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A New Approach to Screening for Methionine-Producing Bacteria

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Research Article

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ABSTRACT

Aim: To find a method of screening for active Methionine-producing organisms.
Study Design: Examination of cross-section of soil.
Place and Duration of Study: Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka, Nigeria between April 2010 and August 2011.
Methodology: Bacterial isolates (200) from soil were screened for Methionine producers on solid agar medium seeded with Methionine auxotroph, *Escherichia coli*. The agar plates were observed for halo growth of the *E. coli* which indicates Methionine production by the isolate. Methionine production in submerged medium by the isolates was investigated.
Results: A total of 24 bacterial isolates were recovered as Methionine producers. Six of the active isolates used for submerged fermentation accumulated Methionine in a range of 0.46 – 1.40mg/ml. A close relationship was established between the nature of the halo growths of *E. coli* auxotroph on solid agar and the Methionine yields of the active bacterial isolates in submerged medium.
Conclusion: It is a new and fast approach to screening for active Methionine producers.

Keywords: Agar medium; bacteria; halo growth; methionine production; soil; submerged medium.

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1. INTRODUCTION

Methionine is an essential amino acid required for growth and function of the body. It cannot be synthesized internally but must be added to food and feed materials, to improve the protein quality (Pharm et al., 1992).

To successfully establish a commercially viable process for microbial production of Methionine, a high producer organism must be found or generated (Kase and Nakayama, 1975a; Kumar et al., 2003).

Methionine is generally being produced by chemical methods but these processes are expensive and produce racemic mixture that has to be resolved (Fong et al., 1981). Because fermentation processes have been able to inexpensively provide many other amino acids, there is need to develop a microbial process for commercial production of Methionine.

It is, therefore, the objective of this study to find a method of screening for active Methionine-producing organisms.

2. MATERIALS AND METHODS

2.1 Isolation of Bacteria

Soil sample (1.0g) was suspended in 9ml of sterile distilled water and 1ml of the suspension serially diluted tenfold. Nutrient agar plates were inoculated by spreading 0.1ml of 10^{-6} dilution and incubated at 30°C. Duplicate plates were prepared. After 24h incubation, the isolates were purified and stored on Nutrient agar slants at 4°C.

2.1.1 Screening for Methionine-producers on solid agar medium

The isolates were examined for Methionine production in an agar medium composed of glucose, 4.0g; $(NH_4)_2SO_4$, 2.0g; K_2HPO_4, 0.05g; KH_2PO_4. 0.05g; $MgSO_4.7H_2O$, 0.1g; $FeSO_4.7H_2O$, 0.001g; $MnSO_4.4H_2O$, 0.001g; $CaCO_3$, 2.0g; Agar, 15.0g; H_2O, 1litre; pH, 7.0. The agar medium seeded with a 24h broth culture of the Methionine auxotroph, *Escherichia coli* (NCCBI) was spread inoculated with the isolate. An uninoculated agar plate served as control. Halo growth of the *E. coli* after 96h incubation at 30°C indicates Methionine production by the isolate. The active Methionine producers were stored for further studies.

2.1.1.1 Methionine accumulation in submerged culture of the isolate

Methionine production by the isolate in submerged medium was investigated. Medium composition was similar to that of the screening on agar but without agar, while glucose and ammonium sulphate were increased to 20.0g and 10.0g respectively. A 100ml Erlenmeyer flask containing 30ml of the fermentation medium was inoculated with 1ml (ca. $2.3x10^8$ cells/ml) of a 24h broth culture of the isolate grown in a seed medium (yeast extract, 10.0g; peptone, 10.0g; NaCl, 5.0g; H_2O, 1litre; pH 7.0) at 30°C and 120rpm. The flask was incubated for 72h on a rotary shaker at 160rpm and 30°C. Duplicate flasks were prepared and uninoculated flasks served as control. Methionine accumulation in the broth culture was determined.

