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PRODUCTION OF VITAMIN B_{12} BY *Propionibacterium Freudenreichii* AND *Bacillus megaterium*

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ABSTRACT

Effect of some nutritional factors on growth and vitamin B_{12} production by *Propionibacterium freudenreichii* subsp. *freudenreichii* CCM 1857, *Propionibacterium freudenreichii* subsp. *shermanii* P1NRC, *Bacillus megaterium* DSM 2894, and *Bacillus megaterium* 1066 was investigated. Results indicated that sodium lactate was the best carbon source for *Propionibacterium* spp., while glucose was the best carbon source for *B. megaterium*. Yeast extract plus ammonium sulphate was superior nitrogen source for *Propionibacterium* spp., and yeast extract was the best nitrogen source for *B. megaterium*. Use of combination of some compounds ($CoCl_2$ + $CuSO_4$ + $MnSO_4$ + $ZnSO_4$ + $MgSO_4$ + $FeSO_4$) enhanced growth of *Propionibacterium* spp. and *B. megaterium*. Cobalt concentration of 10 mg l^{-1} was favor for *Propionibacterium* spp., however cobalt concentration of 15 mg l^{-1} was favor for *B. megaterium*. High test molasses and corn meal were found to be the best agriculture by-products for *Propionibacterium freudenreichii* subsp. *freudenreichii* CCM 1857 & *Bacillus megaterium* DSM 2894 after being added to the basic medium.

Keywords: Vitamin B_{12} production, Agricultural by-products, Microelements, *Propionibacterium freudenreichii* and *Bacillus megaterium*.

INTRODUCTION

Vitamin B_{12} is an important cofactor for metabolism of carbohydrates, lipids, amino acids and nucleic acids. The vitamin is thus an important additive in animal feeds. Vitamin B_{12} is also used in chemotherapy, especially against pernicious anaemia. Up to now vitamin B_{12} has been produced by fermentation on an industrial scale, since chemical synthesis of the vitamin is very difficult (Florent, 1986; Reynolds *et al.*, 1992 and Kamei *et al.*, 1993).

The microbial production of vitamin B_{12} has been the subject of a many investigations (Abdel-Hafez *et al.*, 1981; Chung and Fields, 1986; Crespo *et al.*, 1991; Magdoub *et al.*, 1992; Quesada –Chanto *et al.*, 1994 and Nakano *et al.*, 1996).

The present work was carried out to study the effect of nutritional requirements on the production of vitamin B_{12} by *Propionibacterium* sp. and *Bacillus megaterium*.

MATERIALS AND METHODS

Microorganisms used

The microorganisms used in this study were namely: *Propionibacterium freudenreichii* subsp. *freudenreichii* CCM 1857 (1), *Propionibacterium freudenreichii* subsp. *shermanii* P1NRC (3), *Bacillus megaterium* DSM 2894 (1) and *Bacillus megaterium* 1066 (3) were provided from Cairo MIRCEN, Fac. Agric., Ain Shams Univ, Cairo, Egypt.

Media used

Medium (1): Semi-solid lactate agar (SLA) recommended by Hettinga *et al.* (1968) was used for the maintenance of *Propionibacterium* sp. It has the following composition: Tryptone 10.0g, yeast extract 10.0g, sodium lactate 16.7ml, $MgSO_4.7H_2O$ 0.5g, KH_2PO_4 0.25g in one liter distilled water and the pH value was adjusted to 7.0 – 7.2.

Medium (2): The medium recommended by Mashhoor, *et al.* (1971) was used for the production of vitamin B_{12} by *Propionibacterium* sp. It has the following composition: Glucose 20.0g, Ammonium sulphate 3.0g, Beef extract 5.0g, KH_2PO_4 2.0g, Yeast extract 5.0g, Cobalt chloride 0.01g in one liter distilled water and the pH value was adjusted to 7.0.

Medium (3): Nutrient Agar (Difco Manual, 1977) was used for the maintenance of *Bacillus megaterium.* Its composition is as follows: Bacto peptone 5.0g, Bacto beef extract 3.0g and agar 15g in one liter Tap water with a pH of 7.0.

Medium (4) Nutrient Glucose (Difco Manual, 1988) supplemented by cobalt chloride was used for the production of vitamin B_{12} by *Bacillus megaterium*, its composition is as follows: Bacto peptone 5.0g, Bacto beef extract 3.0g, Glucose 10.0g and $CoCl_2$ 0.01g in one liter Tap water with a pH of 7.0.

