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Isolation, Cultivation and Study of Methanotrophic Bacteria

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Abstract.

Currently methanotrophic bacteria are the object of intensive biotechnology studies, as those organisms utilize methane or methanol in their metabolism as an only source of organic substances. This feature allows their application in various biotechnology processes. In the study we present the data on methanotrophic bacteria and provide information on their practical applications.

Keywords: methanotrophs, methylotrophs, bacteria, microbiological protein.

Methanotrophs are methylotrophic microorganisms, which functionally and structurally specialize on methane utilization. They are capable to assimilate methane as a single source of organic substances [3]. Methanotrophs inhabit swamps, soil, lakes, seas, waste waters, rice field patches, household solid waste polygons. Some methanotrophs are capable to assimilate atmospheric methane, that provides them an opportunity to occupy diverse ecological niches.

Methanotrophic bacteria are often represented by gram-negative bacilli, vibrioids or cocci, often mobile due to possession of one or a few flagella, or constrain resting forms as Azotobacter-like cysts, lipid cysts or exospores, have sophisticated system of inner cytoplasmatic membranes, consume ammonium, nitrates and nitrites as a source of nitrogen, some fixate molecular nitrogen, reduce nitrates into nitrites, however not oxidizing methane by the nitrates oxygen [1].

On solid nutrient media methanotrophs develop colonies of various shape and colour. Most typical are white, cream, raised shiny colonies, 0.2-0.5 mm in diameter. The colour of methanotrophic is determined by intracellular pigments - carotenoids. It may be red, rosy, or yellow. Pigments protect a bacterial cell from the daylight. With age the colour typically changes. This peculiarity is characteristic for the species intensively forming the residing forms and releasing water-soluble pigments [1]. Related to the unique peculiarities of their metabolism, methanotrophic bacteria has become a potential object of biotechnology studies, being the only biological methane assimilators. For that reason, they are applicable in environmental bioengineering or production of microbiological protein [2, 4].

Purpose of the study

Adjustment of the nutrient media for isolation and cultivation of methanotrophic bacteria.

Materials and methods

For isolation and cultivation of methanotrophic bacteria in the Laboratory of biology and technology of the living systems, L.N. Tolstoy State Pedagogical University, we utilized standard laboratory glassware and devices, microbiology box, sterilization autoclave, and the components of nutrient media. The nutrient media prepared were sterilized under 1.2 atm for 60 min. The bacteria were isolated from their natural habitat, the pond bottom sediment, and from the anaerobic dy from the methane-reactor of the Tula Brewing Plant branch of the Baltica Brewery, Inc. As the control and etalon we used methanotrophic bacteria strains *Methylococcus capsulatus* B-2990 and *Methylomonas*

methanica B-2110from the All-Russian Collection of Microorganisms (VKM)of the federal research centre Pushchino Scientific Center for Biological Research, Russian Academy of Sciences.

For isolation of bacteria into a pure culture the nitrate mineral solution (NMS) was applied, of the following content: – $NaNO_3$ - 8.5 g/l, KH_2PO_4 - 5.3 g/l, $NaHPO_4 \cdot 12H2O$ - 21.6 g/l, K_2SO_4 - 1.7 g/l.,$MgSO_4 \cdot 7H2O$ - 0.37 g/l, $CaCl_2$ (0.07 g/ml -distilled water) - 1 ml, H_2SO_4 - 0.5 ml, $ZnSO_4 \cdot 7H_2O$ - 2.87 g/l, $MnCl_2 \cdot 4H_2O$ - 2.09 g/l, H_3BO_3 - 0.62 г/л, $NaMoO_4 \cdot 2H_2O$ - 0.48 g/l, $CoCl_2 \cdot 6H_2O$ - 0.62 g/l, KI - 0.83 g/l. For comparison, the methanotrophic bacteria strains *Methylococcus capsulatus* B-2990 and *Methylomonas methanica* B-2110 from the VKM were used.