2.1.1.1.1 Methionine assay

The broth culture of the isolate was centrifuged at 5000xg for 15min and the supernatant assayed for the presence of Methionine following the method described by Greenstein and Wintz (1961). To 5ml of the supernatant in a test tube was added 1ml of 5N NaOH and 0.1ml of 10% sodium nitropruside solution. The tube was thoroughly shaken and the mixture allowed to stand for 10min. Then 2ml of 5% aqueous solution of glycine was added to the reaction mixture with frequent shaking over a period of 10min. After an additional 10min interval, 2ml of concentrated orthophosphoric acid was added drop wise to the mixture and the test tube properly shaken. Colour development was allowed to proceed for 5min and colour intensity measured at 540nm in a spectrophotometer (Jenway 6405UV/Vis). The Methionine yield was extrapolated from a standard Methionine curve.

3. RESULTS AND DISCUSSION

Out of 200 bacterial isolates screened for Methionine production on solid agar medium, 24 of them were recovered as Methionine producers. It was observed that only bacterial isolates that released Methionine into agar medium stimulated the growth of the Methionine auxotroph, *E. coli,* seeded in the agar. From the nature of the halo growth of the *E. coli*, 6 of the bacterial isolates (Table 1) were chosen as active Methionine producers and were used in submerged fermentation for Methionine production.

Table 1. Screening test for Methionine production on solid agar and submerged medium

Isolate	Screening on solid agar	Screening in submerged medium
	Nature of halo growth of *E. coli*	mg/ml
CX250	+++	0.81
NU5	+++	0.80
SD170	++	0.51
IM67	++++	1.40
CY109	++	0.46
DH45	+++	0.89

As presented in Table 1, the Methionine yields of the active bacterial isolates range from 0.46mg/ml to 1.40mg/ml. The low yields of Methionine observed among the isolates may have resulted from the use of wild strains. This finding is supported by the work of Rowbury Woods (1961), who noted that wild type strains are not usually capable of producing significant amount of Methionine because their biosynthesis are highly regulated.

As shown in Table 1, a close relationship can be established between the nature of the halo growths of the *E. coli* on solid agar medium and the Methionine accumulation in submerged medium by the bacterial isolates. In essence, the number of colonies of *E. coli* on solid agar is directly proportional to the Methionine yield accumulated by the bacterial isolates in the fermentation medium.

4. CONCLUSION

This study, therefore, has shown a new and fast approach to screening for Methionine-producing bacteria using Methionine auxotroph. Also the very active producers can be speculated from the nature of the halo growths of the auxotroph on solid medium spread with the bacterial isolates. The method saves time and avoids the laborious method being presently used for isolation.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

Fong, C.V., Goldgraben, G.R., Konz, J., Walker, P., Zank, N.S. (1981). Condensation process of DL-Methionine production. In: Goldgraben, A.S. (Ed.), Organic chemicals manufacturing hazards. Ann Arbor Sci publishers, Ann Arbor MI, pp 115–194.

Greenstein, J.P., Wintz, M. (1961). Methionine. in: Chemistry of the amino acid Vol. 3. John Wiley and Sons, New York, pp 2121 -55.

Kase, H., Nakayama, K. (1975a). L-Methionine production by Methionine analog-resistant mutants of *Corynebacterium glutamicum*. Agric. Biol. Chem., 39, 153-160.

Kumar, D., Garg, S., Bisaria, V.S., Streekrishnan, T., Gomes, J. (2003). Production of Methionine by a multi-analogue resistant mutant of *Corynebacterium lilium* M128. Process Biochem., 38(8), 1165-1171.

Pharm, C.B., Galvzez, F.C., Padolina, W.G. (1992). Methionine production by batch fermentation from various carbohydrates. ASEAN Food J., 7, 34–37.

Rowbury, R.J., Woods, D.D. (1961). Further studies of the repression of Methionine synthesis in *Escherichia coli.* J. Gen. Microbiol., 24, 129–144.