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Fermentation Study of *Micrococcus Halobius CH-16* for Lysine Production

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ABSTRACT

Bacterial isolation from compost heaps, refuse dumps and decaying woods were investigated or lysine production. Fifteen of the isolates were recovered as lysine producers, and a gram positive bacterium identified as *Micrococcus halohius* CH-16 was found to accumulate 4.38mg/mi lysine in the broth culture after 96h. Effect of varying concentration of carbon and nitrogen sources on lysine accumulation showed that glucose (4%$^{w}/_{v}$) ammonium sulphate (2%$^{w}/_{..}$) respectively increased [ysine production, influence of B-vitamins and bivalent metals on lysine production by *M. halobius* indicated that nicotinic acid and folic acid stimulated lysine accumulation while Ni^{2+} and Co enhanced lysine yield respectively. All arnino acids and growth stimulators at 0.01%(w/v) respectively improved lysine production. All antibiotics except linomycin inhibited lysine accumulation. Surfactants such as oleic, acid Tween 20 and Tween 80 stimulated lysine production.

Keywords: Fermentation, Micrococcus Hablobius CH-16, lysine, production.

INTRODUCTION

Lysine is an essential amino acid required mainly by children and growing animals. It cannot be synthesized biologically in the body and its breakdown is irreversible [1]. The lack or insufficiency of lysine will result in growth retardation. Lysine also, plays an important role in collagen formulation; a nutrient responsible for the conversion of fatty acid into energy and also helps the immune system ward off viral infections like herpes.- cold sore, mouth ulcers and the associated fever [2].

Lysine is used as food (flavour) enhancer and also food preservation especially with ε-poly-L-lysins [3]. Protein production by microorganisms rich in essential amino acid has been studied by many researchers. [3]; [4], both as food supplements and as sources of amino acids. L-Lysine can bo produced either by a chemical or a biochemical method, which is more economical, even though relatively low yields are obtained during the extraction of L-lysine, requiring specific installations and the use of expensive products [5].

L-lysine was found to be the second most abundant ammo acid recovered in the cell hydrolystate [6], Due to various metabolic regulation mechanisms, most natural strains cannot produce industrially significant amount of L-lysine in the culture. However, alteration of such, regulatory mechanism can lead to over-production of the essential arnino acid for profit maximization [7].

The sterospecificity of amino acids and the steadily increasingly L-lysine demand necessitates indispensably their fermentative production (the L isorner) over synthetic processes [8].

This work was therefore carried out to study the influence of some cultural processes on the production of lysine by a strain of *Micrococcus species.*

MATERIALS AND METHODS

Isolation of bacteria, soil samples from compost heaps, refuse dumps and decaying woods were collected. Soil (10.0g) suspended in 100ml of sterile

distilled water in a 250ml Erlenmeyer flask was agitated with a rotary shaker (Gesellschaft Model D 3006) at 150rpm for 25min. the soil suspension was serially diluted ten fold and 0.1ml of 10^{-5} diluted spread evenly on Tryptone Soy Agar (Oxoid) plates. After 24h incubation at 30^0C, the culture was sub-cultured and pure isolates stored on Nutrient Agar (Oxoid) slants and refrigerated at 4^0C.

Screening of Isolates for Lysine production on solid medium

Following the method described by [9] the isolates were screened on solid medium for lysine production. Sterile minimal agar medium containing (g/l): Glucose, 2.0; KH_2PO_4, 1.36; $(NH_4)2\ SO_4$, 2.0; $MgSO_4$, $7H_2O$, 0.2; $CaCl_2$ 0,01; $FeSO_4$, $7H_2O$, 0-C005; Agar, 12.0, H_2O, IL; pH 7.4, was seeded with an auxotroph, *E coli* 1099 or *E. coli* 5210 and the plates spread inoculated with the isolates- Alter 72h incubation of the plates at 30°C, isolates showing halo growth of the auxotroph were sub-cultured and used for further studies. These isolates produced lysine on solid medium and were termed lysine producers.

Table 1: Screening of lysine producers in submerged medium containing different carbon and nitrogen sources

Medium (Carbon/Nitrogen)	**Lysine** (mg/ml)
Glucose/Ammonium Sulphate Glucose/Ammonium Chloride Glucose/Potassium Nitrate Glucose/Ammonium Phosphate	1.95 1.16 1.08 0.78
Sucrose/Ammonium Sulphate Sucrose/Ammonium Chloride Sucrose/Potassium Nitrate Sucrose/Ammonium Phosphate	1.02 0.72 0.85 0.68
Fructose/Ammonium Sulpnate Fructose/Ammonium Chloride Fructose/Potassium Nitrate Fructose/Ammonium Phosphate	1.12 0.91 0.64 0.86
Maltose/Ammonium Sulphate Maltose/Ammonium Chloride Maltose/Potassium Nitrate Maltose/Ammonium Phosphate	0.72 0.60 1.34 1.06
CMC/Ammonium Sulphate CMC/Ammonium Chloride CMC/Potassium Nitrate CMC/Ammonium Phosphate	1.21 1.00 0.98 1.22

CMC = Carboxymethylcellulose

Screening lysine producers in submerged medium with diilerent carbon and nitrogen sources

Seed inoculums: A loopful of a 24h culture of the lysins producer was inoculated into a test tube containing 5ml of the seed .medium (peptone, 10. 0g; NaCl, 5.0g; yeast extract, 10.0g; H_2O, culture was used for fermentation after 18h incubation on a shaker (120rprn) at 30°C.

