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STUDY OF POSSIBLE USING OF *Streptomyces baarnensis* SW1 AS A NATURAL SOURCE OF VITAMIN B_{12}

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ABSTRACT

Streptomyces baarnensis strain SW1was the most efficient strain in vitamin B_{12} production. The effects of different concentrations of oatmeal extract, some environmental factors i.e. incubation period, initial pH, temperature and shaking rate at different levels were studied to obtain the maximum yield of vitamin B_{12} production. The results of the present study revealed that the maximum yield of vitamin B_{12} production by *Streptomyces baarnensis* strain SW1was reached under submerged culture conditions in modified fermentation medium containing 15 g L^{-1} of the most suitable raw material (oatmeal extract) with initial pH value 7 and temperature degree at 32 °C after 6 days on a rotary shaker at 200 rpm, being 61.08µg ml^{-1}.The ability of S. baarnensis SW1 dried mycelium as natural source of vitamin B_{12} was studied , the obtained results in vivo revealed that vitamin B_{12} derived from the dried mycelium is available .

Keywords: *S.baarnensis*, HPLC, cyanocobalamin (vitaminB_{12}), oatmeal extract.

INTRODUCTION

Vitamin B_{12} is an important cofactor for metabolism of carbohydrates, lipids and synthesis of proteins (Gottschalk, 1986), thus it is used in animal feeds. The vitamin is also applied in chemotherapy, especially against pernicious anemia (Scott, 1992) and involved in the manufacture of myelin sheath, a fatty layer which insulates cranial, spinal and peripheral nerves (Shane, 2000). Cobalamin is synthesized exclusively by bacteria but it is present in normal animal liver, where it exists as methylcobalamin, adenosylcobalamin and hydroxocobalamin, while plants and fungi are thought to neither synthesize nor use vitamin B_{12} in their metabolism (Duda *et al.*, 1967). Streptomycetes are very potent producers of useful metabolites, such as antibiotics, vitamins and other bioactive compounds (Bergey's Manual, 1984 and Abu El-Wafa *et al.*, 2005). Many reports pointed that vitamin B_{12} produced by *Streptomyces* spp, such as *S. albidoflavus*, *S. antibioticus*, *S. aureofaciens*, *S. colombiensis*, *S. fradiae*, *S. olivaceus*, *S. griseus* and *S. roseochromogenus* (Wood & Hendlin, 1952; Pfeifer *et al.*, 1954; Abou-Zied & Yousef, 1971; Salama & Kamal, 1983; Ibrahim, 1989 and Abd El-Meguid, 2000). Rauma *et al.* (1995) reported that some seaweeds could supply adequate amounts of bioavailabile vitamin B_{12} when consumed by strict vegetarians. Thus, it is still unclear whether the algal vitamin B_{12} is available to mammals.

MATERIALS AND METHODS

Strain used

Streptomyces baarninses SW1 was isolated from Egyptian soil and identified according to Shirlig & Gottlieb (1968) by Abu El-Wafa *et al.* (2005).

Media used

Modified starch nitrate medium contained: 20 g starch, 2 g KNO_3, 1 g K_2HPO_4, 0.5 g NaCl, 0.5 g $MgSO_4$, 0.01g $FeSO_4$, 1 g $CaCO_3$, 0.008 Co Cl_2. 6 H_2O g L^{-1} and 1 ml trace salts solution (Pridham *et al.*, 1958). This medium was used for preservation, growing and preparation of standard inoculum of *Streptomyces baarninses* SW1 (Abu El-Wafa *et al.*,2005).

Standard inoculum preparation

For preparation of the standard inoculum, the spores obtained from *Streptomyces baarninses* SW1 grown on starch nitrate agar medium (Waksman, 1961) for 14 days of incubation at 28±2 °C, were suspended with 10 ml of sterile distilled water, centrifuged for 10 min. at 3000 rpm (Hopwood & Ferguson, 1985). One ml of the suspension containing about $16x10^9$ spore ml^{-1} was inoculated in 50 ml of modified starch nitrate broth medium in 250 ml Erlenmeyer flask. The inoculated flasks were incubated at 28±2°C on a rotary shaker at 180 rpm for 4 days. The vegetative growth from each flask was used as standard inoculum. The mycelium growth obtained from each flask was washed by sterile distilled water for many times, dried at 70°C for 24 h and weighted.

