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

Comentado [s.1]: The title name should be rewrite. This tittle is not appropriated

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

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 Ɛ- position (Tome and Bos, 2007; Rao *et al*., 2011; Malothu *et al*., 2012). L-Lysine is generally deficient in the food supply of man and meat producing animals (Pellett and Ghosh, 2004). 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 feed to provide a balanced diet (Nasab *et al*., 2007). Agricultural sub~~by~~ products may be used as low-cost carbohydrate sources for microbial production of high value added products such as amino acids (Buzzini and Martini, 1999). Microbial fermentation provides 100% L-amino acids whereas by chemical method 50% D and 50% L- amino acids are obtained (Khan *et al*., 2006). Anastassiadis (2007) revealed that fermentation process is more economical, optical active and the ~~sterospecificity~~stereospecificity (the L-isomer) make it more advantageous compared with synthetic processes. The present report demonstrated a comparative studies between synthetic and agricultural products on lysine production which demonstrated ~~show~~ ~~tha~~t that banana and soyabean gave the maximum lysine yield (1.158mg/mL and 1.279mg/mL) for the fermentation period of 96h.

MATERIALS AND METHODS

Microorganism

Alcaligenes aquatilis was isolated from soil in Unizik Awka, Nigeria. It was maintained on starch casein agar slants at 4^{o}C. The medium for seed culture consisted of peptone, 10.0g; yeast extract, 10.0g; NaCl, 5.0g; distilled water, 1L; pH adjusted to 7.2 with 1N NaOH. The medium was sterilized at 121^{o}C. Two loopful of a 24h culture of the isolate on nutrient agar was inoculated into 2ml of the sterile seed medium in a test tube and incubated on a Searchtech HY-2A orbital shaker at 160rpm and 30°C for 48h.

Fermentation

The basal medium for fermentation experiments was composed of KH_2PO_4, 0.5g; K_2HPO_4, 0.5g; $MgSO_4.7H_2O$, 0.001g; $MnSO_4.H_2O$, 0.001g; $FeSO_4.7H_2O$, 0.001g; $CaCO_3$, 0.02g; $(NH_4)_2SO_4$, 10g; glucose, 20g; water, 1 ~~litre~~liter while the pH was adjusted to 7.2, was used for lysine production. After sterilization the flask was cooled to room temperature and 1mL (ca 1.8 x 10^7) of a 24h seed inoculum of the isolate was 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 (160 rpm) at 30^{o}C for 96h. Bacterial growth and lysine production were determined from the broth culture.

Comparative difference between Glucose and Banana

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, 0.05g; K_2HPO_4, 0.05g; $MgSO_4.7H_2O$, 0.1g; $MnSO_4.4H_2O$, 0.001g; $FeSO_4.7H_2O$, 0.001g; $CaCO_3$, 2.0g; carbon source, 20g; $(NH_4)_2SO_4$, 10g; distilled water, 1L, pH 7.2. A 100 ml Erlenmeyer flask containing 20ml of the fermentation medium was inoculated with 2ml of seed inoculum (two loopful) and the flask incubated on an orbital shaker (160rpm) at 30°C. Duplicate flasks were used and uninoculated flasks served as control. After 96h fermentation, lysine production was determined as previously described. Thus, sorghum gave the maximum lysine accumulation.

Comparative difference between ammonium sulphate and soyabean

Two nitrogen sources [NH_4SO_4 and soyabean (*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, 0.05g; K_2HPO_4, 0.05g; $MgSO_4.7H_2O$, 0.1g; $MnSO_4.4H_2O$, 0.001g; $FeSO_4.7H_2O$, 0.001g; $CaCO_3$, 2.0g; glucose, 20g; nitrogen source, 10g; distilled water, 1L, pH 7.2. A 100ml

Erlenmeyer flask containing 20ml of the fermentation medium was inoculated with 2ml of the inoculums (two loopful) and the flask incubated on an orbital shaker (160rpm) at 30°C. Duplicate flasks were used and uninoculated flasks served as control. After 96h fermentation, lysine production was determined as previously described.