Fermentation: The basal medium for fermentation consists of (g/l): $KHPO_4$, 1.0; MgSO. $7H_2$6, 0.4; $MnSO_4$. H_2O, 0.002; $FeSO_4$,

7HA 0.002; $CaCO_3$, 50.0; H_2O, !L; pH 7.2. Glucose, 20.0g and $(NH_4)_2SO_4$, 10.0g served as a carbon and nitrogen sources respectively, and the medium sterilized at 115^{0}C for 10min. One milliliter volume of the seed culture (ca. 5,6 x 10^8 cells/ml) was inoculated into 100ml Erlenmeyer flask containing 20ml of the fermentation medium. Duplicate flask were prepared and incubated at 30°C for 72h in a shaker (160rpm). Uninoculated flasks were used as control.

The carbon and nitrogen sources were replaced and fermentation process carried out as previously described. The most lysine producer was further investigated.

Analysis

culture was determined. The broth culture was centrifuge and 1m! portion of the supernatant was added 1rnl glacial acetic acid and 1ml of a mixture of 0.4ml of 6M orthaphosphoric acid, 0.6ml glacial acetic acid and 25 rng of ninhydrin. A blank test tube was similarly prepared but without ninhydrin. The contents of the test lubes .were mixed, heated to 100°C in a water bath for 1h and then cooled. A -2 ml vol. of glacial acetic acid was added to each test tube and after mixing properly, the optical density of the reacting mixture (tube with ninhydrin) was read against the blank at 515nrn in a spectrophotometer (Jenway spectrophotometer model 6405 uv/vis). Lysine concentration was estimated from a standard lysine curve.

Reducing sugar: Glucose in the fermentation broth was estimated following the method described by [10]. A mixture of 1ml of dinitrosalicyclic acid and 1m; ol supernatant in a test tube was heated for 10min in boiling water The tube was rapidly cooled, the vol. adjusted to 12ml with distilled water and optical density of the mixture read at 540nm in a spectrophotometer. Glucose concentration in the supernatant was estimated from glucose standard curve.

Identification of active lysine producer

Methods described by [11], [12] [13] were used for characterization of the active isolate. Colony morphology on Nutrient agar, biochemical and physiological tests such as gram

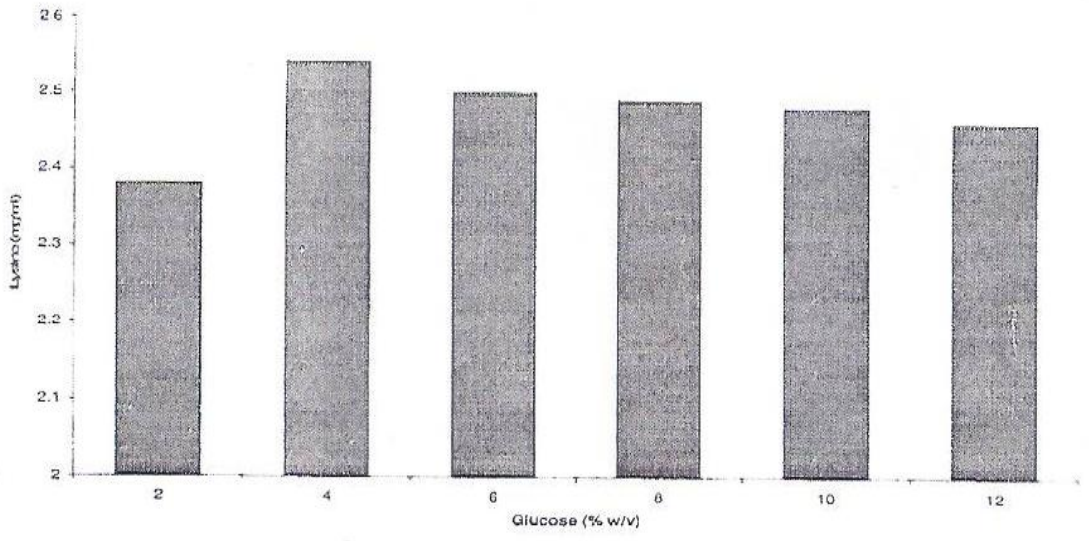

Fig. 1 Effect of varying concentrations oi glucose on lysine production

Reaction, motility, nitrate reduction, sugar, citrate and tyrosine utilization and NaCl tolerance were examined.

Results Identification of active lysine producer

Growth on 24h Nutrient agar plates showed cream coloured circular cells, 0.5-1 mm in diameter. They are gram positive spherical cells occurring in tetrads catalase, oxidase. urease and indole tests were positive, while motility, nitrate reduction, gelatin hydrolysis were negative. Sugars utilized include glucose, galactose, lactose, arabinose, maltose, sucrose, fructose, duicitol, glycerol but mannose and mannitol were not used. Citrate was utilized and sodium chloride tolerance >10% <12%. The organism was identified as a strain of *Micrococcus halobius.*

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Screening lysine producers in submerged medium with different carbon and nitrogen sources

Table 1 shows the influence of different carbon and nitrogen sources on lysine production. The highest lysine accumulation was observed in the glucose-ammonium sulphate medium.

Effect of varying concentrations of glucose on lysine production

The result presented in Fig.1. shows the effect of varying concentrations of glucose on lysine production by M *halobius* strain. At 4% (w/v) glucose concentration, 2.54rng/ml lysine accumulated in the cure broth.

