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PRODUCTION OF VITAMIN B_{12} BY SOME LOCAL ISOLATES OF STREPTOMYCETES

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ABSTRACT

Out of 103 Egyptian local actinomycete isolates, *Streptomyces* SW1 was selected for vitamin B_{12} production. This isolate was completely identified as *Streptomyces baamensis*. The aim of the present study was to select a cheap agricultural or manufactural by-product in the modified starch nitrate fermentation medium for higher vitamin B_{12} production. Results revealed that molasses at 15 g L^{-1} , instead of starch in modified starch nitrate medium, proved to be the best by-product for vitamin B_{12} production by the *S. baamensis* strain SWI. Moreover, some environmental factors i.e. incubation period, initial pH value, temperature and shaking rate at different levels were tested for the optimization of fermentation process to yield the highest cyanocobalamin concentration. Results clearly showed that the optimal production of vitamin B_{12} was reached under submerged culture condition on 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 the rate of 200 rpm, being 53.20 µg ml^{-1}.

Keywords: Cyanocobalamin, *Streptomyces baarnensis*, HPLC, Identification

INTRODUCTION

Vitamin B_{12}, or cobalamin is the largest and the most complex of all the vitamins, consisting of a corrin ring similar to the porphyrins that includes a cobalt ion at its center (Hodgkin *et al.*, 1955). A cyanogroup is usually attached to the cobalt as an artifact of isolation and must be removed in the body before cobalamin can be converted to its active form (Herbert, 1988 and Brody, 1999). Vitamin B_{12} is an important cofactor for metabolism of carbohydrates, lipids and the 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 is present in normal animal liver, where it exists as methylcobalamin, adenosylcobalamin and hydroxycobalamin, while plants and fungi are thought to neither synthesize nor use B_{12} in their metabolism (Duda *et al.*, 1967). Actinomycetes are one of the most important microbial groups in soil, they include streptomycetes which are very potent producers of useful metabolites, such as antibiotics , vitamins and other bioactive compounds (Bergey's Manual, 1984). 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* (Rickes *et al.*, 1948; Hall *et al.*, 1951; Jackson *et al.*,1951; Tarr, 1951; Principe & Thornberry,1952; Wood & Hendlin, 1952; Pfeifer *et al.*,

1954; Abou-Zied & Youssef, 1971; Salama & Kamal, 1983; Ibrahim, 1989 and Abd El Meguid, 2000). The present study was carried out to evaluate the efficiencies of some selected local actinomycete isolates in vitamin B_{12} production. Moreover, the most efficient isolate in this respect was identified up to species. Different raw materials i.e. molasses, oatmeal and cheese whey as well as some environmental factors i.e. incubation period, pH level, temperature degree and shaking rate affecting the fermentation process by the most efficient species were achieved.

MATERIALS AND METHODS

Samples

Soil samples were collected from different localities in Arab Republic of Egypt for isolation of actinomycetes, taken from 10-20 cm depth, dried at room temperature, then mixed with $CaCO_3$ (10%) and incubated at 28±2 °C for 7 days under water saturated condition (Tsao *et al.*, 1960 and Kutzner, 1981).

Isolation of actinomycetes

Starch nitrate agar medium (Waksman, 1961) was inoculated with the pre-treated soil samples using the plate dilution procedure and incubated at 28±2°C for 14 days to isolate the actinomycetes (Kutzner,1981). The actinomycete isolates were picked up and re-cultivated several times under the same conditions of isolation for purity, then, the purified actinomycete isolates were grouped into 6 different series according to the color of the aerial mycelium and preserved on starch nitrate agar medium in a refrigerator at 4°C until used. (Tresner & Backus, 1963; Kutzner, 1972 and Bergey's Manual, 1974). The purified actinomycete isolates were identified up to genus according to their cultural and morphological characteristics (Bergey's Manual, 1984).