The pure culture of methanotrophs was stored on the nutrient media in the tubes with obliqu:e agar in refrigerators set approximately for 5°C. For keeping the strain collection, the cultures were passaged 2-3 times a month. If contaminant microorganisms were detected in the pure cultures, tubes with them were discarded. For studying the growth speed of methanotrophic bacteria, they were re-seeded on the following nutrient media:

- Nitrate Mineral Solution (NMS) - see above;

- Synthetic Nutrient Medium number 2: KNO_3 – 1,0 g/l , $MgSO_4 \cdot 7H_2O$ – 0.2 g/l, $CaCl_2$ – 0.02 g/l, $Na_2HPO_4 \cdot 5H_2O$ – 1.5 g/l, KH_2PO_4 – 0.7 g/l, $CuSO_4 \cdot 5H_2O$ – 0.01 mg/l,$FeSO_4 \cdot 7H_2O$ – 0.02 mg/l, $ZnSO_4 \cdot 7H_2O$ – 0.001 mg/l, $Na_2MoO_4 \cdot 2H_2O$ – 0.003 mg/l, $CoCl_2 \cdot 6H_2O$ – 0.002 mg/l, Trilon B – 0.05 mg/l, $MnCl_2 \cdot 4H_2O$ – 0.003 mg/l, $NiCl_2 \cdot 6H_2O$ – 0.002 mg/l;

- Synthetic Nutrient Medium number 3: H_3PO_4 (70%) - 0.35 mg/l, KCl - 0.125 g/l, $MgSO_4 \cdot 7H_2O$ - 0.105 g/l, $FeSO_4 \cdot 7H_2O$ - 10.75 mg/l,$CuSO_4 \cdot 5H_2O$ - 10 mg/l, $MnSO_4 \cdot 5H_2O$ - 9.5 mg/l, H_3BO_3 - 6.25 mg/l, $ZnSO_4 \cdot 7H_2O$ -1.5 mg/l, $CoSO_4 \cdot 7H_2O$ - 0.25 mg/l, $Na_2MoO_4 \cdot 2H_2O$ - 0.25 mg/l;

- Synthetic Nutrient Medium number 4: KNO_3 - 1.0 g/l, KH_2PO_4 - 0.7 g/l, $MgSO_4 \cdot 7 H_2O$ - 0.2 g/l, $CaCl_2 \cdot 2 H_2O$ - 0.02 g/l, $Na_2HPO_4 \cdot 12 H_2O$ - 1.5 g/l, Trilon B - 5.0 mg/l, $FeSO_4 \cdot 7 H_2O$ - 2.0 mg/l, $ZnSO_4 \cdot 7 H_2O$ - 0.1 mg/l, $MnCl_2 \cdot 4 H_2O$ - 0.03 mg/l, $CoCl_2 \cdot 6 H_2O$ - 0.2 mg/l, $CuCl_2 \cdot 2 H_2O$ - 0.1 mg/l, $NiCl_2 \cdot 6 H_2O$ - 0.02 mg/l, $Na_2MoO_4 \cdot 2 H_2O$ - 0.03 mg/l.

Research results

In result of the conducted studies on the growth speed of methanotrophic bacteria on different nutrient media we found that the Synthetic Nutrient Medium number 3 sustained the best growth of colonies, as it contains most suitable micro- and macro-element concentrations for methanotrophic bacteria.

Conclusions

In result of the performed research we revealed that methanotrophic bacteria can be successfully cultivated on the nutrient media evaluated. The preliminary analysis of metabolites has shown that their considerable fraction is represented by the protein molecules. Thus, methanotrophic bacteria is a distinct group of microorganisms with specific metabolism and unique properties. Efficient utilization of such a bacteria allows to resolve in part series of actual problems, like

decreasing release of methane into the atmosphere and refill the deficit of nutrient protein in the world.

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