

A SURVEY ON THE PRODUCTION OF VITAMIN B₁₂ BY SOME ANTAGONISTIC STRAINS OF *STREPTOMYCES*

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THE production of vitamin B₁₂ by some antagonistic strains of *Streptomyces* sp. isolated from the soils of West Bengal, has been surveyed. Of the strains, 48% showed positive activity for vitamin B₁₂. When tested for antagonistic action, 64% of the strains indicated antibiotic activity against *Staphylococcus aureus*. Strains related to *S. fradiae*, *S. californicus* and 8-80 group were found to elaborate varying amounts of vitamin B₁₂ as well as antibiotic substances. It was also observed that there was no apparent relationship between the production of vitamin B₁₂ and that of antibiotic substances.

INTRODUCTION

The recent report about the ability of the strains of *Streptomyces chromogenes*, *S. griseus* and *S. antibioticus* (Rickes *et al.*, 1948) to synthesise vitamin B₁₂ has stimulated further interest in the genus *Streptomyces*. A number of surveys undertaken in different laboratories have already reported the production of vitamin B₁₂ by several species of *Streptomyces* (Darken, 1953). Some of the strains belonging to this group of micro-organisms, isolated in this laboratory mainly as a part of general survey of the microbial flora of Indian soils specially with regard to the production of antibiotic substances were simultaneously screened for the production of vitamin B₁₂ (Mukherjee *et al.*, 1953). The present investigation was undertaken to find out whether micro-organisms producing antibiotic substances were to produce vitamin B₁₂ as well, or whether the synthesis of vitamin B₁₂ had any effect on the yield of antibiotic substances. Of special interest would be the determination of relationship, if there be any, between the production of antibiotic substance and that of a growth factor by a particular group of micro-organisms.

MATERIALS AND METHODS

The strains belonging to *Streptomyces* spp. were maintained on potato-dextrose agar (Kelner, 1947). They were grown in a modified straw-infusion medium (Dey, 1947) in 250 ml. Erlenmeyer flasks for 8 days at 28°C. To obviate the introduction of methionine, (NH₄)₂HPO₄ (0.54%) was used as N-source instead of beef-extract (Lemco).

Prior to inoculation, sterile CoCl₂, H₂O solution (2 ppm.) was added to the medium. Spore-suspension (10 parts by volume) of the strains of *Streptomyces* in sterile water was added as inoculum. After incubation,

the fermented product was adjusted to pH 5.0, autoclaved at 115°C for 10 minutes, filtered and diluted to original volume by means of 1% phosphate buffer. 10 ml. of the test sample were treated with 0.8 mg. of KCN at 60°C. for one hour and assayed for total activity. The excess of cyanide was removed by aeration for 30 minutes.

The potency of the samples was next determined according to the method of Harrison and his co-workers (1951) using a strain of *Escherichia coli* mutant (NCIB 8134) as test organism. The ease of growing the bacteria in a comparatively simple medium together with its specificity for B₁₂ prompted us to select *E. coli* mutant as a test strain for assay. It was reported that the mutant utilised methionine in addition to B₁₂ for growth. It was also observed by Chiao and Peterson (1953) that when the ratio of methionine to B₁₂ on a weight basis was 50,000 or less, the presence of the former did not introduce any significant error in the assay. Since (NH₄)₂HPO₄ was used as N-source, it was thought that methionine, even if synthesised or produced as a result of autolysis, would not be present in amounts sufficient to cause interference with the assay.

A standard curve was drawn using crystalline vitamin B₁₂ (Glaxo) and the activity of samples was determined by extrapolation when required. The antagonistic action of *Streptomyces* spp. was determined against *S. aureus* by agar cup method of assay.

Tests were simultaneously run to check the production of methionine as a metabolite by means of one-dimensional paper-chromatography using phenol-water as a developing solvent.

RESULTS

Of the strains tested for the production of vitamin B₁₂, twenty-four (48%) showed positive activity. Of them, as many as fourteen (28%) strains, produced more than 0.08 μgm./l. When tested by paper-chromatography, methionine was not found to be present at least in detectable amount.

On the other hand when tested for antagonistic action 32 (64%) strains were found to possess positive activity against *S. aureus*.

Table 1 shows the production of vitamin B₁₂ by strains of *Streptomyces* along with their antagonistic action against *S. aureus*. The diameter of the zone of inhibition in each case was recorded to the nearest mm.

Of the isolates, strains belonging to *S. fradiae*, *S. californicus* and 8-80 group, characterised according to Pridham and Gottlieb (1948), and marked with asterisks in Table 1, were found to elaborate varying amounts of vitamin B₁₂ under identical conditions of fermentation.