Media used

Four different media were used throughout this investigation. The composition of each medium is given in gram per liter distilled water or as otherwise stated.The activation medium contained: 5 g corn steep solids, 5 g peptone, 10 g glucose, 5 g NaCl and 1 g $CaCO_3$, with a pH before autoclaving of 6.8 (Saunders *et al*, 1952). This medium was used for activation of streptomycete isolates . The screening medium (Saunders *et al*, 1952) containing: 15 g soybean meal, 5 g corn steep solids, 15 g glucose, 2 g yeast extract, 3 g NaCl, 1 g NH_4NO_3, 2 g $CaCO_3$, 5 g glycerol and 0.0025 g $CoCl_2.6H_2O$. The pH before autoclaving was 7. This medium was used for screening of actinomycete isolates producing vitamin B_{12}. Starch nitrate medium (Waksman, 1961) 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 $CaCO_3$ and 1 ml trace salts solution (Pridham et al., 1958). This medium was used for preservation, growing and preparation of standard inoculum of actinomycete isolates. The modified starch nitrate fermentation medium employed for vitamin B_{12} production contained the same previous components but $CoCl_2.6H_2O$ was added at the rate of 0.008 g L^{-1}.

Screening of *Streptomyces* isolates producing vitamin B_{12}.

The heavy spores obtained from the slant culture of each isolate of purified 103 isolates, which grown on starch nitrate agar medium for 14 days at 28±2°C, were inoculated into 50 ml of the activation medium in 250 Erlenmeyer flask and incubated on a rotary shaker 180 rpm at 24±2°C for 2 days . Two ml of previous vegetative inoculum medium were transferred into 50 ml screening medium in 250 Erlenmeyer flask and incubated under the same conditions for 7 days .After incubation period vitamin B_{12} was determined according to the method described by Saunders *et al*, (1952).

Assay of vitamin B_{12}

Vegetative growth in each flask were centrifuged at 3000 rpm for 5 min. and washed thereafter with sterilize distilled water for many times. The mycelium growth was suspended with 10 ml of 0.2 M acetate buffer (pH 5.5) containing 0.001% KCN , then the suspension was autoclaved for 20 min. at 121°C and filtered by filter No.1 (Hafez, 1993). The vitamin B_{12} probability produced was identified and assayed (µg ml^{-1}) using HPLC (Perkin Elmer 785 A UV/V15 detector and equipped with Zorbax SB C18 column, 4.6 x 300 mm) according to the method described by Li *et al* (2000). Cyanocobalamin from USP was used as reference standard. The most efficient isolates in vitamin B_{12} production in screening medium (Saunders *et al.*, 1952) were retested under the same conditions in modified starch nitrate fermentation medium. The yield factor of vitamin B_{12} (µg g^{-1} dry mycelium) was also determined.

Identification of the most efficient *Streptomyces* isolate in vitamin B_{12} production

The identification of *Streptomyces* isolate up to species was originally based on cultural, morphological and physiological characteristics according to the International *Streptomyces* Project (ISP) methods as described by Shirling and Gottlieb (1966).

Cultural and morphological characteristics:

The color of the aerial mycelium was observed by the naked eye after 7,14 and 21 days on 4 standard media including oatmeal agar, yeast and malt extract agar, glycerol-asparagine agar and inorganic salts-starch agar (Shirling and Gottlieb,1966). The color of substrate mycelium and those of the soluble pigment were also examined on the same media. Spore chain morphology and spore surface ornamentation were examined by light and transmission electron microscopes, respectively on inorganic salts starch agar medium after 14 days of incubation at 28±2°C (Shirling and Gottlieb,1966).

