysine is one of the amino acids that are not originally synthesized in the body even though it is quite important nutritionally to man and animals. Hence it is supplemented in food and food materials most especially cereal products so as to boost its protein quality. The banana and pineapple fruits were purchased from 'New market' in Amorji-nike, Enugu State, a total of 10 fruits were used. Some pieces of decayed tissues from the banana and pineapple fruits were transferred by means of sterile techniques, to the nutrient agar contained in covered dishes (Petri-dishes) 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 were indicated as lysine producers, and they are *Bacillus* spp and *Acetobacter aceti*.

**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. <sup>[1]</sup> 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.<sup>[1]</sup> 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.<sup>[2]</sup> 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.<sup>[2]</sup> 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.<sup>[3]</sup>

Banana (*Musa acuminata*) 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.<sup>[4]</sup>

Originally, Banana is grown in South and Southeast Asia and cultivated in so many tropical and subtropical countries and ranks among second highest fruits and vegetables that are being exported worldwide. The top ten countries which grow and provide substantial quantities of banana are India, the Philippines, China, Ecuador, Brazil, Indonesia, Mexico, Costa Rica, Colombia, and Thailand.<sup>[4]</sup> The cultivation of bananas is important to developing countries in tropical and subtropical areas, since it provides a promising food source for the domestic market to meet local nutritional requirements, as well as for people around the world. In addition, the cultivation of bananas creates job opportunities for local people, and also plays a vital role in the economy of exporting countries. Most of the bananas used in developed countries are imported. The two largest importers are the United States of America and the European Union where each region imports an average of 32% of the total world production of bananas. Japan (9%), Eastern Europe (6%), South America and Canada (8%) are the other major Importing countries. It is due to the unfavorable state of the climate of these countries that makes banana not produced in these countries hence the importation. Also due to the continuous quest for healthy living, the populace of these countries take banana too satisfy their nutritious needs.<sup>[5]</sup>

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 are 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.

Pineapples are equally very famous fruit and it can be said to be the second harvest of importance after banana. It contributes over 20% of world population of tropical fruits.<sup>[4]</sup> The origin of pineapple is traced to be from Brazil and Paraguay in the Amazonic basin where the fruit was domesticated. It has been defined as the most probable area of origin the zone comprised from upper Panama and Brazil, Paraguay and Argentina, including the northern Amazonian forest and the semi-arid regions of Brazil, Venezuela and Guyanas.<sup>[4]</sup> The worldwide production of pineapple started by 1500 when it was transferred to Europe and tropical regions of the world through propagation. The most popular variety is the Cayenalisa (Smooth Cayenne) which was first introduced in Europe from French Guyana and in the late XIX century, the canned was introduced commercially in Hawaii. Thailand, Philippines, Brazil and China are the main pineapple producers in the world supplying nearly 50 % of the total output.<sup>[6]</sup> Other important producers include (India, Nigeria, and Kenya)

## 1.2 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.

## 1.3 STATEMENT OF PROBLEM

Lysine is actively incorporated in the pharmaceutical industries in the development of balanced 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.

## 2.0 METHODS AND MATERIALS

### 2.1 MATERIALS

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

### 2.2 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°C. Culture of the lysine auxotroph was maintained on Nutrient agar (Oxoid) slants stored at 4°C.

### 2.3 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°C for 1h. The various growth media used were sterilized with the autoclave at 121°C and 15p.s.i. (pounds per square inch) pressure for 15min; and 115°C and 10p.s.i. for 10min.

### 2.4 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.

### 2.5 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°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°C.

### 2.6 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.<sup>[6]</sup> The minimal agar used has the following composition glucose, 4.0g; (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 2.0; K<sub>2</sub>HPO<sub>4</sub>, 0.05g; KH<sub>2</sub>PO<sub>4</sub>, 0.05; MgSO<sub>4</sub>.7H<sub>2</sub>O, 0.1g; FeSO<sub>4</sub>.7H<sub>2</sub>O, 0.01g; MnSO<sub>4</sub>.H<sub>2</sub>O, 0.01g; CaCO<sub>3</sub>, 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°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.

### 2.7 LYSINE PRODUCTION IN SHAKE FLASK FERMENTATION

#### INNOCULUM DEVELOPMENT

A seed medium of the following composition was used to build up the inoculums.<sup>[8]</sup> 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°C for 48 hours on a rotary shaker at 160 rpm.

### 2.8 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<sub>2</sub>PO<sub>4</sub>, 0.05g; K<sub>2</sub>HPO<sub>4</sub>, 0.05g; MgSO<sub>4</sub>.7H<sub>2</sub>O, 0.1g; MnSO<sub>4</sub>.H<sub>2</sub>O, 0.01g; FeSO<sub>4</sub>.7HO, 0.01g; CaCO<sub>3</sub>, 20g); (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 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°C for 72h. At the end of the fermentation period, the broth culture was used for lysine determination.

### 2.9 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 515nm.

### 2.10 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

### 3.0 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.

### 3.1 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 C6 was the most active lysine producer.

### 3.2 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 <i>Escherichia coli</i>
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

### 3.3 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, CHIDERA C7, CHIDERA C8.

**3.4 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	-	-	-	+	+	-	+	+

**3.3 TABLE 4: CONFIRMATORY TEST OF BACTERIA ISOLATES**

Isolates	Motility Test	Catalase Test	Indole Test	Ureas Test	Citrate Test	Oxidase Test	Probable Organisms
CHIDERA A3	-	+	-	-	+	-	<i>Bacillus spp</i>
CHIDERA C6	-	+	-	-	+	-	<i>Acetobacter aceti</i>

Key: Positive (+) Negative (-)

## DISCUSSION, CONCLUSION AND RECOMMENDATION

### 4.0 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 0.36mg/ml concentration and CHIDERA C6 gave the least lysine yield 0.15mg/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.

#### 4.1 CONCLUSION

From this study it can be deduced 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.

#### 4.2 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.

#### REFERENCES

1. Zara, R.C. and Dresden, D. What are the benefits of Lysine?. Medical New Today. 2018.
2. Divya, J. and Pallavi, S.U. What is L-lysine Good for?. Medicine Net. 2021
3. Ezemba, C.C., Ekwealor, I.A., Nwagbo, I.A. Optimization of Fermentation Conditions of *Bacillus thuringiensis* EC1 for Enhanced Methionine Production. *Advances in Microbiology* 2014; 4:344–352.
4. Armstrong, W. P. "Identification Of Major Fruit Types". Wayne's Word: An On-Line Textbook of Natural History. 2013.
5. Natalie, B. and Megan, W. Health Benefits of Pineapple. MedicalNews Today. 2018.
- 6 Food and Agricultural Organization of the United Nations. Sustainable Development. Statistical database. 2019.
7. Ozulu, A., Kahveci, M. and Troncoso, J. Abnormalities in the formol titration method. *Journal of American Chemistry Society. Kluwer academic publisher, New York* 2012; pp 43:152–163.
8. Ezemba, C.C., Ekwealor, C.C., Ekwealor, I.A., Ozokpo, C.A., Chukwujekwu, C.E., Anakwenze, V.N., Archibong, E. J. and Anaukwu, G.C. *Microbacterium lacticum*; a lysine producing Bacterium isolated from Oil contaminated soil in South East Nigeria. *British Microbiology Research Journal*, 2015; **9**(2):1–8.
9. Chinard, F.B. Photometric estimation of proline and ornithine. *Journal of Biology and chemistry* 1952; **199**: 95–991.