Effect of $(NH_4)_2SO_4$ concentrations on lysine Effect of production

Fig.2. shows the effect of $(NH_4)_2S_2O_4$ concentrations on lysine production. At 2% level of $(NH_4)_2SO_4$, 2.73mg/ml lysine was produced. Influence of B-vitamins on lysine production. As presented in Fig.3., nicotinic acid and folic acid stimulated lysine accumulation. The highest lysine yield of 2.87mg/ml was produced when nicotinic acid at l.0µg/ml was added to the broth culture of M. *halobius* strain. Biotin and riboflavin had no effect on lysine production.

Bivalent metals effect on lysine production

The effects of bivalent metals on lysine yield are presented in Fig. 4 Ni^{2+} and Co^{2+} enhance lysine accumulation while lysine production.

Accumulation while $Ca^{2+}Cu^{2+}$ and Sr^{2+} did not stimulate lysine production.

Influence of amino acids on lysine production

The result in Fig.5 shows that all amino acids improved lysine accumulation, Phenylalanine stimulated a lysine yield of 2.94mg/ml.

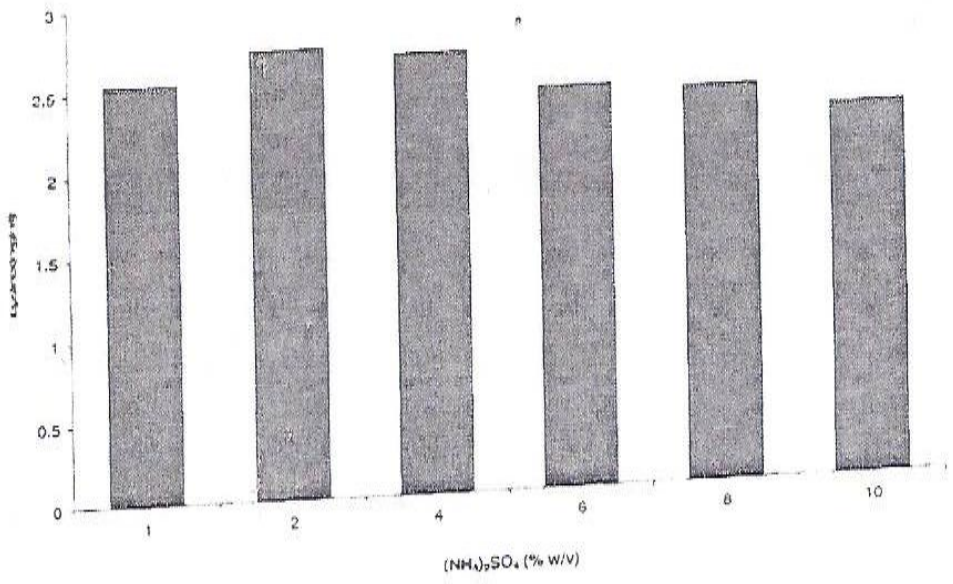

Fig .2. Effect of nitrogen concentration on lysine production

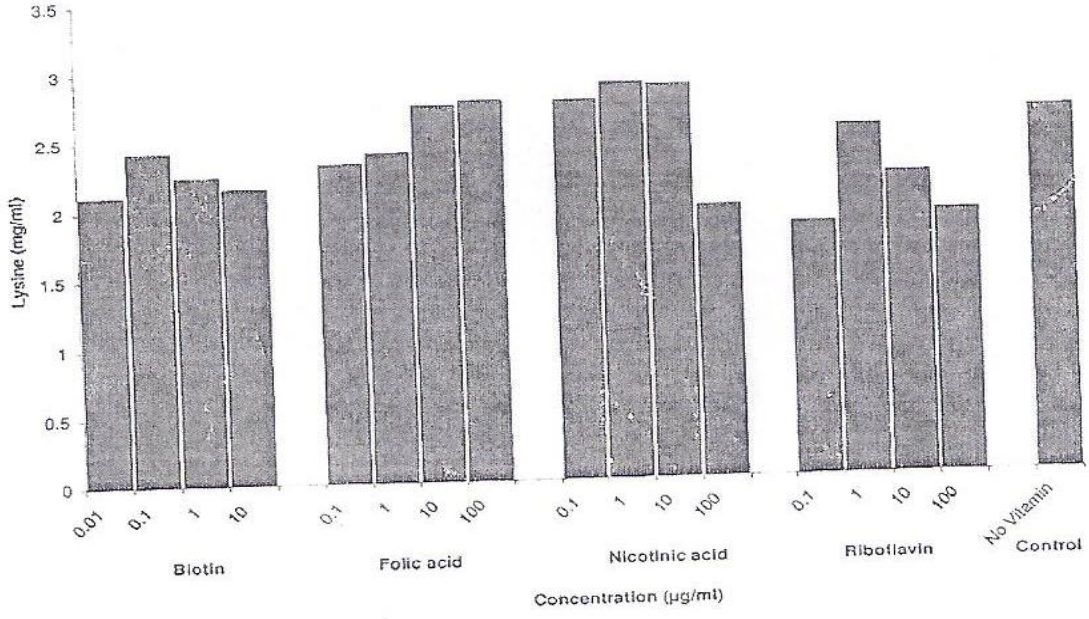

Fig. 3. Influence of B-vitamins on lysine production.

Effect of growth stimulators on lysine production

The stimulatory effects of casein, peptone and yeast extract on lysine accumulation by *M. halobuis* strain are presented in Fig. 6. A mixture of the three growth stimulators gave a lysine yield of 2.95mg/ml.

Antibiotics effects on lysine production

The effects of varying concentrations of penicillin lincomycin, chloramphanicol, iotracyc!ine and erythromycin on lysine production by a strain of *M. Halobius* are presented in Fig.7. All antibiotics except lincomycin inhibited lysine accumulation.