The physiological characteristics:

The selected *Streptomyces* isolate was investigated for its ability to produce melanoid pigment on tyrosine agar and peptone–yeast extract iron agar media after 2 and 4 days of incubation at 28 ±2°C, ability to grow on Czapek´s agar medium (Prauser and Folta, 1968), tolerance to different concentrations of sodium chloride (Tresner *et al*, 1968) on inorganic salt starch agar medium (Shirling and Gottlieb,1966), sensitivity for streptomycin

sulphate (100 µg ml^{-1}) on Bennet's agar medium (Jones, 1949), antimicrobial activity against 10 test organisms including bacteria, fungi and yeasts according to the method described by British Pharmacopoeia (2000) and utilization of 11 carbon sources (1%) on inorganic salt starch agar medium (Shirling and Gottlieb,1966).Identification of *Streptomyces* isolate up to species was carried out using the keys of Shirling and Gottlieb, 1968 and Bergey's Manual (1974).

Standard inoculum preparation

For preparation of the standard inoculum, the spores obtained from the selected *Streptomyces* isolate SW1 (that gave highly production of vitamin B_{12}) 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 *et al*, 1985). One ml of the suspension containing about $16x10^9$ spore ml^{-1} was inoculated in 50 ml of 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 and 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 different raw materials for vitamin B_{12} production

Molasses and oatmeal extract at the different concentrations of 5, 10, 15 and 20 g L^{-1} and cheese whey at various rates of 20, 30, 40 and 50 ml 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 the efficient selected *Streptomyces* isolate obtained from each flask was washed by sterile distilled water for many times, dried at 70°C for 24 h and then weighted. Vitamin B_{12} was extracted according to Hafez (1993) and 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 done.

RESULTS AND DISCUSSION

Screening of actinomycete isolates for vitamin B_{12} production

One hundred and three actinomycete isolates were isolated 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* as they form well developed branching, non-septate, non-fragmented aerial mycelia bearing a long spore chains and non-motile spores which not borne in verticillate sporophores. *Streptomyces* isolates were divided into 6 groups according to their color of aerial mycelium (Tresner and Backus, 1963). Data in Table (1) clearly show 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 by *Streptomyces* isolates SW7, SR1A and SR1 being 1.45, 1.40 and 1.38 µg ml^{-1}, respectively .Data are in confirmation with the findings of Salama and Kamal (1983), who reported that 11% of *Streptomyces* isolates obtained from soil were vitamin B_{12} producers. However, Abd El Meguid (2000) found that, 33% of *Streptomyces* isolates produced vitamin B_{12}.

Table (1): Screening of *Streptomyces* isolates according to their efficiencies in producing vitamin B_{12} using Saunders medium.

Color of *Streptomyces* series.	Number and percentage of *Streptomyces* isolates		*Streptomyces* (produced vit. B_{12})		Total and percentage of vit. B_{12} producers	
	No.	%	Isolate No.	Vit. B_{12} (µg/ml)	Total isolates producers	Percentage of vit. B_{12} producers
Gray	58	56.32%	SR1 SR1A SR31 SR11 SR16 SR45	1.38 1.40 1.07 0.90 1.20 0.78	6	5.83%
Green	11	10.68%	-	-	-	0.0%
Red	4	3.88%	SD1 SD2	1.08 1.30	2	1.94%
Violet	2	1.94%	-	-	-	0.0%
Yellow	15	14.56%	SY8 Sy11	1.05 0.78	2	1.94%
White	13	12.62%	SW1 SW7	1.70 1.45	2	1.94%
Total	103	100%	12	-	12	11.65%

The previous four *Streptomyces* isolates were retested in modified starch nitrate fermentation medium to select the most efficient one in vitamin B_{12} production. Data presented in Table (2) clearly show that the level of vitamin B_{12} yield in modified starch nitrate fermentation medium was at the range of 0.8 to 9.87 µg ml^{-1}, total vitamin productivity was about of 8 to 98.7 µg per 50 ml culture and yield factor was between 3.38 to 37.25 µg g^{-1}dry mycelium. The maximum production of the vitamin was produced by the *Streptomyces* isolate SW1, being 9.87 µg $ml^{-1}$with total vitamin yield of 98.7 µg per 50 ml culture and yield factor of 37.25 µg g^{-1} dry mycelium. However, the vitamin yield per ml, total production of vitamin and yield factor for *Streptomyces* isolates SR1, SR1A and SW7 were 2.22, 1.44 and 0.80 µg ml^{-1} ; 22.2, 14.4 and 8.0 µg per 50 ml culture and 7.60, 7.72 and 3.38 µg g^{-1} dry mycelium, respectively .Therefore, *Streptomyces* isolate SW1 proved to be the most efficient *Streptomyces* isolate for vitamin B_{12} production, thus it was selected for subsequent study.

