THE PRODUCTION OF VITAMIN B_{12} BY VARIOUS STRAINS OF ACTINOMYCETES

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The production of vitamin B_{12} by strains of *Streptomyces chromogenus*, *S. griseus*, and *S. antibioticus* was reported first by Rickes and co-workers (1948). Since that time a number of surveys have been made in which actinomycetes, particularly those of the *Streptomyces* genus, have been reported as good producers of the vitamin (Garey and Downing, 1951; Hall *et al.*, 1950*a*, *b*, 1951; Pridham *et al.*, 1951; Saunders *et al.*, 1951; Shull and Routien, 1951).

During studies on isolates of soil actinomycetes for antibiotic production in our laboratories, a routine screening procedure was developed for the detection of vitamin B_{12}-producing strains. The details of the method and results obtained in the screening of a representative group of soil isolates are given here.

METHODS

Cultures. The cultures of actinomycetes used in these studies were isolated originally from soil samples (soil sample number indicated by first line of code) for antibiotic investigations. Fourteen of the cultures were characterized as *S. griseus* types through morphological and growth characteristics. An industrial strain of *S. griseus* was run as control throughout the twelve runs in which soil isolates were tested.

Inoculum. Soil stocks were employed with all cultures. Transfers from soil tubes were made to an agar medium of beef extract 0.5 per cent, peptone 0.5 per cent, cerelose 1.0 per cent, NaCl 0.5 per cent, and agar 2.0 per cent. After 7 days' incubation at 24 C, spores from the slant cultures were transferred by loop to a submerged culture medium consisting of corn steep solids 0.5 per cent, peptone 0.5 per cent, cerelose 1.0 per cent, NaCl 0.5 per cent, and $CaCO_3$ 0.1 per cent, with the initial pH adjusted to 6.8.

Cultures in the vegetative inoculum medium were incubated on a rotary shaker at 24 to 26 C. Two 48-hour transfers were made. The final fermentation flasks were inoculated with 4 per cent by volume of the vegetative inoculum cultures.

Fermentation methods. The medium employed for vitamin B_{12} production was of the following composition: soybean meal, 1.5 per cent; corn steep solids, 0.5 per cent; no. 2019 yeast, 0.2 per cent; cerelose, 1.5 per cent; NaCl, 0.3 per cent; NH_4NO_3, 0.1 per cent; $CaCO_3$, 0.2 per cent; glycerol, 0.5 per cent; and Co^{++} (as $CoCl_2 \cdot 6H_2O$), 0.00025 per cent.

All fermentations were run in triplicate with 100 ml volumes of medium in 500 ml Erlenmeyer flasks. Incubation was carried out for 7 days at 24 to 26 C on a Gump rotary shaker operating at a rate of 180 rpm.

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Samples for B_{12} assay were taken daily after 3, 4, 5, 6, and 7 days' incubation. B_{12} assays were run by a modification of the microbiological method of Skeggs *et al.* (1948). In the modified method, sodium thioglycolate was used in place of ascorbic acid.

TABLE 1

Vitamin B_{12} yields with ninety isolates of soil actinomycetes

CULTURE	MAX AVG YIELD μG/ML	CULTURE	MAX AVG YIELD μG/ML	CULTURE	MAX AVG YIELD μG/ML
2-1	0.16	NA137-C1	0.48	NA185-PV9	0.57
2-2	0.21	NA137-M11	0.39	NA192-B12	0.01
2-3	0.18	NA137-M12	0.32	NA192-B13	0.09
NA81-B26	0.35	NA137-M24	0.19	NA192-B15	0.32
NA81-B31	0.49	NA137-M27	0.26	NA192-B28	0.11
NA81-M18	0.44	NA137-P13	0.34	NA192-B34	0.26
NA81-M23	0.76	NA162-B3	<0.01	NA192-M4	0.72
NA81-M26	0.48	NA162-B8	0.64	NA192-M5	0.49
NA81-P13	0.08	NA162-B13	0.09	NA192-M6	0.13
NA81-P14	0.03	NA162-M1	0.37	NA192-M11	0.58
NA81-P25	0.36	NA162-M2	0.53	NA192-M13	0.55
NA81-P26	0.03	NA162-M4	0.20	NA192-M14	0.33
NA81-P30	0.50	NA162-M7	0.44	NA192-M21	0.04
NA81-P31	0.05	NA162-PV4	0.41	NA192-M27	0.22
NA137-B19	0.31	NA185-M13	0.22	NA192-M29	0.34
NA192-PV3	0.02	NA524-A17	0.07	NA524-X17	0.65
NA192-PV9	0.18	NA524-A18	0.07	NA524-X22	0.37
NA192-PV14	0.18	NA524-M3	0.78	NA524-X26	0.04
NA192-PV16	0.26	NA524-M4	0.53	NA524-X33	0.23
NA192-PV21	0.16	NA524-M6	0.91	NA524-X49	0.64
NA192-PV24	0.27	NA524-M7	0.73	NA538-A2	1.17
NA484-M4	0.56	NA524-M10	0.66	NA538-A8	0.06
NA500-A3	0.76	NA524-M14	0.12	NA538-A10	0.69
NA500-A11	0.58	NA524-PV5	0.76	NA538-A11	0.73
NA500-A16	0.23	NA524-X3	0.69	NA538-M1	1.67
NA500-M8	0.68	NA524-X5	0.08	NA538-M2	0.03
NA500-X50	0.07	NA524-X9	0.62	NA538-M6	0.70
NA524-A6	0.54	NA524-X11	0.62	NA538-M13	1.63
NA524-A14	0.06	NA524-X13	0.07	NA538-PV2	0.27
NA524-A16	0.06	NA524-X15	0.43	NA538-X3	1.84