Determination of the suitable concentration of oatmeal extract for vitamin B_{12} production

Oatmeal extract at the different concentrations of 5, 10, 15 and 20 g L^{-1} were added separately instead of starch in modified starch nitrate medium. The initial pH rate adjusted to 7 by phosphate buffer (British Pharmacopoeia, 2000). Fifty ml of every treatment in 250 ml Erlenmeyer flask was sterilized. After the sterilization, the inoculated flasks were incubated at 28±2°C on a rotary shaker at 180 rpm for 7 days. After incubation period, the vegetative growth of *Streptomyces baarninses* SW1 obtained from each flask was washed by sterile distilled water for many times, dried at 70°C for 24 h and then weighted. Vitamin B12 was extracted according to Hafez (1993), determined by HPLC method (Li *et al.*, 2000) and the average of the amount of vitamin B_{12} production was determined. Production of vitamin B_{12} using different environmental conditions such as incubation period, pH value, temperature degree and shaking rate were also determined.

In vivo availability of vitamin B_{12} derived from dried mycelium of *Streptomyces baarnensis* SW1

In vivo study was carried out on three groups of rats: first group was fed on diet (vitamin B_{12} free), the second group was fed on diet mixed with vitamin B_{12} supplement (5μg vitamin B_{12} / Kg diet) and the third group fed on diet mixed with dried mycelium of the selected *S. baarnensis* SW1 (equivalent to 5μg vitamin B_{12} per Kg diet). After 8 weeks, the rats groups were anesthetized then the peritoneal cavity was opened, liver was removed,

washed by 9 g L^{-1} sodium chloride solution and stored at -80 °C until analyzed. A portion of liver (one gram) was cut into small pieces, homogenized in 10 ml acetate buffer (pH 5). Vitamin B_{12} was extracted from liver homogenate by boiling with 20mg potassium cyanide for 30 min in the dark. The solution centrifuged at 10000 rpm for 10 min, filtration according to the method described by Watanabe *et al.* (1998) and the vitamin B_{12} assayed by the method described by Li *et al.* (2000).

Data were statistically analyzed by t test according to statistical methods described by Snedecor and Cochran (1980). (L S D. at 5%).

RESULTS AND DISCUSSION

Results recorded in Table (1) reveal that the mass production of vitamin B_{12} by *S. baarnensis* strain SW1 gradually increased with increasing oatmeal extract concentration. The highest yields of vitamin B_{12} production were 396.5 and 393.4 µg per 50ml culture in modified fermentation medium containing 20 and 15 g L^{-1} oatmeal extract, respectively, followed in decreasing order by 187.5 and 120.3 µg per 50ml in the same medium containing 10 and 5 g L^{-1} oatmeal extract , respectively . Although, the amount of vitamin B_{12} produced in modified fermentation medium containing 20 g L^{-1} oatmeal extract was higher than that containing 15 g L^{-1} oatmeal extract, the concentration of 15 g L^{-1} oatmeal extract was selected for subsequent study because the increase in vitamin B_{12} in medium containing 20 g L^{-1} did not exceed 0.78%.

Table (1): Production of vitamin B_{12} by *S. baarnensis* strain SW1 in modified fermentation medium containing oatmeal extract at different concentrations

Parameters*	Control medium**	Oatmeal extract concentrations (g /L)			
		5.0	10.0	15.0	20.0
Mycelium dry wt. (g per 50ml)	2.65	1.28	1.44	1.36	1.52
Vit. B_{12} yield (µg ml^{-1}) in extract	9.87	12.03	18.75	39.34	39.65
Total vit. B_{12} yield (µg / 50 ml culture)	98.70	120.3	187.5	393.4	396.5
Yield factor (µg g^{-1} dry mycelium)	37.25	93.98	130.21	289.26	260.86

*After the 7th day of incubation at 28±2 °C and pH 7.0, under submerged culture condition (180 rpm).