Table (2): Production of vitamin B_{12} by the most efficient isolates of *Streptomyces* in modified starch nitrate fermentation medium.

Parameters	*Streptomyces* isolates			
	SW1	SW 7	SR1	SR1A
Mycelium dry weight(g / 50ml)	2.65	2.37	2.92	1.98
Vitamin B_{12} yield (µg ml^{-1}) in extract	9.87	0.80	2.22	1.44
Total vitamin B_{12} yield (µg / 50 ml culture)	98.70	8.0	22.2	14.4
Yield factor (µg g^{-1} dry mycelium)	37.25	3.38	7.60	7.72

Identification of *Streptomyces* isolate SW1

The most efficient *Streptomyces* isolate SW1 in vitamin B_{12} production was identified up to species by studying their cultural, morphological and physiological characteristics according to the keys proposed by Shirling and Gottlieb (1968) and Bergey's Manual (1974) for the complete identification of *Streptomyces*. Data in Table (3) and Figures 1 and 2 clearly show that the experimental *Streptomyces* isolate SW1 seemed to be related to *Streptomyces baarnensis* (Shirling and Gottlieb, 1968) and *Streptomyces clavifer* (Bergey's Manual, 1974)

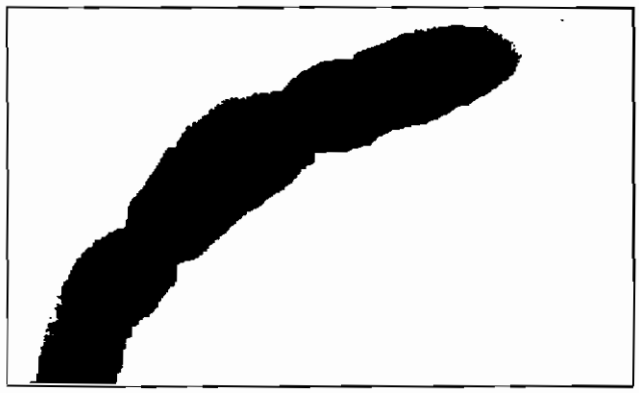

Fig (1): **Electron micrograph of spore morphology of *Streptomyces* isolate SW1 (Transmission x – 4000).**

Fig (2): Micrograph of spore chain morphology of Streptomyces isolate SW1 (x- 400).

Table (3): Cultural, morphological and physiological characteristics of the selected *Streptomyces* isolate SW1 as compared with those of similar species reported in different identification keys

Characteristics	Local *Streptomyces* isolate SW1	*S. baarnensis*[1]	*S. clavifer*[2]
(1)Cultural characteristics Color of aerial mycelium	White	White	White
Color of substrate mycelium	Colorless	Colorless	Colorless
Diffusible pigments	-	-	-
(2)Morphological characteristics Spore chain morphology	Flexuous	Flexuous	Flexuous
Spore surface ornamentation	Smooth	Smooth	Smooth
(3)Physiological characteristics Melanoid pigment produced	-	-	-
Growth on Czapek`s medium	Excellent	Excellent	Poor
Sodium chloride tolerance	≥ 4	ND	≥ 10
Sensitivity to streptomycin	Sensitive	Sensitive	ND
Antimicrobial activity:	-	ND	ND
Utilization of different carbon sources No Carbon	-	-	-
D-Glucose	+	+	+
D-Xylose	+	+	+
L-Arabinose	+	+	-
L-Rhamnose	-	+	+
D-Fructose	+	+	+
Galactose	+	+	+
Raffinose	ND	-	-
D-Mannitol	+	+	+
Inositol	+	±	-
Salicin	+	ND	-
Sucrose	+	±	ND