EXPERIMENTAL RESULTS

Ninety cultures of actinomycetes selected at random were screened for vitamin B_{12} activity and all were found to produce the vitamin. The maximum average yields of the cultures tested are given in table 1.

The highest yields were obtained with cultures NA538-A2 (1.17 μg/ml),

NA538-M1 (1.67 μg/ml), NA538-M13 (1.63 μg/ml), and NA538-X3 (1.84 μg/ml). It should be noted that all four cultures were isolated from the same soil sample and that one of them, NA538-A2, was characterized as *S. griseus*. Bacterial spectrum data on the antibiotics produced by NA538-M1, NA538-M13, and NA538-X3 indicated that these cultures were similar and therefore may represent one species.

TABLE 2

Summary of data on vitamin B_{12} production of ninety isolates

B_{12} YIELDS, μG/ML	NUMBER OF STRAINS	PER CENT OF TOTAL
0.25 or less	35	39
0.26–0.50	25	28
0.51–0.75	21	23
0.76–1.00	5	6
1.01 or greater	4	4

TABLE 3

Summary of data on fourteen strains of Streptomyces griseus

CULTURE	MAX AVG YIELD μG/ML	RATIO*
NA81-B26	0.35	0.55
NA81-M18	0.44	0.77
NA81-M23	0.76	1.33
NA81-P30	0.50	0.78
NA137-C1	0.48	0.64
NA162-B8	0.64	1.00
NA162-M1	0.37	0.49
NA162-M7	0.44	0.69
NA162-PV4	0.41	0.64
NA185-PV9	0.57	0.76
NA192-B15	0.32	0.67
NA484-M4	0.56	0.84
NA524-X11	0.62	0.93
NA538-A2	1.17	1.27

* Ratio = Maximum average yield of isolate/maximum average yield of control.

In table 2 is given a summary of the range of B_{12} production by the cultures tested. It was found that the largest single group of cultures (39 per cent) produced the vitamin at a level of 0.25 μg/ml or less and that the majority of the cultures (67 per cent) produced within the range of 0.50 μg/ml or less. Only 10 per cent of the culture gave yields of 0.76 μg/ml or greater.

Fourteen of the cultures under study were characterized as strains of *S. griseus*. A summary of the data on these strains is given in table 3. Moderate levels of vitamin B_{12} production (0.32 to 0.76 μg/ml) were noted with all cultures except NA538-A2, which gave a maximum average yield of 1.17 μg/ml. The control culture of *S. griseus* gave an average yield of 0.74 μg/ml over a period of 10 runs.

SUMMARY

Ninety cultures of actinomycetes were screened for vitamin B_{12} production in a soybean meal medium fortified with cobalt. Four of the cultures were found to produce significantly higher yields of the vitamin than those of the control culture of *Streptomyces griseus*. One of these, characterized as *S. griseus*, gave yields of approximately 1.0 μg/ml, and the three nongriseus strains gave yields of 1.63 to 1.84 μg/ml.

Data on fourteen *S. griseus* strains indicated a narrow range of vitamin B_{12} production with these cultures, and none of these cultures gave as high yields as the best nongriseus strain.

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