PRODUCTION OF VITAMIN B_{12} BY ACTINOMYCETES

BY

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B. Sc. Agric. Sc. (Plant Pathology), Asyut University, 1994

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إنتاج فيتامين ب$_{12}$ بواسطة ألأكتينوميسيتات

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ABSTRACT

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Production of vitamin B_{12} by actinomycetes

The aim of the present study was to produce vitamin B_{12} by some actinomycetes using different raw materials such as molasses, oatmeal and cheese whey, moreover some environmental factors i.e. incubation period, initial pH, temperature and shaking rate at different levels were also studied.

One hundred and three actinomycete isolates obtained from different soils at various localities in Egypt and were purified. The isolates were morphologically investigated as described by **Bergey's Manual (1984)**. Results revealed that they are belonging to the genus *Streptomyces*.

Screening results revealed that only twelve of them were vitamin B_{12} producers representing 11.65% of total number of *Streptomyces* isolates and the level of vitamin B_{12} yield ranged between 0.78 to 1.70 µg ml^{-1}. The maximum production of the vitamin was produced by the *Streptomyces* isolate SW1, being 1.70 µg ml^{-1}, followed in descending order by *Streptomyces* isolates SW7, SR1A and SR1, being 1.45, 1.40 and 1.38 µg ml^{-1}, respectively . The aforementioned four isolates were completely identified up to species according to the keys proposed by **Shirling and Gottlieb ($1968_{a,b}$)** and **Bergey's Manual (1974)** as *Streptomyces baarnensis* strain SW1; *Streptomyces clavifer* strain SW7; *Streptomyces halstedii* strain SR1A and *Streptomyces nigrifaciens* strain SR1.

The four efficient *Streptomyces* strains SW1, SW7, SR1A and SR1 retested in modified starch nitrate fermentation medium to select the most efficient one in vitamin B_{12} production. Results revealed that the *Streptomyces baarnensis* SW1 proved to be the most efficient strain in vitamin B_{12} production, thus it was selected for subsequent study.

The obtained results revealed that the optimal production of vitamin B_{12} was reached under submerged culture condition in the modified fermentation medium containing 15 g L^{-1} molasses with initial pH value of 6 and a temperature of 28 ^{o}C after 4 days on a rotary shaker

at 200 rpm, being 53.20 µg ml^{-1}. Results also revealed that the optimal production of vitamin B_{12} was reached under submerged culture condition in the modified fermentation medium containing 15 g L^{-1} oatmeal extract with initial pH value of 7 and a temperature of 32 ^{o}C after 6 days on a rotary shaker at 200 rpm, being 61.08µg ml^{-1}.

Concerning the addition of cheese whey to the modified fermentation medium, results indicated that the optimal production of vitamin B_{12} was reached 2.79 µg ml^{-1} under submerged culture condition in the modified fermentation medium containing 40 ml L^{-1} cheese whey with initial pH value of 7 and a temperature of 32 ^{o}C after 5 days on a rotary shaker at 200 rpm.

In the light of aforementioned results, it could be concluded that the yield of vitamin B_{12} production by the *Streptomyces baarnensis* SW1 was affected by the type of raw material. The most efficient *Streptomyces baarnensis* strain SW1 produced the highest yield of the vitamin (61.08 µg ml^{-1}) in modified fermentation medium containing 15 g L^{-1} oatmeal extract, but, the yield decreased to 53.20 µg ml^{-1} in the same medium containing 15 g L^{-1} molasses, while the lowest yield of the vitamin was 2.79 µg ml^{-1} in the same medium containing 40 ml L^{-1} cheese whey under the same condition.

In vivo, the availability of vitamin B_{12}, derived from *Streptomyces baarnensis* SW1, was studied. Results indicated that vitamin B_{12} in dried mycelium of *Streptomyces baarnensis* SW1 was bioavailable.

Keywords: Identification, *Streptomyces baarnensis*, HPLC, Cyanocobalamin (B_{12}) , bioavailability

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