1- According to Shirling and Gottlieb (1968)
2- According to Bergey's Manual (1974) ND: Not determined

However, the experimental isolate differed from *Streptomyces clavifer*, where the later species could utilize L-rhamnose, but it could not utilize L-arabinose, l-inositol and salicin and the growth on Czapek´s medium was poor. On the other hand, the various properties of the experimental isolate appeared to be in harmony with those of *Streptomyces baarnensis* with slight difference in L-rhamnose utilization. Therefore, the experimental isolate SW1 was identified as a strain of *Streptomyces baarnensis*. This isolate was selected for the following experiments.

Production of vitamin B_{12} by *Streptomyces baarnensis* SW1 in fermentation medium containing different raw materials

Data in Table (4) clearly show that the optimum concentrations for vitamin B_{12} production using different raw materials such as molasses, oatmeal and cheese whey in modified starch nitrate fermentation medium (pH7) after the 7th day of incubation at 28±2°C under submerged culture condition (180 rpm) were 15, 15 g L^{-1} and 40 ml L^{-1}, respectively.

Table (4): Production of vitamin B_{12} by *Streptomyces baarnensis* strain SW1 in fermentation medium containing different concentrations of raw materials

Mass production	Control medium Starch nitrate	Raw materials concentrations ($g\ L^{-1}$) 5.0 Molasses	5.0 Oatmeal	10.0 Molasses	10.0 Oatmeal	15.0 Molasses	15.0 Oatmeal	20.0 Molasses	20.0 Oatmeal	Cheese whey ($ml\ L^{-1}$) 20	30	40	50
Mycelium dry wt. (g per 50ml)	2.65	1.92	1.28	2.66	1.44	3.49	1.36	3.27	1.52	1.23	1.61	2.52	2.97
Vit. B_{12} yield ($\mu g\ ml^{-1}$) in extract	9.87	28.36	12.03	34.88	18.75	45.80	39.34	45.61	39.65	1.15	1.30	1.85	1.75
Total vit. B_{12} yield (μg) in 50ml medium	98.70	283.6	120.3	348.8	187.5	458.0	393.4	456.1	396.5	11.5	13.0	18.5	17.5
Yield factor ($\mu g\ g^{-1}$ dry mycelium)	37.25	147.71	93.98	131.13	130.21	131.23	289.26	139.48	260.86	9.35	8.07	7.34	5.89

The highest yield of vitamin B_{12} produced by *Streptomyces baarnensis* SW1 was obtained in modified starch nitrate fermentation medium containing 15 g L^{-1} molasses being 458.0 µg per 50 ml culture following in decreasing order by 20 and 10 g L^{-1} being 456.1 and 348.8 µg per 50 ml culture, respectively The mass production of *Streptomyces baarnensis* increased gradually with increasing of molasses concentrations in modified starch nitrate fermentation medium. It was found that the optimum concentration of molasses was 15 g L^{-1} for producing 45.8 µg ml^{-1} of vitamin B_{12} with 3.49 g mycelium dry weight per 50 ml culture (Table 4). White and Demain (1971) and Shteinbery and Datsiuk (1985) reported that molasses has contained betaine and this component stimulated the vitamin B_{12} production by microorganisms.

Environmental factors affecting vitamin B_{12} production

From the previous results, it could be confirmed that modified starch nitrate fermentation medium containing 15 g L^{-1} molasses was the most suitable medium for vitamin B_{12} production by *Streptomyces baarnensis* SW1. Environmental conditions affecting vitamin B_{12} production such as incubation period, initial pH of medium, incubation temperature and shaking rate were studied.