Surfactants effects on lysine production

Fig. 8 shows the influence of surfactant on lysine production by a strain of *M. halobius.* Oleic acid, Tween 20 and Tween 80 enhanced lysine accumulation.

Time course of fermentation for lysine production

Time course of growth, glucose utilization, pH and lysine production by *M. halobius* CH-16 are shown in Fig. 9. Maximum growth and lysine accumulation of 4.39mg/ml was observed after 96h of fermentation and at a pH of 6,8- The percentage glucose utilization within this period was 69%.

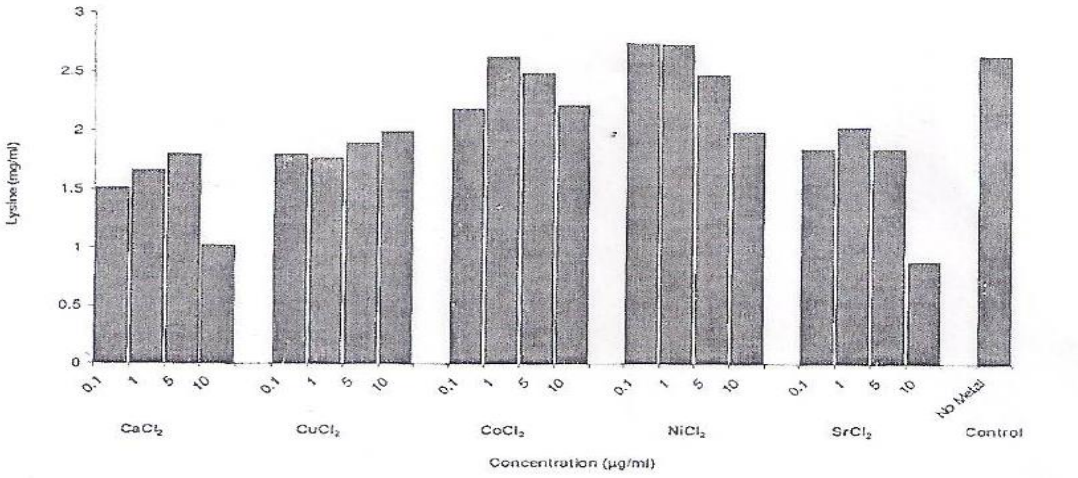

Fig 4. Bivalent metals effect on lysine production

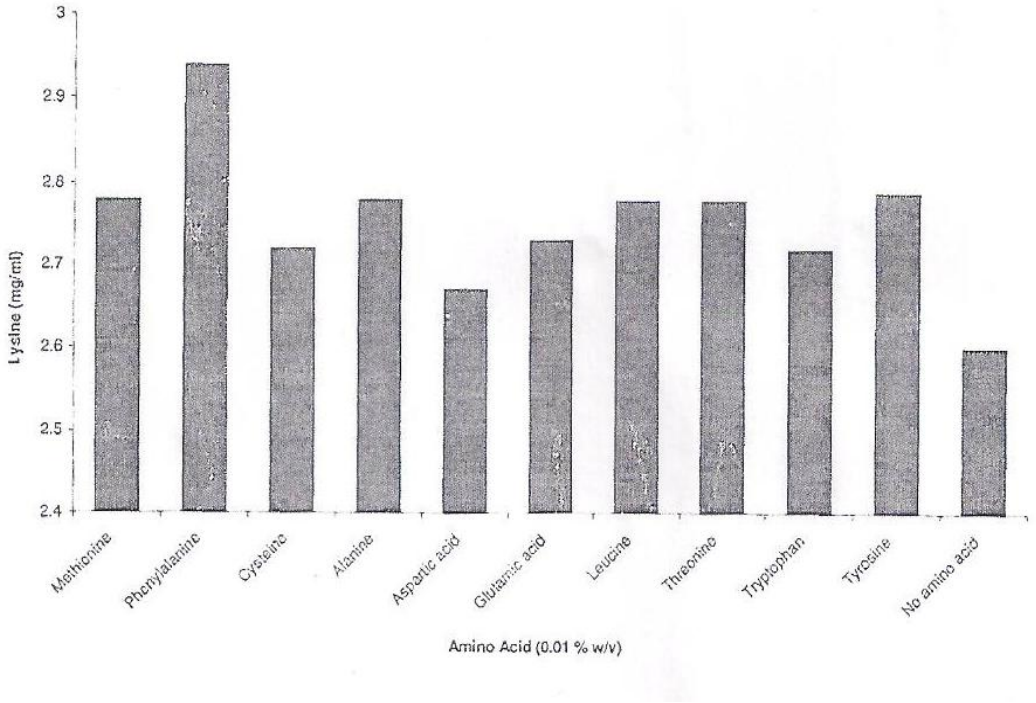

Fig. 5: Influence of amino acids on lysine production

DISCUSSION

Bacterial Isolates from compost heaps, decaying woods and refuse dumps were investigated for lysine production. Fifteen of the isolates, both gram positive and gram negative bacteria were recovered as iysine producers.

Under submerged fermentation, an active isolate identified as a strain of *Micrococcus halobius* was recovered and then studied.

Production of lysine by *Micrococcus* species has been reported by many researches [14]; [15]; [16]; [17]; [18]; [19], although [20] noted that the high yielding strains are found mostly among species of *Arthrobacter,. Corynebacterium* and *Brevibacterium.*

The influence of medium composition on iysine accumulation in a culture broth was reported by [21]. [22], however, noted that in addition to physiochemical parameters (like pH, agitation and aeration rate, air saturation, temperature, dissolved CO_2 and foaming), medium composition is a very important factor strongly influencing fermentation processes, often being of extensive process development and optimization. According to [23].