Fermentation process

Fifty ml of the medium were dispensed into 250ml cotton plugged Erlenmeyer flasks. Each flask was inoculated with a suitable inoculum of *Propionibacterium* sp. (3.5×10^5) and incubated at 30°C for 4 days as static culture then 4 days as submerged culture, while flasks of *Bacillus megaterium* inoculated with (5.5×10^5) were incubated at 30°C on rotary shaker (150 rpm) for 4 days. 5,6 Dimethylbenzimidazole (DMB) was added, as a vitamin B_{12} precursor, to the fermentation cultures 24 hr before the end of incubation period as recommended by Pedziwilk *et al.* (1979) and Marwaha *et al.* (1983a).

Effect of different carbon sources

Effect of different carbon sources on the production of vitamin B_{12} was studied. The original carbon source of the basic medium (medium No.2 & No.4) was replaced by equivalent carbon amount of each of the tested carbon sources.

Effect of different nitrogen sources

The original nitrogen source of the basic medium (medium No.2 & No.4) was replaced by equivalent nitrogen amount of each of the tested nitrogen sources, to study the effect of different nitrogen sources on vitamin B_{12} production.

Effect of different elements

An experiment was performed to study the effect of different elements on the production of vitamin B_{12}. Six elements (0.001 %) i.e. Cu, Mn, Zn, Mg, Fe, Co and mixture of them were tested in this study.

Effect of different agricultural wastes and by-products

The effect of different agricultural wastes and by-products on the production of vitamin B_{12} was investigated. Five wastes and by-products namely: whey (whey with lactic acid and sweet whey), Corn meal, Potato

starchy waste, Molasses (Black strap molasses and High test molasses) and Corn steep liquor were tested for the production of vitamin B_{12}.

Determination of bacterial growth

Growth of tested organisms was determined by separating the biomass from culture broth using centrifugation at 10000 rpm for 30 min and drying at 80°C for 48 hr. (Quesada-Chanto, *et al.*, 1994).

Determination of vitamin B_{12}

The production of vitamin B_{12} was determined in the culture and in the cells according to the method of Mazumder, *et al.*, (1987).

RESULTS AND DISCUSSION

Effect of different carbon sources

Data presented in Table (1) show that the ability of *P. freudenreichii* subsp. *freudenreichii* (1) and *P. freudenreichii* subsp. *shermanii* (3) to produce vitamin B_{12} which was markedly affected by the carbon source of the medium. Sodium lactate and glucose were the most suitable carbon sources giving 70 & 60 µg l^{-1} in culture and 3.17 & 3.13 µg g^{-1} in cells for *P. freudenreichii* subsp. *freudenreichii* (1) and 57 & 42 µg l^{-1} in cultures and 2.59 & 2.51 µg g^{-1} in cells for *P. freudenreichii* subsp. *shermanii* (3), respectively. These values were followed, in descending order by other sugars. However the addition of raffinose, sucrose, maltose, starch, mannitol and sorbitol as a sole carbon source gave a drastic effect on the cultures, since no growth was detected in any of these treatments.

These results are in line with Abdel-Hafez, *et al.*, (1981) who found that sodium lactate and glucose were the best suitable carbon sources for vitamin B_{12} production by *Propionibacterium shermanii* P-16.

Regarding the effect of different carbon sources on the production of vitamin B_{12} by *Bacillus megaterium*, data presented in Table (1) show that the production of vitamin B_{12} also varied according the type of carbon source added to the culture medium. Glucose and starch as a sole carbon source were found to be as superior compared to the other carbon sources giving 48 & 46 µg l^{-1} in cultures for *Bacillus megaterium* (1) and 34 & 33 µg l^{-1} in cultures for *Bacillus megaterium* (3), respectively.

It is noteworthy to state that there was no obvious relationship between the production of vitamin B_{12} and the biomass production.

An experiment was carried out to study the effect of different concentrations of sodium lactate and glucose which exhibited superiority among other tested carbon sources for *Propionibacterium* sp. & *Bacillus* sp., respectively.

Data in Fig (1) clearly show that 4.0% sodium lactate gave the highest yield of vitamin B_{12} in the cells of *P. freudenreichii* subsp. *freudenreichii* (1) and *P. freudenreichii* subsp. *shermanii* (3) being 250 & 139 µg l^{-1} in cultures and 7.8 & 4.5 µg g^{-1} in cells, respectively. While, 2% glucose gave the highest yield of vitamin B_{12} in the cells of *Bacillus megaterium* (1) and *Bacillus megaterium* (3) being 119 & 93 µg l^{-1} in cultures and 6.8 & 6.2 µg g^{-1}, respectively.

