STUDIES ON VITAMIN B_{12} PRODUCTION OF MICROORGANISM FROM ENVIRONMENTS

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ABSTRACT

A total of 421 microbial strains isolated from the soil and water samples were undertake in this study. Forty vitamin B_{12} producing strains were obtained by microbiological assay method, in which, five strains were capable of producing vitamin B_{12} more than 0.08 mg / L. And strain CCU-045 possess highest vitamin B_{12} producing ability, its yield high up to 0.174 mg / L.

The composition of optimal synthetic medium for CCU 045 to produce vitamin B_{12} was 3 % methanol (by volume); 1.5 g/L $(NH_2)_2CO$; 1.5 g/L $(NH_4)_2SO_4$; 3 mg / L $CoCl_2 \cdot 6H_2O$; 5 mg/L $FeSO_4 \cdot 7H_2O$; 5 mg/L $CaCl_2 \cdot 2H_2O$; 0.15 mg/L $MgSO_4 \cdot 7H_2O$; 0.2 mg /L yeast extract and 1.5 g /L for each of KH_2PO_4 and K_2HPO_4(pH 7.0). The optimal cultural conditions for vitamin B_{12} production were as follows: incubation temperature, 30°C; agitation, 115 rpm and 100mL medium in 500mL Hinton flask. The yield of vitamin B_{12} of CCU 045 cultivated for 7 days under this condition was 0.184 mg / L.

The products of vitamin B_{12}-producing strains including CCU 045 were analyzed by UV spectrophotometry, thin-layer chromatography and high performance liquid chromatography. All of these strains were confirmed to produce vitamin B_{12}.

KEY WORDS: *vitamin B_{12}, UV spectrophotometry, thin-layer chromatography, high performance liquid chromatograph.*

INTRODUCTION

In 1926, Minot and Murphy found that liver extracts could cure human pernicious anemia, a discovery for which they were awarded the Nobel Prize in medicine in 1934. Independently, Ricke and Smith isolated crystalline vitamin B_{12} from liver extracts in 1948. The vitamin B_{12} needs of animals are covered by food intake or by absorption of vitamin B_{12} produced by intestinal microorganisms. However, humans obtain vitamin B_{12} only from food, since vitamin B_{12} synthesized by microorganisms in the large intestinal tract cannot be assimilated[1)].

The concentrations of vitamin B_{12} which are present in animal tissues are too low to be used in commercial production. Activated sludge from sewage treatment contains 4-10 mg B_{12} / kg, but isolation from this source is expensive due to the difficulties in separating the various B_{12} analogues. Chemical synthesis is also impractical, since it requires 70 reaction steps. Vitamin B_{12} was first obtained commercially as a byproduct of streptomycete fermentations for the production of the antibiotics streptomycin, chloramphenicol, or neomycin, with a yield of about 1 mg/L. As the demand for vitamin B_{12} increased, fermentation processes were developed with high yield strains.

Most of the vitamin B_{12} fermentation processes use glucose as the carbon source. But the price of carbohydrate becomes higher and higher. In the past 10 years, various alcohol and hydrocarbon substrates as carbon source have been tested in a variety of strains for vitamin