RESULTS AND DISCUSSION

Lysine producing bacteria need ample supply of a suitable carbon and nitrogen source. Trifonova *et al.* (1993) and Javed *et al*. (2011) used agricultural product as substrate for production of lysine by *Brevibacterium* spp. and citric acid by *Aspergillus niger*, respectively. The ability of these bacteria to utilize these agricultural products is supported by the work of Umerie *et al.* (2000), who used agricultural by-products as carbon and nitrogen sources for lysine production. Krishnamurth (1980), Nicoloni *et al. (*1987) and Nigam (2000) used agricultural by-products for single cell protein production. For this purpose, the concentrations were the same. As shown in Figures 1 and 2, maximum lysine of 1.158mg/ml was accumulated when sorghum was used while 1.279mg/ml was obtained when soyabean was used. This is in consistent with the report of Adnan *et al.* (2011), who studied the selection of substrates for L-lysine production by *Brevibacterium linens* DSN 20158 and observed maximum L-lysine production (2.213g/kg) with soyabean meal. Umerie *et al.*(2000) and Ekwealor and Orafu (2003), similarly, observed that defatted soyabean meal stimulated the highest amount of lysine in *Bacillus laterosporus* and *Bacillus* species, respectively. Also, Ikpeme *et al.* (2006) reported that soyabean and peanut meal were more effective in promoting antibiotic production in mutant strains of *Bacillus pumilus* Bpu 32. The result is also in agreement with the work of Pham *et al*. (1992), who reported the use of carbohydrate sugar cane juice, molasses, banana, cassava and coconut water as source of carbon for methionine production.

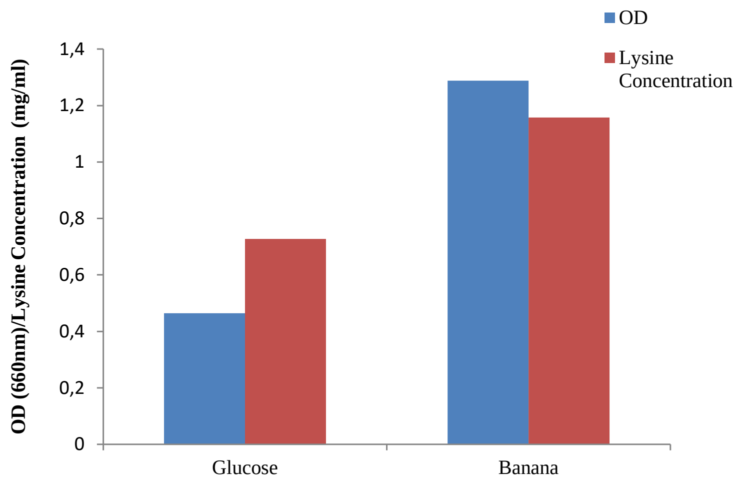

Fig. 1: Effect of Carbon Sources on Lysine Production *Alcaligenes aquatilis*

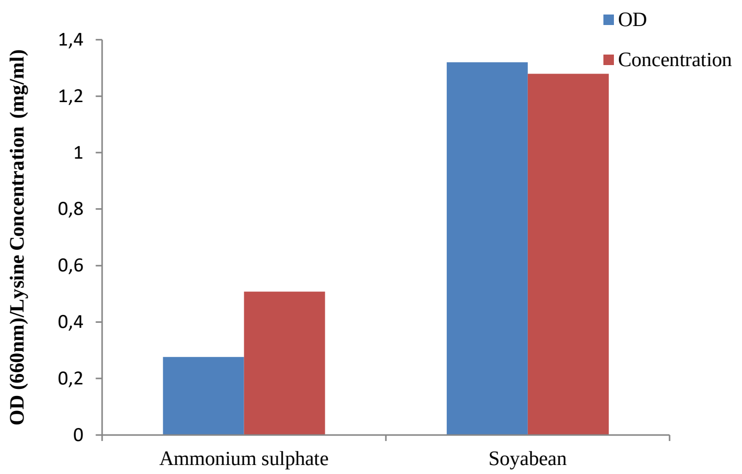

Fig. 2: Effect of Nitrogen Sources on Lysine Production *Alcaligenes aquatilis*

CONCLUSION

It was found that the fermentative method has the important advantage of yielding the optically active L-form of lysine directly. It was also established that agricultural products can be used for lysine production by fermentation and if well developed will reduce the importation of this product into the country and make it more readily available.