Table (1).Effect of different carbon sources on the production of vitamin B_{12} by *P. freudenreichii* subspp. and ***Bacillus megaterium.***

Carbon sources	*P. freudenreichii* subsp. *freudenreichii*(1)			*P. freudenreichii* subsp. *shermanii* (3)			*Bacillus megaterium* DSM 2894 (1)			Bacillus megaterium 1066 (3)		
	Biomass g l^{-1}	Vit.B_{12} µg l^{-1} in cultures	Vit.B_{12} µg g^{-1} in cells	Biomass g l^{-1}	Vit.B_{12} µg l^{-1} in cultures	Vit.B_{12} µg g^{-1} in cells	Biomass g l^{-1}	Vit.B_{12} µg l^{-1} in cultures	Vit.B_{12} µg g^{-1} in cells	Biomass g l^{-1}	Vit.B_{12} µg l^{-1} in cultures	Vit.B_{12} µg g^{-1} in cells
Arabinose	18.6	52	2.8	16.3	40	2.45	5.3	13	2.45	4.5	18	4.00
Fructose	13.0	36	2.77	12.6	30	2.38	6.3	28	4.44	4.8	19	3.96
Galactose	15.2	39	2.57	13.6	32	2.35	7.3	44	6.03	6.6	28	4.24
Glucose (control)	19.2	60	3.13	16.7	42	2.51	7.7	48	6.23	7.2	34	4.72
Mannose	15.4	40	2.60	10.2	36	3.53	7.1	41	5.77	7.1	33	4.65
Lactose	5.1	1	0.20	14.3	39	2.73	7.4	45	6.08	7.0	30	4.29
Maltose	0	0	0	0	0	0	7.4	45	6.08	6.7	30	4.48
Sucrose	0	0	0	0	0	0	7.5	46	6.13	7.1	33	4.65
Raffinose	0	0	0	0	0	0	2.2	3	1.36	2.2	3	1.36
Dextrin	12.5	25	2.00	12.1	14	1.16	6.6	31	4.70	6.1	26	4.26
Strach	0	0	0	0	0	0	7.7	46	5.97	7.2	33	4.58
Glycerol	17.9	41	2.29	15.8	38	2.41	5.1	11	2.16	3.5	9	2.57
Mannitol	0	0	0	0	0	0	6.8	43	6.32	7.0	31	4.43
Sorbitol	0	0	0	0	0	0	7.1	38	5.35	5.1	22	4.31
α Keto glutric acid	12.1	20	1.65	11.8	13	1.10	0.9	3	3.33	0.7	0.2	0.29
Succinic acid	11.9	9	0.76	10.7	8	0.75	1	1	1	0.9	0.7	0.78
Sodium citrate	10.5	6	0.57	10.0	4	0.40	0.9	3	3.33	1	1	1
Sodium lactate	22.1	70	3.17	22.0	57	2.59	7	34	4.86	6.6	28	4.24