DISCUSSION

The present investigation confirms the previous observation that the species of *Streptomyces* can produce vitamin B₁₂, when grown on media containing cobalt salts (Darken, 1953). Strains related to a particular group of *Streptomyces* spp., were found to elaborate varying amounts of B₁₂ as well as antibiotic substances (Table 1). It was also observed that

Table 1. Production of vitamin B₁₂ and the antagonistic activity of different strains of Streptomyces spp.

| Isolate No. | Identity | Average yield of vitamin B ₁₂ (μgm./l) | Antagonistic activity against <i>S. aureus</i> as measured by zone of inhibition (mm.) |
|------------------------|---|---|--|
| Ac ₇ (8) | <i>S. erythrochromogenes</i> | 20 | 23.0 |
| Ac ₃ (9) | " | — | 23.5 |
| Ac ₁ (34) | " | 70 | 24.0 |
| Ac ₃ (177) | " | — | 19.5 |
| Ac ₁₄ (193) | " | — | 14.0 |
| Ac _{x2} (9) | <i>S. albosporus</i> | — | 20.0 |
| Ac ₃ (195) | " | — | 20.0 |
| *Ac ₄ (10) | <i>S. californicus</i> | 150 | 13.0 |
| *Ac ₄ (198) | " | 210 | — |
| *Ac ₁ (218) | " | — | 15.0 |
| Ac ₁₀ (193) | <i>S. alboflavus</i> | 70 | 20.0 |
| Ac ₁ (27) | <i>Streptomyces</i> sp. related to <i>S. alboflavus</i> . | 160 | — |
| Ac ₁ (214) | <i>S. viridochromogenes</i> | 20 | — |
| Ac _{x1} (32) | <i>Streptomyces</i> sp. related to <i>S. viridochromogenes</i> . | 170 | — |
| *Ac ₁ (46) | <i>S. fradiae</i> (mutant ?) | 60 | 14.0 |
| Ac ₂ (51) | <i>Streptomyces</i> sp. related to <i>S. griseus</i> . | 250 | — |
| Ac ₆ (125) | " | 280 | — |
| Ac ₆ (177) | " | — | 10.0 |
| Ac ₁ (64) | <i>S. flavovirens</i> | — | — |
| Ac ₁ (67) | <i>S. erythreus</i> | 10 | 15.0 |
| Ac ₂ (95) | <i>S. cellulosa</i> | — | 16.0 |
| Ac ₃ (185) | <i>S. halstedii</i> | — | 29.5 |
| Ac ₄ (195) | " | 10 | 29.5 |
| Ac ₁₂ (B1) | " | — | 28.0 |
| Ac ₆ (118) | <i>Streptomyces</i> sp. related to <i>S. halstedii</i> . | — | 23.0 |
| Ac ₁ (82) | <i>Streptomyces</i> sp. related to <i>S. albus</i> | 140 | — |
| Ac ₈ (118) | " | 90 | — |
| Ac ₂ (177) | <i>S. rutgersensis</i> | — | 25.0 |
| Ac ₃ (183) | " | — | 23.0 |
| Ac ₂ (197) | " | — | 25.0 |
| Ac ₃ (177) | <i>S. albidoflavus</i> | 120 | — |
| Ac ₂ (185) | <i>Streptomyces</i> sp. related to <i>S. albidoflavus</i> . | 90 | 16.0 |
| Ac ₃ (194) | <i>S. griseolus</i> | 180 | — |
| Ac ₁₁ (195) | <i>Streptomyces</i> sp. related to <i>S. scabies</i> . | — | 24.0 |
| Ac ₃ (196) | <i>S. lavendulae</i> (mutant ?) | — | — |
| Ac ₁₂ (217) | <i>S. purpeochromogenes</i> | — | 12.0 |
| Ac ₈ (185) | <i>Streptomyces</i> sp. related to <i>S. flaveolus</i> . | — | 23.0 |
| *Ac ₃ (203) | <i>Streptomyces</i> sp. related to <i>S. fradiae</i> and <i>S. californicus</i> . | 70 | 24.0 |
| Ac ₁ (9) | <i>Streptomyces</i> sp. (Unidentified) | 80 | — |
| Ac ₁ (60) | " | — | 14.0 |
| Ac ₃ (BG) | " | — | 15.0 |
| Ac ₉ (177) | " | 120 | — |
| Ac ₄ (193) | " | 150 | — |
| Ac ₆ (193) | " | 140 | — |
| Ac _{x3} (194) | " | — | 30.0 |
| Ac ₄ (203) | " | 20 | 24.0 |
| Ac ₆ (217) | " | — | — |
| Ac ₄ (B1) | " | — | 23.0 |
| Ac ₅ (B1) | " | — | 20.0 |

several strains which did not produce antibiotic substance were able to synthesise vitamin B₁₂. The reverse was also true. The synthesis of these complex organic metabolites, one growth inhibiting and the other growth accessory, by a group of micro-organisms, often a single species, presents a fascinating problem. From the preliminary observations presented here, it appears that the production of B₁₂ does not bear even a qualitative relationship with that of antibiotic substances. It has been found that there is a much closer correlation between growth and the production of B₁₂ than there is between growth and the production of the antibiotic substance (Dulaney and Williams, 1953). On the other hand, the porphyrin-like nature of grisein—an antibiotic substance produced by a strain of *S. griseus* a similar nature of a part of the B₁₂ molecule, the observation that the most probable action of stimulators for the production of B₁₂ lies in their being competitive inhibitors (Dulaney and Williams, 1953), the use of some of the antibiotics as growth stimulators in the form of food-supplement—all these suggest some relationship between production of vitamin B₁₂ and that of antibiotic substance by a particular genus. It is of interest that in a conjunctive fermentation, the trend of the curve of vitamin B₁₂ production was found to be parallel to that of the production of the antibiotic substance throughout the fermentation cycle (Buchanan *et al.*, 1950). No final word can, however, be said at the present state of our knowledge, until the mechanism of biosynthesis of these complex molecules is clearly understood.

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