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Comparative Studies on Synthetic and Agricultural Product on Lysine Production by *Alcaligenes aquatilis*

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Authors' contributions

This work was carried out in collaboration among all authors. Author CBN carried out the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors NNU, CCE and CTE assisted in the performance of the statistical analysis and the literature searches. Author IAE supervised the study. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Production of lysine by *Alcaligenes aquatilis* from agricultural sub-products (banana and soybean) was compared to glucose and ammonium sulphate as a carbon and nitrogen source. Ammonium sulphate was constant as a nitrogen source when the two carbon sources were investigated and glucose constant as a carbon source when the nitrogen sources were investigated. The production of lysine was examined quantitatively by acidic ninhydrin method. The results showed that banana and soybean improved the maximum lysine yield (1.158 mg/ml and 1.279 mg/ml) for the fermentation period of 96 hrs.

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1. INTRODUCTION

L-lysine, 2, 6 diaminohexanoic acid ($C_6H_{14}N_2O_2$) is a basic amino acid having two amino groups, one, on α- position and other at - position [1,2]. L-Lysine is generally deficient in the food supply of man and meat producing animals [3]. Since animal feeds such as grain and defatted oil seeds contain only a small quantity of lysine, poultry, cattle and other livestock are unable to synthesize these amino acids. It must be added in the feed to provide a balanced diet [4]. Agricultural sub-products may be used as low-cost carbohydrate sources for microbial production of high value-added products such as amino acids [3,5]. Microbial fermentation provides 100% L-amino acids whereas by chemical method 50% D and 50% L- amino acids are obtained [6,7] revealed that the fermentation process is more economical, optically active and the stereospecificity (the L-isomer) make it more advantageous compared with synthetic processes. The present report is on comparative studies between synthetic and agricultural products on lysine production which demonstrated that banana and soybean gave the maximum lysine yield (1.158 mg/mL and 1.279 mg/mL) for the fermentation period of 96 h.

It is, therefore, the objective of this study to explore the agricultural sub-products for the process optimization for the laboratory scale production of L-lysine.

2. MATERIALS AND METHODS

2.1 Microorganism

Soil samples were collected from various spots at Science Village, Nnamdi Azikiwe University, Anambra State at 10-15 cm depth. Starch Casein Agar [8] and Starch Ammonium Sulphate Agar. The plates were incubated at 30^{o}C for 7 days. Colonies that developed were subcultured and pure cultures preserved at 4^{o}C on sterile starch casein agar slant. The isolates were screened for lysine production using a minimal medium. The active isolates were sent to CABI International Organization, the United Kingdom, by 16S rDNA sequence analysis using the FASTA algorithm with the prokaryote database from EBI for identification and the result came out to be *Alcaligenes aquatilis*. It was maintained on starch casein agar slants at 4^{o}C. The medium for seed culture consists of peptone, 10.0 g; yeast extract, 10.0 g; NaCl, 5.0 g; distilled water, 1 liter; pH adjusted to 7.2 with 1N NaOH. The medium was sterilized at 121^{o}C. Two loopful (ca 2.4 × 10^7) of a 24 h culture of the isolate on nutrient agar was inoculated into 2 ml of the sterile seed medium in a test tube and incubated on a Searchtech HY-2A orbital shaker at 160 rpm and 30°C for 48 hrs.

2.2 Production of Lysine in a Submerged Culture

2.2.1 Seed inoculum preparation

The medium for seed inoculum consists of peptone, 10.0 g; yeast extract, 10.0 g; NaCl, 5.0 g; distilled water, 1 liter. The pH was adjusted to 7.2 with 1N NaOH. Two loopful (ca 2.4 × 10^7) of a 24h culture of the isolate on nutrient agar was inoculated into 2 ml of the sterile seed medium in a test tube and incubated on a Searchtech HY-2A orbital shaker at 160 rpm and 30°C for 48 hrs.

