

SHORT
COMMUNICATIONS

The Ability of Some Strains of Propionic Acid Bacteria Isolated in Uzbekistan to Synthesize Vitamin B₁₂

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Vitamin B₁₂ (cobalamin) is widely used to cure disorders of the nervous system, arthritis, and pernicious anemia. This vitamin is produced by microbiological synthesis, using propionic acid bacteria [1].

To develop the biotechnology of vitamin B₁₂ production in Uzbekistan, we attempted to isolate propionic acid bacteria from natural sources and to assess their cobalamin-synthesizing ability.

Propionic acid bacteria were isolated from rancid kaimak (an Uzbek fermented dairy product) and hard cheese, using routine isolation procedures.

Samples (1 g) were placed in Voitkevich medium [2] and incubated at 30°C for 30 days. An aliquot (5 ml) of this medium with the obvious signs of propionic acid fermentation (a turbidity of the medium and the specific flavor of propionic acid) was transferred to the fresh medium, and incubation was continued for the next two weeks. Pure cultures were obtained by the method of serial dilutions using an agar medium of the following composition (g/l): glucose, 10; (NH₄)₂SO₄, 5, K₂HPO₄, 2, CaCO₃, 10; peptone, 5; agar, 15; and corn extract, 20 ml (pH 7.0). Inoculated agar plates were incubated at 37°C.

Individual colonies were transferred to a liquid medium containing (g/l) glucose, 20; (NH₄)₂SO₄, 3; K₂HPO₄, 2; and corn extract, 20 ml (pH 7.0). Morphological characteristics were studied using cultures grown in nutrient broth and on nutrient agar. The ability of the cultures to synthesize vitamin B₁₂ was evaluated from the formation of corrinoids in a medium containing (g/l): glucose, 20; sodium lactate, 4; casein hydrolysate, 10; (NH₄)₂SO₄, 3; CoCl₂ · 6H₂O, 0.005; and MnSO₄ · 4H₂O, 0.005 (pH 6.8–7.0). The corrinoids were determined spectrophotometrically [3].

Thirty bacterial strains that we isolated showed phenotypic characteristics common to the genus *Propionibacterium* [4]. Cells were from 0.5 × 1–1.2 μm to

0.8 × 1.6 μm in size, pleiomorphic, often branched or bifurcated, produced V-type and Y-type forms, and occurred singly, in pairs, in short chains, and often in hieroglyph-like aggregates. Cells were nonmotile, gram-positive, non-spore-forming, facultatively anaerobic. When grown in liquid media, the strains produced a grayish powdered precipitate. Colonies grown on agar media were pinpoint, convex, yellowish or cream-colored, shiny or dull. All strains were catalase-positive and produced acid from glucose.

Two strains, 30 and 33, produced corrinoids in amounts typical of active producers of vitamin B₁₂. Strain 30, isolated from hard cheese, produced 19–21 mg corrinoids/l. Strain 33, isolated from 40% kaimak, produced 29–30 mg corrinoids/l. Taking into account the fact that the known industrial producers of vitamin B₁₂ have a productivity of 25–40 mg corrinoids per l of optimized medium [5], strains 30 and 33 can be considered to be promising producers of vitamin B₁₂.

Further studies will be concerned with the identification of the strains to a species level and the analysis of the corrinoid composition.

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