Stefan Ratering
Max Plank Institut fuer terrestrische Mikrobiologie
Karl Frisch Str.
350041 Marburg

Isolation of a methane utilizing phototrophic bacteria

Phototrophic bacteria are wide spread in nature. The ecological niches are the anoxic parts of waters and sediments. They are found in freshwater, marine, and hypersaline environments, hot springs and arctic lakes, as well as elsewhere (Pfennig, 1989). The phototrophic bacteria are not only physiologically an extremely heterogeneous group, they are also distributed taxonomically in a number of distinct families and groups.

Anoxygenic phototrophic bacteria can use several inorganic and small organic compounds as an electron donor. The inorganic electron donors mostly are sulfide and other reduced sulfur compounds, but also compounds like iron(II) and hydrogen conserve the same function. To get energy the phototrophic bacteria transform light into metabolic useful chemical energy by bacteriochlorophyll-mediated processes (Imhoff, 1992).

To use the inorganic and the small organic compounds as electron donor is less energy necessary than to use H_2O. The phototrophic bacteria need only 1/8 of the energy to use sulfide as to use H_2O. Purple bacteria and Chloroflexus use PS II-type reaction center. Heliobacteria and green sulfur bacteria use PS I-type reaction center (Ormerod, 1992).

One reduced compound that is common in anoxic freshwater environments is methane. Two roles of methane in bacterial metabolism have been studied: 1 as product of methanogenic bacteria and 2. as an electron donor and carbon source in methanotrophic bacteria. Use of methane as an electron donor for phototrophic bacteria is a third possibility. Thermodynamically it is possible to use methane as electron donor with one photosynthesic reaction center. Wertlieb and Vishniac (1967) claimed to show utilization of methane by a strain of Rhodopseudomonas gelatinosa, but they could not unequivocally demonstrated the ability to grow with methane.

This report describes the attempt to isolate a methane utilizing bacteria form an environment that contains a high number of methane producing bacteria and therefore a large amount of methane.

Material & Methods

AT-Media for nonsulfur purple bacteria (1000 ml)

Trace elements SL10	10 ml
Vitamin solution	1 ml
KH_2PO_4	1 g
$MgCl_2$ $6H_2O$	0.5 g
$CaCl_2$ $2H_20$	0.1 g
NH_4Cl	1.0 g
$NaHCO_3$	3.0 g
NaCl	1.0 g
TiNTA	25 ml
Adjust to pH 6.9	

Vitamin solution (100 ml)

Biotin	10 mg
Niacin	35 mg
Thiaminedichlorid	30 mg
p-Aminobenzoic acid	20 mg
Pyridoxoliumhydrochloride	10 mg
Ca-panthothenate	10 mg
Vit B12	5 mg

Trace element solution

$FeCl_2$ $4H_2O$	1800 mg
$CoCl_2$ $6H_2O$	250 mg
$NiCl_2$ $6H_2O$	10 mg
$CuCl_2$ $2H_2O$	10 mg
$MnCl_2$ $4H_2O$	70 mg
$ZnCl_2$	100 mg
$H3BO_3$	500 mg
Na_2MoO_4 $2H_2O$	30 mg
Na_2SeO_3 5 H_2O	10 mg

Reducing agent (TiNTA)

Stock solution: 80 mM TiCl in 160 mM NTA. Neutralization with saturated $NaCO_2$

The media was prepared and filled up with a Widdel flasks. 100 ml serumbottles were filled with 40 ml media and the headspace were changed to N_2/CO_2 (80/20). The headspace of several bottles were change for methane. In several other bottles were injected 10 ml of 100 % methane.

The inoculum of the swamp at the Devils lane was inoculated in a amount of 1 ml. The incubation temperature was room temperature and the bottle stood 30 cm from a 100 watt bulb.

Results

After 2 weeks it was no growth in any bottle with methane as electron donor. Also in the control with thiosulfate (10 mM) and acetat (5 mM) it could not determinated any growth.

Discussion

The results show not that growth with methan as electron donor is unpossible. Two weeks may be to short for visible growth under this conditions. Wertlieb and Vishniac (1967) has also very few growth yield. Furthermore because it was no growth in the control it could be that the media with TiNTA was not correct.
For that reason it is necessary to try different kinds of media and to wait a longer time to see visible growth. Perhaps it is also necessary to try inoculums form different places with methan production.

References

Imhoff, J. J (1992) Taxonomy, Phylogeny, and Gernaeral Ecology of Anoxygenic Phototrophic Bacteria, In: Photosynthetic Prokaryotes, edited by H. Mann and Noel G. Carr, Plenum Press, New York, 1992, p. 53-92

Ormerod, J. G. (1992) Physiology of the Photosynthetic Prokaryotes In: Photosynthetic Prokaryotes, edited by H. Mann and Noel G. Carr, Plenum Press, New York, 1992, p. 93-120

Pfennig, N. (1989) Ecology of photrophic purple and green sulfur bacteria. In: Autrotrophic Bacteria, edited by H. G. Schlegel and B. Bowien, Springer Verlag Heidelberg, p. 97-116

Werlieb and Vishniac (1967) Methane ultization by a strain of Rhodopseudomonas gelatinosa, J. Bacteriol. 93, p.1722