Comentado [s.2]: The conclusion should be rewrite and improve

REFERENCES

Adnan, A., Zareena, M., Quratulain, S. and Khadija, S. (2011). Optimization of Fermentation parameters for the production of L-lysine from *Brevibacterium linens* DSM 20158, using statistical approach. *World Applied Sciences Journal* **13**(5):1132- 1140

Anastassiadis, S. (2007). L-lysine fermentation. Recent Patents on Biotechnology **1**:11-24

Buzzini, P. and Martini, A. (1999). Production of carotenoids by strains of Rhodotorula glutinis cultured in raw materials by agro-industrial origin. *Bioresource Technology* **71**:41-44

Ekwealor, I. A. and Orafu, A. E. (2003). Preliminary study of L-lysine production by *Bacillus* species using various agricultural by-products. *Nahrung/Food* **47**:226-227

Ikpeme, E. M., Etim, L. B. and Arikpo, G. (2006). Optimum cultural conditions for the production of antibiotics by mutant strain of *Bacillus pumilus* Bpu32. *Nigeria Journal of Microbiology* **20**(3):1232-1237

Javed, A., Jamil, A. and Rezaei-Zarchi, S. (2011). Optimization and hyper-expressed production of lysine through chemical mutagenesis of *Brevibacterium flavum* by N-nitroso Nethylurea. *African Journal of Microbiology Research* **5**(29): 5230-5238

Khan, S. H., Rasool, G. and Nadeem, S. (2006). Bioconversion of cane molasses into amino acids. Pakistan Journal of Agricultural Science **43**(3- 4): 156- 161

Kirshnamurth, G. U. (1980). In proceedings of the symposium on waste from food processing Industry: Utilization and disposal. CFTRI, Mysone, India

Malothu, R., Dowlathabad, M. R. and Balanarasaiah, T. (2012). Bioprocess Technology Development and Convalescing Production of L-Lysine in *Corynebacteriumglutamicum.* International Conference on Chemical Engineering and its Applications, Bangkok (Thailand)

Nasab, M. N., Ansaris, S. and Montazer, Z. (2007). Fermentative production of lysine by *Corynebacterium glutamicum* from different carbon sources. *Iran Journal Agricultural Research*. **26**(1-2): 99- 105

Nicoloni, L., Humolstein, V. C and Corilli, A. (1987). Solid state fermentation of orange peed and grape stalk by *Pleurotus ostreatus*. Agrocy be Aegerita and armilloriella mellea. *Journal of Applied Microbiology and Biotechnology*, **26**:95-98

Nigam, J. N. (2000). Cultivation of *Candida langeronii* in sugar cane bagasse hemicellulosic hydrolyzate for the production of single cell protein. *World Journal of Microbiology Biotechnology*. **16**:367-372

Pellet, P. L. and Ghosh, S. (2004). Lysine fortification: past, present, and future. *Journal of Food Nutrition* **25(**2):107-113

Pham, C. B., Galvez, C. F. and Padolina, W. G. (1992). Methionine fermentation by batch fermentation from various carbohydrates. *ASEAN Food Journal.* **7**:34–37

Rao, B. S., Muralidhararao, A. and Swamy, V. N. (2011). Studies on Continuous Production Kinetics of LLysine by Immobilized *Corynebacterium glutamicum*13032. *Middle-East Journal of Science Research* **7**(2): 235-240

Tome, D. and Bos, C. (2007). Lysine Requirement through the HumanLife Cycle. Journal of Nutrition **137**:1642S–1645S

Trifonova, V. V., Ignatora, N. I., Milyukova, T. B., Overchenko, M. B. and Rimareva, L. V. (1993). Possible application of various types of fruit and vegetable raw material to microbial synthesis of lysine. *Prikladnaya Biokhimiyai Mikrobiologiya* **29**:475 -479

Umerie, S. C., Ekwealor, I. A. and Nwagbo, I. O. (2000). Lysine production by *Bacillus laterosporus* from various carbohydrates and seed meals. *Bioresource Technology* **75**:249-252