** Modified starch nitrate fermentation medium

Data recorded in Table (2) clearly indicate that the mycelium dry weight (g per 50ml culture) and the yield of vitamin B_{12} (µg ml^{-1}) of *S. baarnensis* SW1 increased gradually in modified fermentation medium containing 15 g L^{-1} oatmeal extract with the increasing of incubation period until the 6th day, then decreased thereafter. A maximal productivity of vitamin B_{12} was 46.54 µg ml^{-1} obtained from 2.02 g dry mycelium and total vitamin B_{12} yield was 465.4 µg per 50 ml culture after the 6th day. The percentage increase of vitamin B_{12} produced after 6 days of incubation was 18.27% from control treatment. Thus, this treatment was selected for the subsequent study. The obtained results confirmed those of Hall *et al.* (1953) they found that the highest yield

of vitamin B_{12} produced by *Streptomyces olivaceus* NRRL –B-1125 after 4 days of incubation. Abd El-Meguid (2000) reported that the best day of incubation period for production vitamin B_{12} by *Streptomyces griseus* in fermentation medium was the 5th day .On the other hand, Sultanova and Shechelkova (1971) determined the vitamin B_{12} produced by actinomycetes after 10 days of incubation.

Table (2): Effect of incubation period on the growth and production of vitamin B_{12} by *Streptomyces baarnensis* SW1* in modified fermentation medium containing 15 g L^{-1} oatmeal extract

Parameters	Incubation period (days)				
	3	4	5	6	7**
Mycelium dry weight (g / 50ml culture)	0.47	0.69	1.39	2.02	1.36
Vitamin B_{12} yield(µg ml^{-1}) in extract	9.87	10.83	14.68	46.54	39.35
Total vit. B_{12} yield (µg / 50 ml culture)	98.7	108.3	146.8	465.4	393.5
Yield factor (µg g^{-1} dry mycelium)	210.0	157.0	105.6	230.4	289.3

* At pH 7, temperature 28±2 °C and shaking rate at 180 rpm.
**Control treatment.

The modified fermentation medium containing 15 g L^{-1} oatmeal extract and incubated for 6 days (Table 3) gave the maximal production of vitamin B_{12} at pH 7. The amount of vitamin B_{12} was at the peak, being 46.54 µg ml^{-1} with 2.02 g dry mycelium per 50 ml culture and total vitamin B_{12} yield of 465.4 µg per 50 ml culture. These results are in agreement with those obtained by Saunders *et al.* (1952) who mentioned that the optimum pH for vitamin B_{12} production by various species of actinomycetes ranged from 6.0 to 7.0. Hester and Ward (1954) and Pfeifer *et al.* (1954), also found that pH 7-8 were the optimum for vitamin B_{12} production by *Streptomyces olivaceus* NRRLB-1125. Ibrahim (1989) reported that the maximum production of vitamin B_{12} was around pH 7, but pH 4 and 9 resulted in a very marked effect almost nil production of the vitamin, while Abd El-Meguid (2000) found that the range of pH from 5 to 8 was suitable for producing a high yield of vitamin B_{12} by *Streptomyces griseus* in fermentation medium.

Table (3): Effect of initial pH value on the growth and production of vitamin B_{12} by *S. baarnensis* SW1* in modified fermentation medium containing 15 g L^{-1} oatmeal

Parameters	Initial pH value					
	4	5	6	7**	8	9
Mycelium dry weight (g / 50ml culture)	0.31	1.13	2.4	2.02	1.75	0.145
Vitamin B_{12} yield (µg ml^{-1}) in extract	0.00	10.68	32.39	46.54	19.64	0.00
Total vit. B_{12} yield (µg / 50 ml culture)	0.00	106.8	323.9	465.4	196.4	0.00
Yield factor (µg g^{-1} dry mycelium)	0.00	94.51	135.0	230.4	112.2	0.00