2.3 Fermentation

The basal medium for fermentation experiments was composed of KH_2PO_4, 0.5 g; K_2HPO_4, 0.5 g; $MgSO_4.7H_2O$, 0.001 g; $MnSO_4.H_2O$, 0.001 g; $FeSO_4.7H_2O$, 0.001 g; $CaCO_3$, 0.02 g; $(NH_4)_2SO_4$, 10 g; glucose, 20 g; water, 1 liter; pH adjusted to 7.2, was used for lysine production. After sterilization, the flask was cooled to room temperature and 1 ml (ca 1.8 x 10^7) of a 24 h seed inoculum of the isolate was inoculated into the fermentation medium. The experiment was performed in duplicate, with uninoculated flask serving as a control. The flask was incubated on a rotary shaker (160 rpm) at 30^{o}C for 96h. Bacterial growth and lysine production were determined from the broth culture.

2.4 Comparative Difference between Glucose and Banana, Ammonium Sulphate and Soybean

Two carbon sources [glucose and banana (*Musa acuminata*)] were studied for their effects on lysine accumulation by the isolate. Fermentation was carried out in a medium consisting of KH_2PO_4, 0.05 g; K_2HPO_4, 0.05 g; $MgSO_4.7H_2O$, 0.1 g; $MnSO_4.4H_2O$, 0.001 g; $FeSO_4.7H_2O$, 0.001 g; $CaCO_3$, 2.0 g; carbon source, 20 g; $(NH_4)_2SO_4$, 10 g; distilled water, 1L, pH 7.2. A 100 ml Erlenmeyer flask containing 20ml of the fermentation medium was inoculated with 2 ml (ca 2.4 × 10^7) of seed inoculum and the flask incubated on an orbital shaker (160 rpm) at