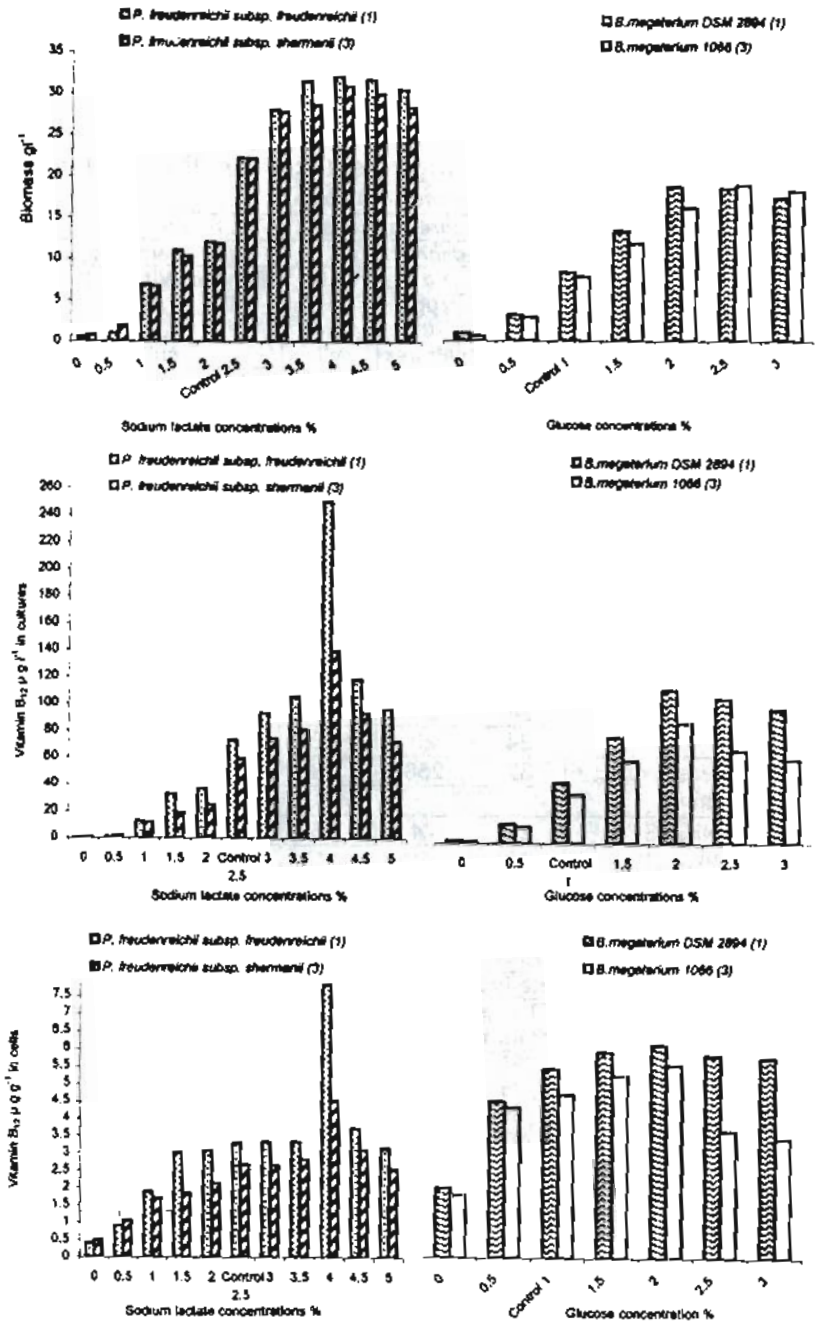

Fig (1): Effect of different concentrations of sodium lactate and glucose on the production of vitamin B_{12} by *Propionibacterium* sp. AND *B. megaterium*

Effect of different nitrogen sources

The nitrogen source in the basic medium was replaced by different nitrogen sources. Results recorded in the Tables (2 & 3) clearly show that the sources of nitrogen greatly affected the production of vitamin B_{12}.

Table (2): Effect of different nitrogen sources on the production of vitamin B_{12} by *P.freudenreichii* subspp.

Nitrogen sources	*P. freudenreichii* subsp. *f reudenreichii (1)*			*P. freudenreichii subsp. shermanii (3)*		
	Biomass g l^{-1}	Vit.B_{12} µg l^{-1} in cultures	Vit.B_{12} µg g^{-1} in cells	Biomass g l^{-1}	Vit.B_{12} µg l^{-1} In cultures	Vit.B_{12} µg g^{-1} in cells
Beef extract	30	16	0.53	29	51	1.8
Casein	28	64	2.3	26	49	1.9
Malt extract	30	90	3	30	87	2.9
Peptone	30	84	2.8	29	77	2.7
Proteose peptone	29	81	2.8	24	78	3.3
Trypton	31	96	3.1	26.2	92	1.87
Yeast extract	31	107	3.5	30	95	3.2
Ammonium citrate	19	20	1.1	20	16	0.8
$(NH_4)_2HPO_4$	26	35	1.3	26	34	1.3
NH_4NO_3	26	34	1.3	25	26	1.0
$(NH_4)_2SO_4$	24	21	0.9	24	17	0.7
$NaNO_3$	24	21	0.9	23	20	0.9
NH_4Cl	24	29	1.2	24	26	1.1
Beef extract+ Yeast xtract + $(NH_4)_2SO_4$ (Control)	32	250	7.8	31	140	4.5
Beef extract + $(NH_4)_2SO_4$	28	74	2.6	29	62	2.1
Yeast extract+ $NH_4)_2SO_4$	32	352	11	32	242	7.6

Table (3): Effect of different nitrogen sources on the production of vitamin B_{12} by *Bacillus megaterium*.