* At 28±2 °C for 6 days and shaking rate at 180 rpm
** Control treatment

Data recorded in Tables (4) clearly indicate that the optimum incubation temperature for high production of vitamin B_{12} by *S. baarnensis* SW1 grown in modified fermentation medium containing 15 g L^{-1} oatmeal extract was 32 °C. This temperature was suitable for producing a high yield of vitamin B_{12} at

the rate of 54.48 µg ml^{-1} obtained from 1.89 g dry mycelium and total vitamin B_{12} yield of 544.8 µg per 50 ml culture for oatmeal extract medium. The percentage increase of vitamin B_{12} produced at 32 °C of incubation was 17.06 % in modified fermentation medium containing 15 g L^{-1} oatmeal as compared with control treatment (incubation temperature at 28 °C). Thus, it was selected for subsequent study. Many investigators studied the range of temperature for the process of vitamin B_{12} production by actinomycetes and they found that it extends from 24 to 30 °C (Abou-Zeid and Yousef 1971).These optimum temperatures are near those for production of the vitamin by other bacteria. Merck and Co-Inc (1971) found that the optimum incubation temperature was 28- 30°C, for *Pseudomonas denitrificans* SW1 growing under submerged aerobic condition. Cetin *et al.* (1979) reported that 28°C was the optimal temperature for producing vitamin B_{12} by *Propionibacterium freudenreichii.* Abd El-Meguid (2000) mentioned that optimum temperature for B_{12} production was 30°C by *Streptomyces griseus* and *Bacillus megaterium.*

Table (4): Effect of temperature on the growth and production of vitamin B_{12} by *S. baarnensis* SW1* in modified fermentation medium containing 15 g L^{-1} oatmeal extract

Parameters	Temperature degree (°C)				
	20	24	28**	32	36
Mycelium dry weight (g / 50ml culture)	0.25	1.56	2.02	1.89	1.50
Vitamin B_{12} yield (µg ml^{-1}) in mycel. ext.	0.00	12.97	46.54	54.48	10.18
Total vit. B_{12} yield (µg / 50 ml culture)	0.00	129.7	465.4	544.8	101.8
Yield factor (µg g^{-1} dry mycelium)	0.00	83.41	230.4	288.25	67.78

* After 6 days of incubation period, pH 7 and shaking rate at 180 rpm

** Control treatment

Concerning the effect of shaking rate on mycelium dry weight and vitamin B_{12} yield, the maximum production of the vitamin B_{12} for oatmeal medium was 61.08 µg ml^{-1} (total vitamin B_{12} yield 610.8 µg per 50 ml culture) , obtained at the shaking rate of 200 rpm. Dry mycelium mass, being 2.3 g per 50 ml culture, respectively for each raw material (Table 5). The percentage increase of vitamin B_{12} produced at the rate of 200 rpm as compared to 180 rpm (control) was 12.2 in modified fermentation medium containing 15 g L^{-1} oatmeal . Data are in agreement with the findings of Hall *et al* (1951) who studied the effect of aeration on the productivity of vitamin B_{12} by *Streptomyces griseus*, they found that the average yield of vitamin B_{12} was nearly 100% greater in flasks incubated on a reciprocal shaker (88-92 strokes min^{-1}). Abd El-Meguid (2000) reported that the maximum productivity of vitamin B_{12} was at 200 rpm for *Streptomyces griseus*, while static incubation resulted in almost no production of the vitamin.

It is of important to note that, from the above mentioned results, there is no clear correlation between the amount of vitamin B_{12} (µg) and mycelial dry weight (g) of *S. baarnensis* strain SW1. Therefore, yield factor (µg g^{-1} dry mycelium) could not be considered as a criterion for the total amount of vitamin B_{12} produced.

Table (5): Effect of shaking rate on the growth and production of vitamin B_{12} by *S. baarnensis* SW1* in modified fermentation medium containing 15 g L^{-1} oat meal extract

Parameters	Shaking rate (rpm)				
	0	140	160	180	200
Mycelium dry weight (g / 50ml culture)	1.91	1.04	1.24	1.89	2.3
Vitamin B_{12} yield (µg ml^{-1}) in extract	0.00	10.02	25.97	54.48	61.08
Total vit. B_{12} yield (µg / 50 ml culture)	0.00	100.2	259.7	544.8	610.8
Yield factor (µg g^{-1} dry mycelium)	0.00	93.35	209.4	288.25	265.6

* Incubation at 32±2 °C for 6 days and pH 7

** Control treatment

In vivo availability of vitamin B_{12} derived from *Strptomyces baarnensis* strain SW1