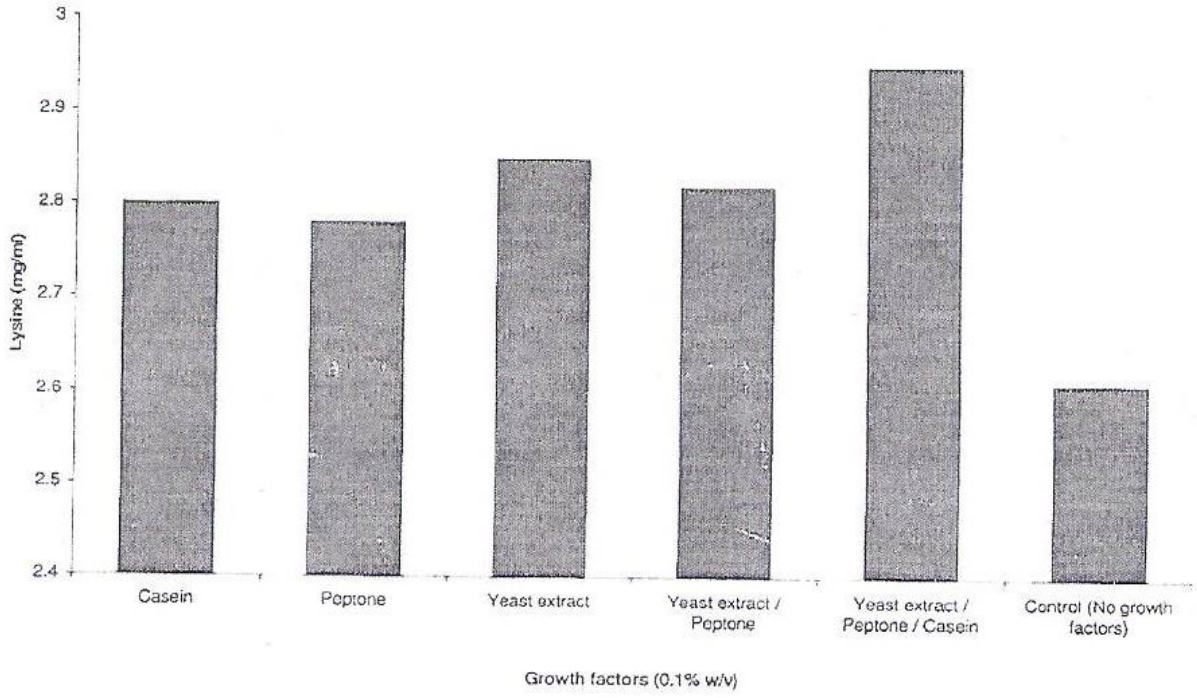

Fig. 6. Effect of growth stimulators on lysine production

Culture medium must satisfy in a suitable manner the requirement of microbial growth and production.Effect of carbon and nitrogen sources on lysine production is presented in Table 1. Glucose and ammonium sulphate added to the culture medium of *M. halobius* CH-16 gave the highest lysine yield, and as suggested by [23], the carbon and nitrogen sources may have satisfied the requirement for growth and the high lysine production.

The effect of varying concentration of glucose as presented in Fig. 1. indicates that lysine production is a function of the initial carbon source in the fermentation medium; This view is supported by the works of [24] [25]. [26] also reported that glucose has a repressive effect on growth and metabolite production at a concentration higher than 5%.

Fig. 2 shows the effect of $(NH_4)_2SO_4$ concentrations on lysine accumulation by *M. halobius* CH-16. $(NH_4)_2S04$ at 2% ($^w/_v$) enhanced lysine yield and suggests that lysine production could be a function of initial nitrogen , concentration present in the medium. This view is support^ by the work of [27], They reported that a high lysine yield by *Brevibactenum* sp P1-15 was obtained when 2% $(NH_4)_2SO_4$ was maintained in the medium throughout the fermentation process.

Influence of B-vitamins on lysine production by *Micrococcus halobius* CH-16 presented in Fig. 3., showed that folic acid and nicotinic acid improved lysine yield. The stimulatory effect of folic acid on lysine accumulation by *Bacillus megatetium* SP 86 was reported by [28]. However, like other vitamins, they are likely to play catalytic roles within the bacterial cell, usually as components of coenzyrnes or as prosthetic groups of enzymes, bui their involvement in amino acid production are not yet known.

The effect of bivalent metals on lysine accumulation by *M. halobius* CH-16, as shown in Fig. 4., indicates that Co^{2+} and Ni^{2+} increased lysine yield. This finding is in line with the observation of [29]. They noted the new role of Ni^{2+} as an essential metal for several enzyme-catalysed reactions in microorganisms observed by [30].

[31] in their study on divalent cation transport system of *Rhodopseudomonas capsulata* found Co^{2+} to be essential for growth but [32] reported the stimulatory effect on grov/th and iysine production by *Bacillus megaterium.*

These bivalent metals probably act as activators or inhibitors of enzymes involved in the synthetic steps of metabolites [33]; [34] but the actual mechanism of stimulation or inhibition of metabolite formation is still not known.

However, [35] noted that the mineral constituents of a medium play an influential part in growth and quantitative yield of metabolites.