Nitrogen sources	*Bacillus megaterium DSM 2894 (1)*			*Bacillus megaterium 1066 (3)*		
	Biomass g l^{-1}	Vit.B_{12} µg l^{-1} In cultures	Vit.B_{12} µg g^{-1} in cells	Biomass g l^{-1}	Vit.B_{12} µg l^{-1} In cultures	Vit.B_{12} µg g^{-1} in cells
Beef extract	17	95	5.6	15	83	5.5
Casein	14	38	2.7	14	32	2.3
Malt extract	17	89	5.2	15	93	6.2
Peptone	17	83	4.9	15	73	4.9
Proteose peptone	15	40	2.7	14	34	2.4
Trypton	18	120	6.7	17	104	6.1
Yeast extract	18	132	7.3	18	111	6.2
Ammonium citrate	11	8	0.7	8	8	1
$(NH_4)_2 HPO_4$	17	65	3.8	15	57	3.8
NH_4NO_3	14	37	2.6	13	31	2.4
$(NH_4)_2SO_4$	16	63	3.9	15	43	3.3
$NaNO_3$	13	22	1.7	12	14	1.2
NH_4Cl	13	12	0.9	12	12	1
Beef extract+ Peptone (Control)	18	122	6.8	15	97	6.5

The mixture of ammonium sulphate and yeast extract were the best nitrogen source for *Propionibacterium* sp. giving 11.0 and 7.6 µg g^{-1} in the cells for *P. freudenreichii* subsp.*freudenreichii* (1) and *P. freudenreichii* subsp. *shermanii* (3), respectively. While, yeast extract was the best nitrogen source for *Bacillus megaterium* giving 7.3 and 6.2 µg g^{-1} in cells for *Bacillus megaterium* (1) and *Bacillus megaterium* (3), respectively.

To study the effect of nitrogen concentrations added as a mixture of yeast extract and ammonium sulphate, different yeast extract concentrations ranged from 0.5 to 4.0% were used with constant level of ammonium sulphate (0.3%), and different ammonium sulphate concentrations ranged from 0.1 to 0.7% were used with constant level of yeast extract (2.5%).

Results recorded in Table (4) clearly show that suitable concentration of yeast extract plus ammonium sulphate was found to be (2.5 +0.5 %), respectively, which gave the highest vitamin B_{12} in culture being 358 µg l^{-1} and in cells being 10.85 µg g^{-1} for *P. freudenreichii* subsp. *freudenreichii* (1), *P. freudenreichii* subsp. *shermanii* (3) gave the highest vitamin B_{12} in culture being 251 µg l^{-1} and in cells being 7.84 µg g^{-1}, using the above nitrogen mixture concentration.

Table (4): Effect of yeast extract + Ammonium sulphate concentration as nitrogen source on production of vitamin B_{12} by *P.freudenreichii* subspp.

Yeast extract + $(NH_4)_2.SO_4$ (concentration%)	*P. freudenreichii* subsp *freudenreichii (1)*			*P. freudenreichii* subsp *shermanii (3)*		
	Biomass g l^{-1}	Vit.B_{12} µg l^{-1} in cultures	Vit.B_{12} µg g^{-1} in cells	Biomass g l^{-1}	Vit.B_{12} µg l^{-1} in cultures	Vit.B_{12} µg g^{-1} in cells
0.5+0.3	30	60	2.00	24	58	2.42
1.0+0.3 (control)	32	352	11.00	32	243	7.59
1.5+0 3	32	353	11.03	32	243	7.59
2.0+0.3	33	354	10.73	32	243.4	7 61
2.5+0.3	33	356	10.79	32	244.3	7.64
3.0+0.3	30	132	4.40	30	131	4.37
3.5+0.3	30	106	3.53	27	111	4.11
4.0+0.3	30	102	3.40	24	92	3.83
2.5+0 1	32	122	3.81	30	116	3.87
2.5+0.2	32	122	3.81	31	122	3.94
2.5+0.3 (control)	33	355	10.76	32	245	7.66
2.5+0.4	33	356	10.79	32	246	7.69
2.5+0.5	33	358	10.85	32	251	7 84
2 5+0.6	32	151	4.72	32	137	4.82
2.5+0 7	32	124	3.88	31	117	3.77

With regard to *B. megaterium*, results in Table (5) show that 1.5 % yeast extract gave the highest yield of vitamin B_{12} in cultures 141 & 122 µg l^{-1} and vitamin B_{12} in the cells 7.42 and 6.78 µg g^{-1} for *Bacillus megaterium* (1) and *Bacillus megaterium* (3), respectively.