The statistical analysis of chromatographic separation of vitamin B_{12} assayed in livers extraction of rats groups, revealed that there are significant differences between the treatments. Diet mixed with vitamin B_{12} supplement (2.59µg ml^{-1}) or diet mixed with dried mycelium of *Streptomyces baarnensis* strain SW1 (2.21µg ml^{-1}) was significantly higher than the diet without addition of vitamin B_{12} (1.6 µg ml^{-1}). On the other hand, diet mixed with vitamin B_{12} supplement (2.59µg ml^{-1}) and diet mixed with dried mycelium of *Streptomyces baarnensis* strain SW1 (2.21µg ml^{-1}) showed no significant difference. These results indicated that vitamin B_{12} produced by *Streptomyces baarnensis* strain SW1 was available to rats.

Further studies are needed for the application of dried mycelium of *Streptomyces baarnensis* strain SW1 in animals nutrition.

REFERENCES

Abd El Meguid, Mona F. (2000). Study on the improvement of the production of cyanocobalamin by certain local isolates of gram-positive bacilli. M.Sc. Thesis, Faculty of Pharmacy, Cairo University,pp.110-126.

Abou-Zeid, A. and A. A. Yousef (1971). Fermentation production of tetracycilin, the anti-fungal antibiotic and vitamin B_{12} by *Streptomyces aureofaciens*. Pak. J. Sci. Ind. Res. 4 (12): 126-128. (Cited from Chem. Abst. 75 (11) 128478c ,1971).

Abu El-Wafa, W. M.; F.H. Ahmed; M.S. Sharaf and E. A. Saleh (2005). Production of vitamin B_{12} by some local isolates of streptomycetes J. Agric. Sci. Mansoura Univ., 30 (2): 1069 – 1082.

Bergey's Manual of Systematic Bacteriology (1984). Streptomycetes and related genera (Vol. 4). Williams, S. T. (ed.) Williams and Wilkins. Baltimore, USA.,: 2451-2469.

British Phrmacopeia (2000). Magesty's stationary office, P.(77-17), P. (86-1).

Cetin, E. T.; S. Tankan; T. B. Gurler and A. Filize (1979). The preparation of vitamin B_{12} by fermentation. Arstima a kurmi 6 : pp.805-812. (Cited from Chem. Abst. 92 (21) 1429703, 1980).

Duda, J.; Z. Pedziwilk and K. Zodrow (1967). Studies on the vitamin B_{12} content of the leguminous plants. Acta Microbiol. Pol., 6: 233-238.

Gottschalk, G. (1986). Bacterial metabolism, 2 ed. pp.244-246. New York, Springer Verlag ISBN 3-54096153-4.

Hafez, M. M. (1993). Studies on isolation of vitamin B_{12} from certain local microbial isolates. M. Sc. Thesis, Faculty of Pharmacy, Zagazig University,Egypt.

Hall, H. H.; J. C. Benjamin; C. F. Wiksen and H. M. Tsuchiya (1951). Production of vitamin B_{12} by microorganisms especially *Streptomyces olivaceus*. Abstract paper of the Am. Chem. Soc., 119th Meeting, 22A.

Hall, H. H.; R. G. Benedict; C. F. Wiesen; C. E. Smith and R. W. Jackson (1953). Studies on vitamin B_{12} production with *Streptomyces olivaceus*. Appl. Microbiol., 1:124.

Hester, A. S. and G. E. Ward (1954). Vitamin B_{12}. Feed supplement. Ind. Eng. Chem., 46: 238-243.

Hopwood, D. A. and H. M. Ferguson (1985). A rapid method for lyophilization *Streptomyces* cultures. J. Appl. Bacteriol., 32: 434-436.

Ibrahim, H. K. (1989). A study on cyanocobalamin activity of certain isolated local microorganisms. Ph. D. Thesis, Faculty of Pharmacy, Zagazig University, Egypt.

Li, H. B.; F. Chem and Y. Jiang (2000). Determination of vitamin B_{12} in multivitamin tablets and fermentation medium by high performance liquid chromatography with fluorescence detection. J. Chromatogr. A., 891 (2): 243: 247.