All amino acids enhanced lysine yield by *M. halobius* CH-16 (Fig.5). The stimulatory effects of amino acids have been reported by many authors [36]; [37]; [38]; [39]. They noted that various amino acids in the range of 100-5QOu,g/rnl improved lysine accumulation in *M. glutamicus, Srevibacterium LactofetmenWrn* No 2256-213, *Brevibactenum fiavum* LT-1 ATCC 21258, *Corynebactenum glutamicum* BL-25 ATCC 21526 and *Bacillus megaterium* Growth

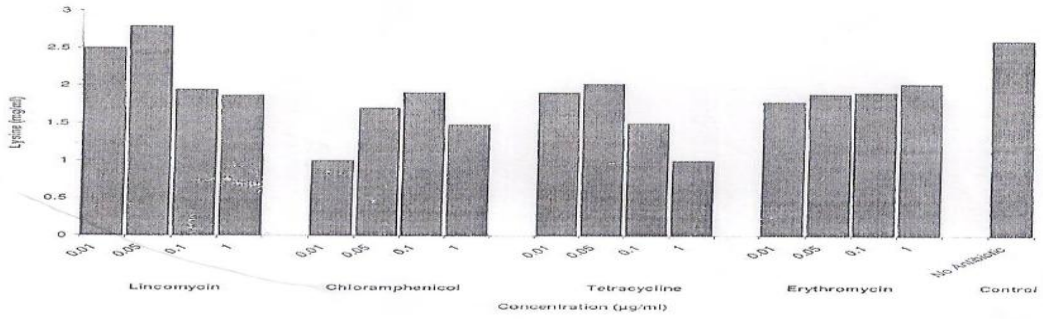

Fig.7. Antibiotics effect on lysine production

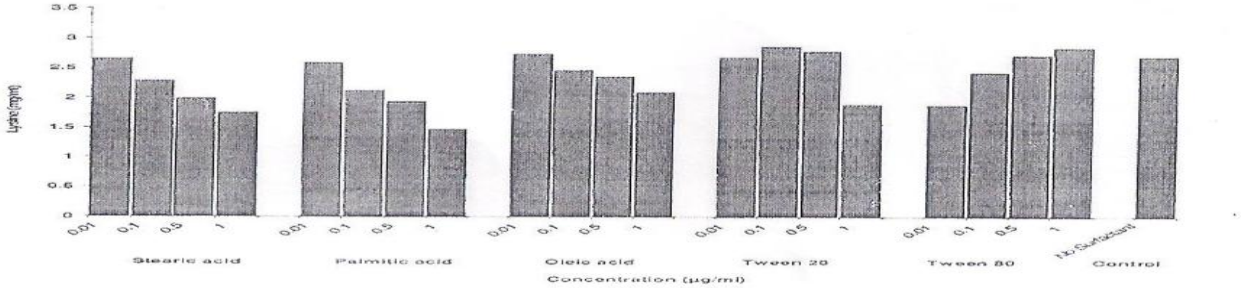

Fig. 8. Surfactants effect on lysine production.

stimulators at 1mg/ml increased lysine accumulation by *M. halobius* CH-16 (Fig.6). This observation is similar to the findings of [40]. They reported an enhanced lysine yield by *B. lactofermentation, C. glutamicum* ATCC 21513 and *B. megaterium* respectively at 0.1-2% yeast extract.

All antibiotics except lincomycin inhibited lysine accumulation (Fig.7). The stimulatory effect of lincornycin Is supported by the findings of Ekwealor and Obeta (2008). They reported the improved lysine yield by this antibiotic in *B. megaterium* strains. The inhibitory effect of other antibiotics as suggested by Isralides *et al.* (1989), may probably be due to great decrease in cell viability of *M. halobius* CH-16 which also prevented the production of lysine.

Surface active agents promotes cell permeability in microorganism and as observed in fig, 8, lysine