Effect of different elements

It is well known that metabolic ions play an important role in the production of vitamin B_{12} (Abdel-Hafez, *et al.*, 1981, Marwaha, *et al.*, 1983 b and Czaczyk, *et al.*, 1997). Therefore, to investigate the effect of some

elements namely, $CoCl_2$, $CuSO_4$, $MnSO_4$, $ZnSO_4$, $MgSO_4$, $FeSO_4$ and $CoSO_4$ on the productivity of the tested organisms. Each element was added to give a final concentration of 0.001%, cobalt salt was used either singly or coupled with other element or in combination with the different elements.

Table (5): Effect of yeast extract concentrations as nitrogen source on production of vitamin B_{12} by *Bacillus megaterium*.

Yeast extract (concentration%)	*Bacillus megaterium* DSM 2894 (1)			*Bacillus megaterium* 1066 (3)		
	Biomass g l^{-1}	Vit.B_{12} µg l^{-1} in cultures	Vit.B_{12} µg g^{-1} in cells	Biomass g l^{-1}	Vit.B_{12} µg l^{-1} in cultures	Vit.B_{12}µg g^{-1} in cells
0.3	17	112	6.59	17	103	6.06
0.5	18	118	6.56	17	106	6.24
0.8 (control)	19	132	6.95	18	112	6.22
1	19	137	7.21	18	117	6.5
1.3	19	139	7.32	18	120	6.67
1.5	19	141	7.42	18	122	6.78
2	18	129	6.79	18	121	6.72
2.3	18	124	6.89	18	118	6.56
2.5	18	120	6.67	18	109	6.06

Data in Table (6) show that the addition of a combination of elements achieved the highest vitamin B_{12} production being 362, 257, 144 and 124 µg l^{-1} in the cultures and 10.94, 7.28, 7.66 and 6.74 µg g^{-1} in the cells for *P. freudenreichii* subsp. *freudenreichii* (1), *P. freudenreichii* subsp. *shermanii* (3), *Bacillus megaterium* (1) and *Bacillus megaterium* (3), respectively.

These results are in disagreement with those obtained by Abdel-Hafez, *et al.*, (1981) who found that the mixture of micro-elements resulted in an inhibitory effect on the vitamin B_{12} production. While these data are in line with Ramadan and Hazew (1983) who found that the mixtures of trace-elements have a stimulatory effect on vitamin B_{12} production.

Effect of different cobalt concentrations

Cobalt is essential for the production of vitamin B_{12}, since it represents the metallic core of its chemical structure. Therefore, an experiment was conducted to study its effect. Different concentrations of cobalt were used (1, 3, 5, 10, 15, 20, 25 and 30 mg l^{-1}).

Results in Table (7) show that the production of vitamin B_{12} was increased as the cobalt concentration increased to 10 mg l^{-1} by *Propionibacterium* sp. which achieved the highest vitamin B_{12} in the cultures 364 & 258 µg l^{-1} or in the cells 11.03 & 7.82 µg g^{-1} by *P. freudenreichii* subsp. *freudenreichii* (1) and *P. freudenreichii* subsp. *shermanii* (3), respectively. Concerning *B. megaterium*, cobalt concentration of 15 mg l^{-1} gave the highest vitamin B_{12} either in culture 148 & 130 µg l^{-1} or in cells 7.79 & 6.84 µg g^{-1} by *Bacillus megaterium* (1) and *Bacillus megaterium* (3), respectively. Further, increase in cobalt concentration lead to decrease in both vitamin B_{12} production and growth of the organism.

Table (6): Effect of different elements on the production of vitamin B_{12} by *P. freudenreichii* subspp. and *Bacillus megaterium*.