Effect of incubation period

The obtained results in Table (5) clearly show that the vegetative growth of selected isolate (SW1) and its production of vitamin B_{12} increased gradually in modified starch nitrate fermentation medium containing 15 g L^{-1} molasses with the increasing of incubation period until the 4th day, then slightly decreased up to 7th day (Table 5). The highest yield of vitamin B_{12} was 45.81 µg ml^{-1} (total vitamin B_{12} produced in 50ml culture was 458.1 µg) exist in 3.48 g dried mycelium after 4 days of incubation at 28°C. The results confirmed those of Abd El Meguid (2000) who reported that the best day of incubation period for production of vitamin B_{12} by *Streptomyces griseus* in fermentation medium with pH 7 at 30°C on rotary shaker with 200 rpm was the 5th day, but the amount of the vitamin obtained was lower than those produced in the present study. On the other hand, Hall *et al.*, (1953) found that the highest yield of vitamin B_{12} produced by *Streptomyces olivaceus* NRRL –B-1125. after 4 days of incubation. Also, Sultonova and Shchelkova (1971) determined the vitamin B_{12} produced by actinomycetes after 10 days of incubation.

Table (5): Effect of different incubation periods on the production of vitamin B_{12} by *Streptomyces baarnensis* SW1*

Parameters	Incubation period (days)				
	3	4	5	6	7**
Mycelium dry weight (g / 50ml)	1.59	3.48	3.39	3.29	3.49
Vitamin B_{12} yield (µg ml^{-1}) in extract	29.71	45.81	45.80	45.66	45.80
Total vit. B_{12} yield (µg / 50 ml culture)	297.1	458.1	458.0	456.6	458.0
Yield factor (µg g^{-1} dry mycelium)	186.86	131.64	135.10	138.78	131.23

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

Effect of initial pH

It is obvious from data in Table (6) that the mass production of *Streptomyces baarnensis* SW1 gradually increased with increasing pH of the medium until pH 6, then the vegetative growth and the yield of vitamin B_{12} decreased thereafter. The highest values of mycelium dry weight, vitamin B_{12} yield and total vitamin-produced at the level of pH 6 were 3.43 g per 50 ml culture, 51.64 µg ml^{-1} and 516.4 µg per 50 ml culture, respectively. 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 olivcaceus* NRRLB-1125. Ibrahim, (1989), reported that the maximum production of vitamin B_{12} was around pH 7.0, 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 (6): Effect of different pH levels on the production of vitamin B_{12} by *Streptomyces baarnensis* SW1*

Parameters	Initial pH value					
	4	5	6	7**	8	9
Mycelium dry weight (g / 50ml)	0.25	2.42	3.43	3.48	1.69	1.35
Vitamin B_{12} yield (µg ml^{-1}) in extract	0.00	36.92	51.64	45.81	21.51	0.00
Total vit. B_{12} yield (µg / 50 ml culture)	0.00	369.2	516.4	458.1	215.1	0.00
Yield factor (µg g^{-1} dry mycelium)	0.00	152.56	150.55	131.64	127.28	0.00

*** Incubation at 28 °C for 4 days and shaking rate180 rpm**
**** Control treatment**

Effect of temperature

Data in Table (7) obviously show that the optimum temperature was 28°C to produce the highest amount of vitamin B_{12}, being 51.64 µg ml^{-1} (total vitamin was 516.4 µg per 50 ml culture) at the pH 6 . These amount of vitamin B_{12} produced from 3.43 g dry mycelium of *Streptomyces baarnensis* SW1 in 50 ml medium. The increasing of temperature degrees maximized the vitamin production until 28°C, then sharply decreased thereafter.

Table (7): Effect of different temperatures on the production vitamin B_{12} by *Streptomyces baarnensis* SW1*

Parameters	Temperature degree (°C)				
	20	24	28**	32	36
Mycelium dry weight (g / 50ml)	0.21	2.57	3.43	2.91	2.59
Vitamin B_{12} yield (µg ml^{-1}) in extract	0.00	36.69	51.64	41.21	36.93
Total vit. B_{12} yield (µg / 50 ml culture)	0.00	366.9	516.4	412.1	369.3
Yield factor (µg g^{-1} dry mycelium)	0.00	142.76	150.55	141.62	142.59

*** After 4 days of incubation period , pH 6 and shaking rate180 rpm**
**** Control treatment**

These results are nearly confirming to optimum temperature for production of 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*.