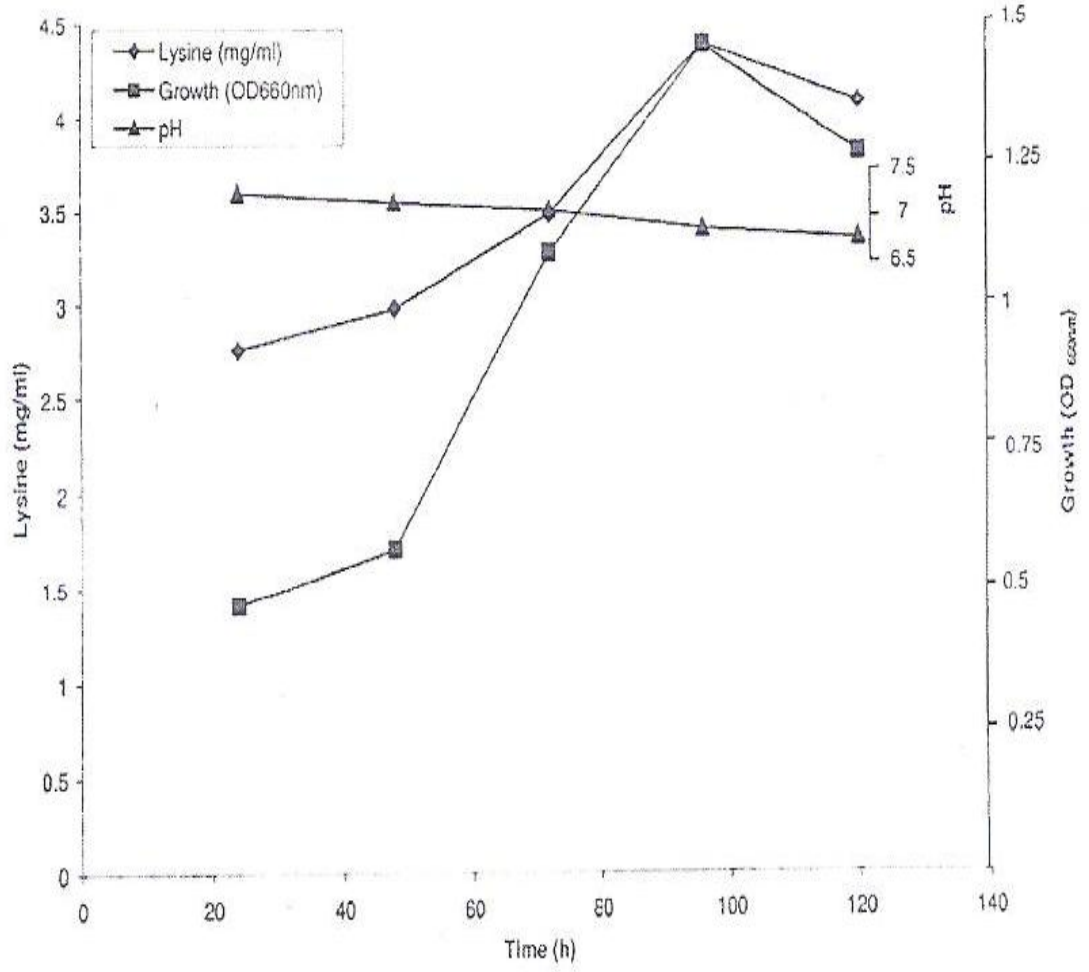

Fig 9. Time course of fermentation for lysine production

Medium (g/L): $KHPO_4$, 1.0; $MgSO_4.7H_20$, 0.4; $MnSO_4.H_2O$, 0.002; $FeSO_4.7H_2O$, 0.002; $CaCO_3$, 50.0; Glucose, 40.0; $(NH_4)_2S0_4$, 20.0; phenylaianine, 0.1; yeast extract, 1.0;

nicotinic acid, 0.001; $NiCl_2$, 0.001, lincomycin, 0.00005; pH 7.2.

Accumulation by *M. haloblus* GH-16 was improved by Oleic acid, Twoen 20 and Tween 80. Similar effects have been reported by [5] [6]. According to [12], the stimulation of Tween 80 observed in *C. giutamicum* is supposed to be caused by its influence on cellular surface structure thus, increasing the lysine yield.

The time course of Iysine production by *M. halobius* CH-16.(Fig.9) was accompanied by utilization of sugar in the medium. [5] reported a similar relationship between sugar consumption and lysine production by *Micrococcus glutamicus.* It is likely that the production of extracellular Iysine by *M. halobius* CH-16 is primarily the result of active assimilation and synthesis as suggested by [9], and not an autotytic or degradation process involving a poiypeptide intermediate.

The increase in Iysine yield (4.39mg/ml) by *M, halobius* CH-16 (Fig.9) as suggested by Kinoshita *et al.* (1957a), Sassi *et al.* (1990) and Anastassiads (2007) may have been influenced by medium composition. It has been shown that optimizing the cultural process improved Iysine accumulation by *M. halobius* CH-16. It is likely that the bacterial strain if improved on will stimulate higher Iysine yield.

REFERENCES

1. Aida, K. (1972). Brief history of research In: The microbial production of amino acids. Yamada, K, S. Kinoshita, T. Tsunda, T.K. Aida (eds). Kodansha, Tokyo pp. 23.
2. Anastassiads, S. (2007). L-Lysine fermentation Recent Patents in Biotechnol 1:11-24.
3. Barrow, G. I and Feltham, R. K. A (1993). Cowan and Steels Manual (or the identification of medical bacteria. Third Edition. Cambridge University Press, Great Britain.
4. Becker, M. J (1982). Product biosynlhetic in continuous fermentation, *Folia Microbiol,* **27:** 315-318.
5. Buchanan, R.E. and Gibbon N.E. (1974). Bergey's Manual of Determinative Bacteriology 8th ed. Williams and Wilkins Co. Baltimore.
6. Chinard, P. P. (1952) Photometric estimation of proline and ornithine. *J. Biol.Chem.* **199:** 91-95.
7. Ekweaior I .A, and Obela, J.A.N. (2005). Siucun; on Iysine production of *Bacillus megatenum.* Afr, J. Biotechnol. 4(7), b33-638.
8. Ekewalor, I .A. and Obeta, J.A.N. (2007). lnfijor.ee of growth factors amino acids and penicillin on iysine accumulation by *Bacillus megatenum* sp76. Nigeria J. Sci. Res. 6;55-i;0,
9. Ekwealor, I. A. and Obeta, J. A. N (2007). Effect o: vitamins and bivalent metsls on Iysine yield in *Bacillus megat &iusn. Afr. J. Biotechnol,* **16**(11): 1348-1351.
10. Ekwealor, I.A, and Obeta, J.A.N. (2008). Antibiotics and surfactant effects on Iysine production of *Bac'tllius megateriurn. Afr. J, Biotechnol.* **7**(10) 1550-1553.
11. Finegoid, S.M. and Baron E.J. (1990). Barley and Scotia diagnostic microbiology 8th ed. The C.V. Mosby Co. U3A.
12. Halsall, O.M. (1975). Overproduction of lysino oy muUant strains of *Escherichia coli* with defective lysine i.r;.-.n sport system. *Biochem Genet,* **13:** 109-125.
13. Hughes, M. N. and Pools, R. K (1989). Metal and microorganisms. Chapman and Hall, USA pp 1-37.
14. Isralides, C.J., Weir, A.N. and Bull, A.T. (1989). Effect ot antibiotic on Iysine production in free and immobilized ceils of *Bacillus subtilis. Appl. Micorbioi. Biotechnol.* **32:**134-136.
15. Jasper, P. and Silver, S. (1978). Divalent cation transport system