Different elements 0.001%	*P. freudenreichii* subsp. *freudenreichii (1)*			*P. freudenreichii* subsp. *shermanii (3)*			*Bacillus megaterium* DSM 2894 (1)			*Bacillus megaterium* 1066 (3)		
	Biomass $g\ l^{-1}$	Vit.B_{12} $\mu g\ l^{-1}$ in cultures	Vit.B_{12} $\mu g\ g^{-1}$ in cells	Biomass $g\ l^{-1}$	Vit.B_{12} $\mu g\ l^{-1}$ in cultures	Vit.B_{12} $\mu g\ g^{-1}$ in cells	Biomass $g\ l^{-1}$	Vit.B_{12} $\mu g\ l^{-1}$ in cultures	Vit.B_{12} $\mu g\ g^{-1}$ In cells	Biomass $g\ l^{-1}$	Vit.B_{12} $\mu g\ l^{-1}$ in cultures	Vit.B_{12} $\mu g\ g^{-1}$ in cells
$CoCl_2$ (control)	33.0	157	4.76	32.4	152	4.69	18.7	141	7.54	18.3	123	6.72
$CoCl_2 + CuSO_4$	33.0	159	4.82	32.5	154	4.74	18.7	142	7.59	18.3	123	6.72
$CoCl_2 + FeSO_4$	32.7	158	4.83	32.4	150	4.63	18.8	143	7.61	18.4	124	6.74
$CoCl_2 + MgSO_4$	33 1	161	4.86	32.4	154	4.75	18.7	142	7.59	18.3	123	6 72
$CoCl_2 + MnSO_4$	33.0	159	4.82	32 4	153	4.72	18.7	141	7.54	18.3	123	6 72
$CoCl_2 + ZnSO_4$	33.0	160	4.85	32.5	155	4.77	18.7	142	7.59	18.4	123	6.68
$CoSO_4$	32.7	152	4.65	31 9	143	4.48	18.7	141	7.54	18.2	122	6.70
Combination of elements	33.1	362	10.94	35 3	257	7 28	18 8	144	7.66	18.4	124	6.74

Table (7): Effect of different cobalt concentrations mg l^{-1} on the production of vitamin B_{12} by ***P. freudenreichii*** **subspp. and** ***Bacillus megaterium.***

Cobalt concentrations	*P. freudenreichii* subsp. *freudenreichii(1)*			*P. freudenreichii subsp. shermanii (3)*			*Bacillus megaterium* DSM *2894* (1)			*Bacillus megaterium* 1066 (3)		
mg/l	Biomass g l^{-1}	Vit.B_{12} µg l^{-1} in cultures	Vit.B_{12} µg g^{-1} in cells	Biomass g l^{-1}	Vit.B_{12} µg l^{-1} in cultures	Vit.B_{12} µg g^{-1} in cells	Biomass g l^{-1}	Vit.B_{12} µg l^{-1} In cultures	Vit.B_{12} µg g^{-1} In cells	Biomass g l^{-1}	Vit.B_{12} µg l^{-1} In cultures	Vit.B_{12} µg g^{-1} in cells
0	16	25	1.56	16	11	0.69	11	10	0.91	11	8	0.73
1	23	62	2.70	22	42	1.90	11	31	2.82	11	12	1.09
3	25	106	4.24	25	95	3.80	15	93	6 20	14	74	5.29
5	28	133	4.75	29	131	4.52	18	116	6.44	16	103	6.44
10 (control)	33	364	11.03	33	258	7.82	19	145	7.63	18.4	124	6.74
15	29	224	7.72	25	86	3.44	19	148	7.79	19	130	6.84
20	25	83	3.32	22	50	2.27	19	122	6.42	18	121	6.72
25	21	32	1.50	18	13	0 72	13	75	5 77	13	56	4.31
30	16	11	0.69	16	10	0.63	13	74	5.69	10	11	1.10

These results are in the same trends of Merck and Co. Inc (1971); Schwartz and Stadtman (1971) and Cetin, *et al.*, (1979) who used cobalt and cyanocobalamin for the production of vitamin. Garey, (1951) pointed out that increase of yields of vitamin B_{12} were reported to be associated with intermittent feeding of five p.p.m cobalt to a synthetic medium inoculated with *Streptomyces*. However obtained results are in accordance with those of Abdel-Hafez, *et al.*, (1981) who reported that the production of vitamin B_{12} increased as the cobalt concentration increased up to 10 p.p.m by propionibacteria.

Use of some industrial and agricultural by-products for vitamin B_{12} production

Some available low price industrial wastes and raw materials such as whey with Lactic acid, Sweet whey, Corn meal, Potato starchy waste, Black strap cane molasses, High test cane molasses and Corn steep liquor were used for vitamin B_{12} production by *Propionibacterium* sp. and *B. megaterium*.