Effect of shaking rate

Concerning the effect of shaking rates on mycelium dry weight and vitamin B_{12} yield, it was found from the current data (Table 8) that the maximum production of the vitamin (53.2 µg ml^{-1}) was obtained at the shaking rate of 200 rpm with dry mycelium mass, being 3.33 g per 50 ml culture (total vitamin was 532 µg per 50 ml culture) with pH 6 at 28°C in the selected fermentation medium. Data were in agreement with the findings of Hall *et al* (1951) who studied the effect of the aeration on the productivity of vitamin B_{12} by *Bacillus lentus* and *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 stokes min). Abd El Meguid (2000) reported that the maximum productivity of vitamin B_{12} was at 200 rpm for either *Bacillus lentus* or *Streptomyces griseus*, static incubation resulted in almost no production of the vitamin for both preceding organisms.

Table (8): Effect of different shaking rates on the production of vitamin B_{12} by *Streptomyces baarnensis* SW1*

Parameters	Shaking rate (rpm)				
	0	140	160	180**	200
Mycelium dry weight (g / 50ml^{-1})	2.00	1.70	2.09	3.43	3.33
Vitamin B_{12} yield (µg ml^{-1}) in extract	0.00	27.93	29.52	51.64	53.20
Total vit. B_{12} yield (µg / 50 ml culture)	0.00	279.3	295.2	516.4	532.0
Yield factor (µg g^{-1} dry mycelium)	0.00	164.29	141.24	150.55	159.76

* Incubation at 28 °C for 4 days and pH 6
** Control treatment

It is thereafter, clear that the highest yield of vitamin B_{12} production was 53.2 µg ml^{-1} by *Streptomyces baarnensis* Sw1 in modified starch nitrate fermentation medium containing 15 g L^{-1} molasses and incubated at 28 °C for 4 days at initial pH level 6 and shaking rate 200 rpm.

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إنتاج فيتامين ب١٢ بواسطة عزلات محلية من الاكتينوميستات

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تم أختيار عزلة محلية من ميكروب *Streptomyces* SW1 لأنتاج فيتامين ب١٢ و ذلك من ١٠٣ عزلة تم الحصول عليها من الأراضى المصرية و تم تعريف هذة العزله تعريفا كاملا، حيث اتضح أنها أحدى سلالات ميكروب *S. baarnensis* وتهدف الدراسة الحالية إلي أختيار أحد المخلفات الزراعية أو النواتج الثانوية الصناعية لأضافتها إلي بيئة التخمير المعدلة للحصول على أعلى كمية منتجة من فيتامين ب ١٢. ولقد أظهرت النتائج أن اضافة المولاس بنسبة ١٥ جم / لتر (بدلا من النشا فى بيئة نترات النشا المعدلـــة) هـــو أفضل المخلفات لأنتاج فيتامين ب١٢ بواسطة السلالة *S. baarnensis* SW1. و بالأضافة الى ذلك فقـــد تم اختبار بعض العوامل البيئية مثل مدة التحضين و أ ل pH الأولى و درجـــة الحـــرارة و معـــدل الـــرج للحصول علي أعلي إنتاج من السيانوكوبالامين. ولقد أوضحت النتائج أن الإنتاج الامثل من فيتـــامين ب١٢ هو ٥٣,٢٠ ميكروجرلم/ مل حيث تم الحصول علية تحت ظروف الرج بأستخدام بيئـــة التخمـــر المعدلـــة و المحتوية على ١٥ جم/لتر مولاس مع ضبط pH البيئة الأولى الى ٦ والتحضين علي درجة حرارة ٢٨ م$^{\circ}$ لمـــدة ٤ أيـــام علـــي الهـــزاز الميكـــانيكي الرحـــوي بمعـــدل ٢٠٠ لفـــة فـــي الدقيقـــة.