of *Rhodopseudmonas capsulata. J. Bacteriol.* **133:** 1323-1328.
16. Kirnura, K., Tanaka, K. and Kinoshita, S. (19G3). The effect of biotin on the amino acid biosynthesis by *Microcociccus glutamicus J. Gen. Appl. Microbiol,* 9:205-212.
17. Kinoshita, S. (1959). The production of arnino acids by fermentation process. *Adv. Appl. Microbiol,* **1:** 201-214.
18. Kinoshita, S. Udoka, S. and Shirnono, M. (1957a). Studies on Hie amino acid fermentation part 1. Production of L-glutamic acid by various microorganisms. *J. Gen, Appl. Microbiol.* **3:** 193-205,
19. Kobayashi, K. and Nishikawa M. (2006). Promotion of E-poly-L-iysine production by iron in *Kitasatospora kifuence. World J. Microbiol Biotechnol.* **10:** 1033-1036.
20. Konicek, J., Smekal, P. and Konickova - Radochova, M. (1931). Effect of Tween 80 and dimethyl sulfoxide on biosynthesis of lysine in regulatory mutants of *Cotynebxcterium giutamicum. Folia Microbiol.* **36:** 587 -589
21. Kubota, K., Maeyashiki, I., Shiro, T Noborn, K.S. and Kamagawa, K.(1970). Method of producing lysine United State Patent No. 3527672.
22. Lakshaman, M. and Rao, M.R.R. (1981). Excretion of lysine by *Micrococcus glutamicus. J. Biosci.* **3** (1): 51 -55
23. Lancaster, J.R. (1988). The bioinorganic chemistry of nickel VCH, Weinheim.
24. Lodha, Ml, Mehla, S.I. and Das, N.B. (1973). Regulation of L-lysine production by aspartic acid family amino acid in homoserine auxolroph of *Micrococcus glutamicus. Indian J. Expt. Biol.* 11: 354-356.
25. Martin, J.E. and McDaniel, L.E. (1977) Production of polyene macrolide anlibiotics. *Adv. Appl, Microbiol.* **21:**1-52.
26. Miller, G.L. (1959). Use of dinistrosalicylic acid reagent for detection of reducing sugars. *Anal. Chem.* **31:**427-431.
27. Mindlin, S.Z. and Zaitseva Z,M. (1966). The action of threonine and isolencine in the biosynthesis of L-lysine by strains of *Micrococcus .^^glutamicus* deficient in homoserine. *Prikl. Biokhim. Microbiol.* 2:128
28. Murgov, I.D. and Zaitsera, Z.M. (1973). The effect of the components of the nutrient medium on the biosynthesis of lysine by a homoserne deficient mutant oi *Brevibacterium ilavum* 178. *Prikl. Biokhim, Mikrobiol,* **9:**356-361.
29. Nakayama, K. (1972). Lysine and diaminopimelic acid, In: The microbial production of arnino acids, Yamada, K., S. Kinoshita, T. Tsunoda and K. Aids (eds). Kodansha, Tokyo, pp 372-380.
30. Nakayama, K., Ssgamihara, K- and Araki, K. (1973). Process for producing L-lysine. United States Patent No. 3708395.
31. Pape, F. (2003). The essential amino acid, lysine, acts as precursor of glutamate in mammalian central nervous system. *FEBS Lett.* 488:34- 38.
32. Pham, C.B., Galvez, F.C.F. and Padolina, W.G. (1992) Methionine production by batch fermentation from various carbohydrates. ASEAN Food J. 7:34-37.
33. Sassi, A.H., Coelio, N., Deschamps, A.M. and Lebeautt, J.M. (1990). Effect of medium composition on L-lysine production by a variant strain of *Corynebacterium gl-jtamicum* ATCC 21513 *Biotechnol. Leit.* **12**(4):295-298.
34. Sen, S. K. and Chatterjee, G. P. (1989). Influence of B-vitamins and trace elements on lysine

production by *Mwoooccus varians* 2fa. Acta Biotechnol. 9: 63 - 67

35. Sen, S. K., Chatterjee, M. and Chatlerjee, S. P. (19S3). L-Lysino production from hydrocarbons by *Mictocuc-Giis varians* 2fa Acta Microbiol, Pol. 32:139-145.
36. Shah, A.M., Hameed, A. and Khan, G.M (2C02), Fermentaiive production of L-lysine: Bacterial fermentation I. J. Med. Sci. 2(3);152-157.
37. Thauer, R.K., Diekert, G and Schnoheit, P, (1980). Biological role of nickel. Trends Biochem. Sci. 5: 304-306.
38. Waksrnan, S.A. (1962). Success and failures in search for antibiotics. *Adv. Appl. Microbiol,* **11:**1-16.
39. Wang, J.S., Yung, C.K., Cheng, W.I. and Liu, L.T (1991) Optimization of culture conditions for L-lysine fermentation by *Brevibacterivni* ap. Rept- Taiwan Sugar Res, Insl. 134:37-48.
40. Young, T.K. and Chipiey, J.R. (1983). Microbiai production of lysine and threonine from whey permeate. Appl. Environ. Microbiol. 45: 610-615,