Merck and Co. Inc. (1971). Compounds with vitamin B_{12} activity by fermentation. (Cited from Chem. Abst., 75 (8): 124326r ,1971).

Pfeifer, V. F.; C. Vojnovich and E. N. Heger (1954). Vitamin B_{12} by fermentation with *Streptomyces olivaceus*. Ind. Eng. Chem., 46: 843-849.

Pridham, T. G.; C. W. Hesseltine and R. G. Benedict (1958). A guide for classification of streptomycetes according to selected groups: Placement strains in morphological sections. Appl. Microbiol., 6: 52-79.

Rauma, A. L.; R. Torroen; O. Hanninen and H. Myakkanen (1995). Vitamin B_{12} status of long-term adherents of a strict uncooled vagan diet (living food diet) in compromised. J. Nutr., (125):2511-2515.

Rudkin, G. O. and R. J. Taylor (1952). Chemical method for determing vitamin B_{12}. Anal. Chem., 24: 1155.

Salama, M. A. and Z. Kamal (1983). Some factors affecting vitamin B_{12} biosynthesis by *Streptomyces*. Proceeding of the V Congress of Microbiology, Cairo, Volume 1, Part 1, Fermentation.

Saunders, A. P.; R. H. Otto and J. C. Sylvester (1952).The production of vitamin B_{12} by various strains of actinomycetes. J. Bacteriol., 64: 725-728.

Scott, J. M. (1992). Folate-vitamin B_{12} interrelationships in the central nervous system. Proc. Nutr. Soc., 51:219-224.

Shane, B. (2000). Folic acid, vitamin B_{12} and vitamin B_6. In: Stipanuk, M. (Ed). Biochemical and physiological aspects of human nutrition. Philadelphia: W. B. Saunders Co., pp.483-518.

Shirling, E. B. and D. Gottlieb (1968). Cooperative description of type cultures of *Streptomyces* III. Additional Species descriptions from first and second studies. Int. J. Syst. Bacteriol., 18: 279.

Snedecor, G. W. and W. G. Cochran (1980). In: Statistical Methods, Iowa State., Univ. Press Ames., p. 233.

Sultanova, I. G. and S. S. Shechelkova (1971). Vitamin B_{12} in cultures of cellulose decomposing actinomycetes isolated from rumen. Dok. Acad. Nauk Uzb. SSR. 28 (4): 69-70.

Waksman, S. A. (1961). The actinomycetes. Vol. II. Classification and Description of Genera and Species. In. The Williams and Wilkins Co. (Eds.). P. 430 Baltimore, U.S.A.

Watanabe, F.; S. Takenaka; K. Abe; Y. Tamura and Y. Nakano (1998). Comparison of a microbiological assay and a fully automated chemiluminescent system for the determination of vitamin B_{12} in food. J. Agric. Food Chem., 46:1433-1436.

Wood, T. R. and D. Hendlin (1952). Process for production of vitamin B_{12}. U. S. Patent, 2, 499,595.

دراسة إمكانية إستخدام *Streptomyces baarnensis* SW1 كمصدر طبيعي لفيتامين ب١٢

فتح الله حسن أحمد[١] ، وائل محمد أبو الوفا[١] ، محمد سعيد شرف[٢] و السيد أحمد صالح[٢]

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لقد أمكن الحصول علي اكبر كمية من فيتامين ب ١٢ بواسطة *Streptomyces baarnensis* SW1 النامية في بيئة التخمير المعدلة والمحتوية علي التركيز الأمثل من مستخلص الشوفان(١٥ جم / لتر) وتحت الظروف المثلي من مدة التحضين (٦ايام) وال pH الأولي (٧) ودرجة الحرارة (٣٢م°) معدل الرج (٢٠٠لفة/دقيقة) كانت ٦١,٠٨ ميكروجرام/ مل مستخلص .وقد تم دراسة مدي قابلية التغذية علي الميسليوم المجفف للسلالة *S. baarnensis* SW1 كمصدر طبيعي لفيتامين ب ١٢ للحيوانات الثديية (الجرذان) مقارنة بمستحضر دوائي لفيتامين ب١٢ وأثبتت النتائج أن الفيتامين الموجود داخل الميسليوم المجفف متاحاً حيويا .