Data recorded in Table (8) indicated that the production of vitamin B_{12} were very low when using by-products and wastes as compared with that produced by the basic medium without wastes. The failure of using by-products and wastes to support vitamin B_{12} production may be due to the insufficient nutrients or to the presence of some inhibitors such as hydroxymethyl furfural in molasses (Burrows, 1970).

Table (8): **Effect of different by-products and wastes on the production of vitamin B_{12} by *P. freudenreichii* subsp. *freudenreichii(1)* and *Bacillus megaterium* DSM 2894 (1).**

By-products	*P. freudenreichii* subsp. *freudenreichii(1)*			*Bacillus megaterium* DSM 2894 (1)		
	Biomass g l^{-1}	Vit.B_{12} µg l^{-1} in cultures	Vit.B_{12} µg g^{-1} in cells	Biomass g l^{-1}	Vit.B_{12} µg l^{-1} n cultures	Vit.B_{12} µg g^{-1} n cells
Control (without wastes)	29.8	301	10.1	10.4	151	14.5
Whey with lactic acid	11.7	11	0.9	0.5	2	4.0
Whey with lactic acid + Medium	20.1	30	1.5	4.9	15	3 1
Sweet whey	2.5	6	2.4	0.2	1	5.0
Sweet whey + Medium	10.7	10	0.9	0.5	3	6.0
Corn meal	13 5	15	1.1	7.9	32	4.1
Potato starchy waste	13.4	15	1 1	5.2	20	3.8
Black strap molasses	5.2	9	1.7	0.7	5	7.1
Black strap molasses + Medium	16.4	27	1.6	4.6	14	3.0
High test molasses	8.5	9	1.1	0.6	4	6.7
High test molasses + Medium	23.9	56	2.3	4.5	12	2.7
Corn steep liquor (CSL)	0.2	0.2	1	0.1	0.1	1
Corn steep liquor(CSL)+Medium	14 6	18	1 2	2.5	1.6	0.64

The yield of vitamin B_{12} by using different by-products and wastes ranged from 0.2 – 56 µg l^{-1} in the cultures and from 0.9 – 2.4 µg g^{-1} in the cells by *P. freudenreichii* subsp. *freudenreichii* (1)and ranged from 0.1 - 32 µg l^{-1} in the cultures and from 0.64 – 7.1 µg g^{-1} in the cells by *Bacillus megaterium* (1).

However, these results need further investigation to optimize the nutritional and environmental factors in order to maximize vitamin B_{12} production from these cheep agri-industrial by-products and wastes.

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إنتاج فيتامين ب١٢ بواسطة *Propionibacterium freudenreichii* و *Bacillus megaterium*

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تم أجراء هذا البحث لدراسة تأثير بعض العوامل الغذائية على نمو و إنتاج فيتامين ب ١٢ بواسطةالميكروبات التالية:
Propionibacterium freudenreichii subsp. *freudenreichii* CCM, *Proioni.Bacterium freudenreichii* subsp. *shermanii* P1NRC 1857, *Bacillus megaterium* 1066 and *Bacillus megaterium* DSM 2894.
و أشارت النتائج الى ان لاكتات الصوديوم كان أفضل مصدر كربون لميكروب *Propionibacterium freudenreichii* ، بينما الجلوكوز كان أفضل مصدر كربونى لميكروب *B. megaterium* . وقد كان أنسب مصدر ديتروجين لميكروب *Propionibacterium freudenreichii* خليط من مستخلص الخميرة و كبريتات الامونيوم، بينما كان مستخلص الخميرة بمفرده هو أفضل مصدر نيتروجينى لميكروب *B. megaterium*. كما أدى استخدام خليط من العناصر المعدنية من (كلوريد الكوبلت+ كبريتات النحاس+ كبريتات المنجنيز+ كبريتات الماغنسيوم+ كبريتات الحديد)إلى تحسين نمو ميكروب *Propionibacterium freudenreichii* و *B. megaterium*. و كذلك كان كلوريد الكوبلت بتركيز ١٠ ملليجرام فى اللتر هو أنسب تركيز لإنتاج فيتامين ب ١٢ بواسطة *Propionibacterium freudenreichii* و بتركيز ١٥ ملليجرام فى اللتر لإنتاج فيتامين ب١٢ بواسطة *B. megaterium* .

و قد وجد أن أفضل منتجات ثانوية زراعية كانت مولاس القصب و جرش الذرة لميكروبى *Propionibacterium freudenreichii* و *B. megaterium* على التوالى و ذلك بعد إضافتهما للبيئة الاساسية.