STUDIES OF METHANOGENIC BACTERIA FROM INTESTINAL TRACTS OF MARINE FISHESES

HELMUT BRANDL
UNIVERSITY OF ZURICH, SWITZERLAND
Introduction

Methane is one of a number of reduced gases present in the oceanic mixed surface layer in amounts higher than would be expected from equilibrium with the atmosphere. Superabundant concentrations of methane in oxygenated surface water have been attributed to both sediment inputs and an in situ biological production. The first is a physical transport of methane-rich coastal water into the open ocean. The source for coastal methane-rich water is anoxic sediments. If physical transport from these sediments is insufficient as an open ocean source, one possible alternative is in situ biological formation by methano-
genic microorganisms. An anaerobic environment in these mixed oxygenated water layers must be postulated, because of the strictly anaerobic conditions required by methane producing bacteria. It appears unlikely that such organisms could survive unprotected in
lightly oxygenated ocean waters. Possible habitats are
fecal pellets or the gastrointestinal tract of marine
fishes. Scranton (1977) has argued convincingly that methane
production in fecal pellets is unlikely to be a
significant source of methane for the mixed water
layer. So the fish gut may be an important
habitat for a methanogenic population of microorganisms.
2. Material & methods

Fishes

5 zup (Stenotomus chrysops)
1 smooth dogfish (Mustelus canis)

The fishes were caught near Woods Hole.

Medium

Enrichment medium (Romesser et al., 1979)
in mixture of 30% distilled water and 70% seawater (wt/vol):

ammonium acetate 0.05
sodium formate 0.1
trypsinase 0.2
yeast extract 0.1
ammonium-2-mercaptoethanol sulphate (HS-OM) 0.001
Na₂CO₃ 0.1
Na₂HCO₃ 0.2
cystine 10
Na₂S · 9 H₂O 0.05
K₃[Fe(CN)₆] 0.0001
Addition of 1% vitamin solution:

(mg/liter distilled H₂O)

- biotin: 2
- folic acid: 2
- pyridoxine hydrochloride: 10
- riboflavin: 5
- thiamine: 5
- nicotinic acid: 5
- pantothenic acid: 5
- vit B₁₂: 0.1
- p-aminobenzoic acid: 5
- thioctic acid: 5

Addition of 1% VFA solution:

- iso-butyric acid
- α-methylisobutyric acid
- isovaleric acid
- valeric acid: each 0.05 (vol/vol)
Addition of 1% trace element solution:

<table>
<thead>
<tr>
<th>Salt</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>NTA</td>
<td>1.5</td>
</tr>
<tr>
<td>MgSO₄</td>
<td>3.0</td>
</tr>
<tr>
<td>MnSO₄</td>
<td>0.5</td>
</tr>
<tr>
<td>NaCl</td>
<td>1.0</td>
</tr>
<tr>
<td>FeSO₄</td>
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</tr>
<tr>
<td>CaCl₂</td>
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</tr>
<tr>
<td>CoCl₂</td>
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<tr>
<td>ZnSO₄</td>
<td>0.1</td>
</tr>
<tr>
<td>CuSO₄</td>
<td>0.01</td>
</tr>
<tr>
<td>Al₂ (SO₄)₃</td>
<td>0.01</td>
</tr>
<tr>
<td>H₃BO₃</td>
<td>0.01</td>
</tr>
<tr>
<td>Na₂MoO₄</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Gas phase: \( \text{H}_2 : \text{CO}_2 \ (80 : 20) \)

Final pH: 7.4

Inoculation temperature: 25°C
The medium was injected through the arms to provide an easier withdrawal of the relatively compact gut contents. Samples of intestinal contents were serially diluted in the enrichment medium and incubated under an atmosphere of H₂ : CO₂ (80 : 20)

The Hungate anaerobic tube technique was employed in order to avoid contact of samples with oxygen. (WOLFE, 1979)

**Oxygen electrode**

To measure the oxygen concentration within the fish gut, an oxygen-micro-electrode (REVSBECH, 1980) was used.

Detection limit of the electrode: 1 μM

**Gas measurements**

Methane in the gas phase was measured using a Carlo 8000 gas chromatograph (thermocconductivity detector; Porapak Q column, 100°C, 20 ml N₂/min). Detection limit was 8 pmol CH₄ / 0.1 ml gas volume injected.
Fluorescence microscopy. The observations were carried out by using an epifluorescence microscope (Olympus model BH-RHL, filter set: Y 455, V, IF 405).
3. Results & discussion

The first step in my work was to verify the anaerobic conditions in the intestinal tract. For this reason the amount of oxygen in the gut of a ray (Stenotomus chrysops) was measured with an oxygen microelectrode. The results showed that the oxygen content is below the detection limit of the electrode so that the assumption of an anaerobic environment can be made.

The gut contents of five rays and one smooth dogfish were examined with respect to populations of methanogenic bacteria. After 3 to 5 days a negative pressure had developed within the enrichment tubes indicating the consumption of H2 and/or CO2. However, at this time methane could not be detected by gas chromatography. Since sulfate-reducing bacteria were found in densities of at least
10⁸ per ml gut contents in both S. chrysops and M. carinii, these bacteria may have consumed H₂ in the enrichments for methanogenic bacteria. After an incubation period of 20 days, methane was detected in the atmosphere above cultures from each of the fish, indicating the presence of a methanogenic population. Normally the sulfate-reducers outcompete the methanogens, but here in the gut one can imagine a certain coexistence. This phenomenon can be explained by two possibilities:

a) there are periods of sulfate depletion in the gut, so that the methane producers have a chance to grow.

b) there are enough substrates in the gut, so that these two groups don’t have to compete.

Under the epifluorescence microscope three different
morphological types of methane producing organisms can be observed in the enrichment cultures:
a round shaped organism, a Methanococcus-like and two types of Methanebacterium, a small rod and a fat rod.

4. Conclusions

The observation of methanogenic bacteria in the fish gut lead one to the questions: How active are the methanogens in the gut? Do they actively produce methane or are they present just because they are swept in with particles from the anaerobic sediment since rays and dogfish are bottom feeders?
Qualitatively these results demonstrate the presence of a methanogenic population in the intestinal tract of fishes. Further work is needed to obtain quantitative results. The next steps in this work will be:

a) the enumeration of methane producers in the gut.

b) the cloning of these organisms to obtain pure cultures and to study their morphology and physiology so that isolates from sediments can be compared with.

c) the investigation of open ocean fishes, which are not bottom feeders in order to answer the question whether the methanogens are part of the normal intestinal flora in these animals.

d) studies of methane production in the intact fish.