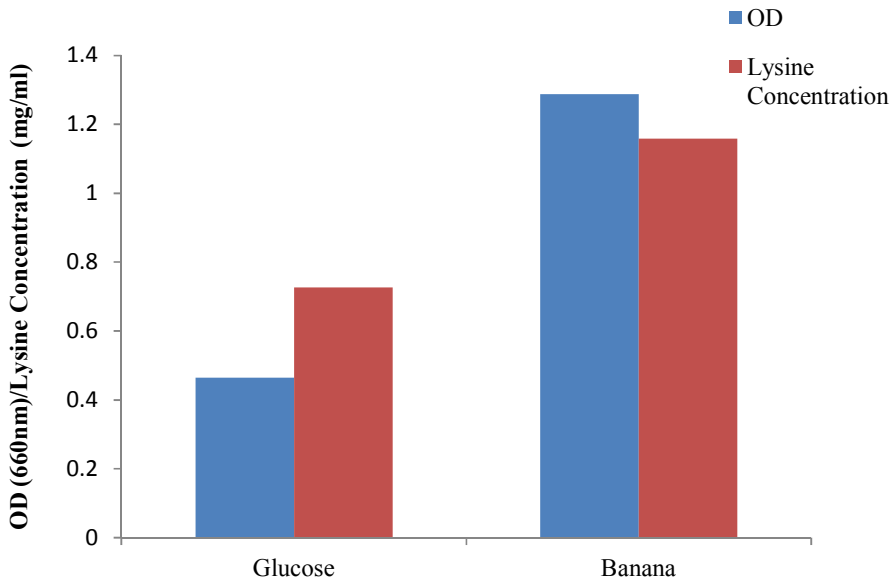

Fig. 1. Effect of carbon sources on lysine production *Alcaligenes aquatilis*

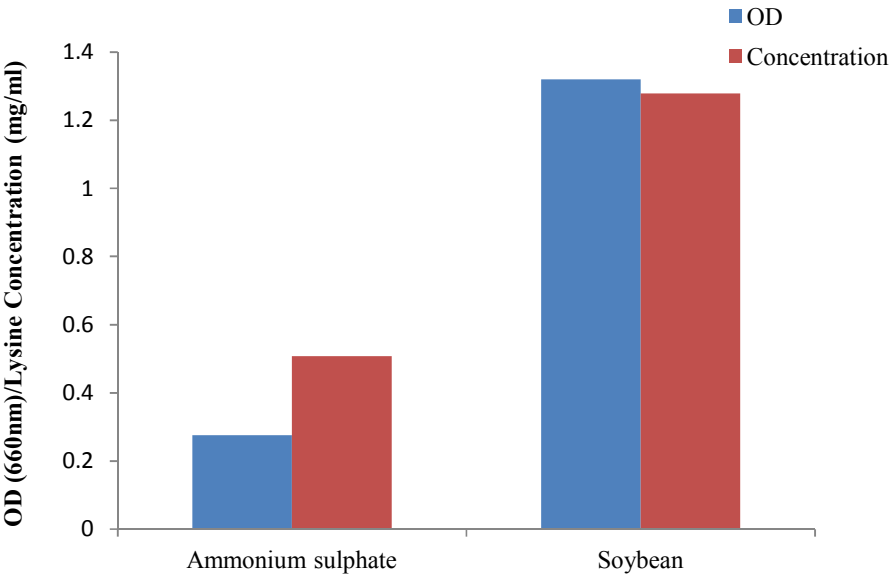

Fig. 2. Effect of nitrogen sources on lysine production *Alcaligenes aquatilis*

30°C. Duplicate flasks were used and uninoculated flasks served as control. After 96h fermentation, lysine production was determined as previously described. Thus, banana gave the maximum lysine accumulation.

Two nitrogen sources [NH_4SO_4 and soybean (*Glycine max*)] were examined for their effects on lysine production by the isolate. Fermentation was carried out in a medium consisting of KH_2PO_4, 0.05 g; K_2HPO_4, 0.05 g; $MgSO_4.7H_2O$, 0.1 g; $MnSO_4.4H_2O$, 0.001 g; $FeSO_4.7H_2O$, 0.001 g; $CaCO_3$, 2.0 g; glucose, 20 g; nitrogen source, 10 g; distilled water, 1 L, pH 7.2. A 100 ml Erlenmeyer flask containing 20 ml of the fermentation medium was inoculated with 2ml (ca 2.4×10^7) of the inoculums and the flask incubated on an orbital shaker (160 rpm) at 30°C. Duplicate flasks were used and uninoculated flasks served as control. After 96h fermentation, lysine production was determined as previously described. Thus, soybean gave the maximum lysine production.

3. RESULTS AND DISCUSSION

Lysine producing bacteria identified as *Alcaligenes aquatilis* was isolated from soil located in Nnamdi Azikiwe University, Awka, Nigeria. That this organism, a lysine producer is present in the soil and is in line with the findings of [9], they noted that some lysine producing bacteria may be found in soil. [10], also isolated lysine producers which are capable of utilizing hydrocarbon from both oil- contaminated and uncontaminated soil in south-east of Nigeria. [11] also were able to isolate lysine producer *Bacillus megaterium* sp14 from the soil. The production of lysine by *Alcaligenes aquatilis* agrees with the work of [12] who noted a novel lysine producing bacterium from oil-contaminated soil. The influence of glucose on growth and lysine production was compared to that of banana. Glucose in a basal medium was replaced by the equivalent concentration of banana. Banana proved to be the best carbon source for lysine production (Fig. 1). The result presented in Fig. 2, show that soybean was the best medium for lysine production and was chosen as the nitrogen source for shake flask culture.

4. CONCLUSION

This research has shown has agricultural products can be used as substrates for the production of lysine. It is comparatively economical and practicable. The fermentative method has the important advantage of yielding the optically active L-form of lysine directly. It has also established that agricultural products can be used for appreciable lysine production by fermentation and if well developed will reduce the importation of this product into the country and make it more readily available.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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