

PRODUCTION OF VITAMIN B₁₂ BY ACTINOMYCETES

BY

Wael Mohamed Abu El-Wafa

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

A thesis submitted in partial fulfillment
of
the requirements for the degree of

MASTER OF SCIENCE

in

Agricultural Science
(Agricultural Microbiology)

Department of Agricultural Microbiology
Faculty of Agriculture-Ain Shams University

2005

Approval Sheet

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Wael Mohamed Abu El-Wafa

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This thesis for M. Sc. degree has been approved by

Prof. Dr. Rabea Mohamed El-Shahawy -----

Prof. Emeritus of Agric. Microbiol., Fac. Agric., Cairo
University. (Fayom Branch)

Prof. Dr. Abd El-Mohsen Ahmed Refaat -----

Prof. Emeritus of Agric. Microbiol., Fac. Agric., Ain Shams
University

Prof. Dr. El-Sayed Ahmed Saleh -----

Prof. Emeritus of Agric. Microbiol., Fac. Agric., Ain Shams
University

Date of examination: 9 / 6 / 2005

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WAEEL MOHAMED ABU EL-WAFA

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Under the supervision of

Prof. Dr. El-Sayed Ahmed Saleh

Prof. Emeritus of Agric. Microbiology, Dept. of Agric. Microbiol., Fac. of Agric., Ain Shams University (principle supervisor)

Dr. Fathalla Hassan Ahmed

Associate Prof. of Microbiology, National Organization for Drug Control and Research

Dr. Mohamed Said Sharaf

Assistant Prof. of Agric. Microbiology, Dept. of Agric. Microbiol., Fac. of Agric., Ain Shams University

ACKNOWLEDGMENT

The author wishes to express his deepest gratitude to Prof. Dr. EL-Sayed A. Saleh, Prof. of Microbiology, Microbiology Dept., Faculty of Agric., Ain Shams University, for suggesting the problems, supervision, progressive criticism and scientific guidance.

Thanks is also to Assoc. Prof. Dr. Fathalla H. Ahmed, Head of General Branch of Medical and Basic Sciences, National Organization for Drug Control and Research (NODCAR) for supervision, encouragement and advices.

A deep thanks is also to Dr. Mohamed S. Sharaf, Assist. Prof. of Microbiology. Microbiology Dept., Faculty of Agric., Ain Shams Univ. for supervision, encouragement and scientific guidance.

Sincere appreciation to Dr. Essam Ezz El-din Hassan, Head of Drug Bioavailability Center, National Organization for Drug Control and Research (NODCAR) for providing the facilities and helpfulness during the study of the vitamin B₁₂ bioavailability.

Deep thanks are also due to the Head of Microbiology Department, National Organization for Drug Control and Research (NODCAR) and all staff members of Microbiology Dept. for the facilities offered and encouragement.

To my parents and my family, I wish to express my warmest thanks for their patience, encouragement and assistance.

إنتاج فيتامين ب12 بواسطة الأكتينوميسيتات

رسالة مقدمة من

وائل محمد أبو الوفا
بكالوريوس علوم زراعية (أمراض النبات) ، جامعة أسيوط، 1994

للحصول على

درجة الماجستير في العلوم الزراعية
(ميكروبيولوجيا زراعية)

قسم الميكروبيولوجيا الزراعية
كلية الزراعة- جامعة عين شمس

2005

صفحة الموافقة على الرسالة

إنتاج فيتامين ب₁₂ بواسطة الأكتينوميسيتات

رسالة مقدمة من

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للحصول على

درجة الماجستير في العلوم الزراعية
(ميكروبيولوجيا زراعية)

وقد تمت مناقشة الرسالة والموافقة عليها

اللجنة:

أ.د. ربيع محمد الشهاوى
أستاذ الميكروبيولوجيا الزراعية المتفرغ، كلية الزراعة ، جامعة القاهرة (فرع الفيوم)

أ.د. عبد المحسن أحمد رفعت
أستاذ الميكروبيولوجيا الزراعية المتفرغ، كلية الزراعة ، جامعة عين شمس

أ.د. السيد أحمد صالح
أستاذ الميكروبيولوجيا الزراعية المتفرغ، كلية الزراعة ، جامعة عين شمس

تاريخ المناقشة

2005 / 6 / 9

رسالة ماجستير

اسم الطالب: وائل محمد أبو الوفا

عنوان الرسالة: إنتاج فيتامين ب₁₂ بواسطة الأكتينوميستات

أسم الدرجة: ماجستير في العلوم الزراعية (ميكروبيولوجيا زراعية)

لجنة الإشراف:

أ.د. السيد أحمد صالح
أستاذ الميكروبيولوجيا الزراعية المتفرغ، قسم الميكروبيولوجيا الزراعية، كلية الزراعة
، جامعة عين شمس (المشرف الرئيسي)

د. فتح الله حسن أحمد
أستاذ مساعد، شعبة الميكروبيولوجي ، الهيئة القومية للرقابة والبحوث الدوائية

د. محمد سعيد شرف
مدرس الميكروبيولوجيا الزراعية، قسم الميكروبيولوجيا الزراعية، كلية الزراعة
، جامعة عين شمس

تاريخ البحث 2001/9

الدراسات العليا

أجيزت الرسالة بتاريخ
2005/ /

موافقة مجلس الجامعة

2005 / /

ختم الإجازة

موافقة مجلس الكلية

2005 / /

ABSTRACT

Wael Mohamed Abu El-Wafa

Production of vitamin B₁₂ by actinomycetes

The aim of the present study was to produce vitamin B₁₂ 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 B₁₂ producers representing 11.65% of total number of *Streptomyces* isolates and the level of vitamin B₁₂ 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 B₁₂ production. Results revealed that the *Streptomyces baarnensis* SW1 proved to be the most efficient strain in vitamin B₁₂ production, thus it was selected for subsequent study.

The obtained results revealed that the optimal production of vitamin B₁₂ 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 °C after 4 days on a rotary shaker

at 200 rpm, being 53.20 $\mu\text{g ml}^{-1}$. Results also revealed that the optimal production of vitamin B₁₂ 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 °C after 6 days on a rotary shaker at 200 rpm, being 61.08 $\mu\text{g ml}^{-1}$.

Concerning the addition of cheese whey to the modified fermentation medium, results indicated that the optimal production of vitamin B₁₂ was reached 2.79 $\mu\text{g ml}^{-1}$ 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 °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 B₁₂ 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 $\mu\text{g ml}^{-1}$) in modified fermentation medium containing 15 g L⁻¹ oatmeal extract, but, the yield decreased to 53.20 $\mu\text{g ml}^{-1}$ in the same medium containing 15 g L⁻¹ molasses, while the lowest yield of the vitamin was 2.79 $\mu\text{g ml}^{-1}$ in the same medium containing 40 ml L⁻¹ cheese whey under the same condition.

In vivo, the availability of vitamin B₁₂, derived from *Streptomyces baarnensis* SW1, was studied. Results indicated that vitamin B₁₂ in dried mycelium of *Streptomyces baarnensis* SW1 was bioavailable.

Keywords: Identification, *Streptomyces baarnensis*, HPLC, Cyanocobalamin (B₁₂) , bioavailability

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