Degradation of methanesulfonate by aerobic bacteria.

Caroline Plugge
Department of Microbiology, WAU,
H. van Suchtelenweg 4,
6703 CT Wageningen
THE NETHERLANDS.
e-mail: Caroline.Plugge@algemeen.micr.wau.nl

Microbial Diversity Course,
MBL, Woods Hole
July 1995.

Supervision: Ed Leadbetter and John d'Elia.

INTRODUCTION.

In the marine organic sulfur cycle Dimethylsulfide (DMS) plays a significant role: over 30% of the total volatile sulfur compounds are due to the production of it. The DMS is derived from the degradation of dimethylsulfoniumpropionate (DMSP), which is produced by marine algae, cyanobacteria and marshplants Kelly et al.,1990) A recent study by Kiene and Hines shows that Sphagnum sp. in peatlands are also a considerable source of DMS..
The occurrence of methanesulfonate (MSA) in the atmosphere is due to the photochemical destruction of DMS. MSA is a stable compound and a strong acid. It does not undergo chemical breakdown in the atmosphere, but is deposited on the Earth's surface in rain and/or snow. There is little known in the literature about the biodegradation of MSA. It can be used as a sulfur source by p.e. Chlorella fusea as well as by some bacteria isolated from soil, sewage and certain enteric bacteria. MSA can be used as a sole source of carbon and energy by a novel Gram- negative methylotrophic bacterium, isolated from the soil. There is a recent report describing two marine strains which can degrade MSA in a similar way. There is, to my knowledge, no report of bacteria which are able to use MSA the sole carbon, energy and sulfur source.
The aim of this study was to obtain more information about the degradation of MSA under aerobic conditions.

MATERIALS AND METHODS.

Media .

For batch cultures a mineral salts medium was used with the following composition per liter of deionized water:
1.2 g K_2HPO_4; 0.6 g KH_2PO_4; 0.05 g $CaCl_2.2H_2O$; 0.16 g $MgCl_2.6H_2O$; 0.5 g NH_4Cl and 2 ml of trace element solution SL12. In some cases 0.02% yeast extract was added.
Phosphates were sterilized separately. The final pH was set at 7.2 with HCl. Methanesulfonate (MSA) and methanol (MeOH) were filter sterilized in stock solutions of 1 M and added to the sterilized media. If necessary, a S-source was added as Na_2SO_4.
In some cases 0.02% yeast extract was added together with the ``six vitamin solution'' and vitamin B12.
When using agarplates, the MSA medium was supplied with 2% washed agar (washed twice) or nutrient agar.
Media for the chemostat had the same composition as described above, except that the $MgCl_2$ and the $CaCl_2$ were sterilized separately and added to the sterilized media.

Type of inoculum.

Different sources of inoculum were used:
soil from the garden near the ``Brick Dorm''; sediment from Cedar Swamp and sediment from School Street Marsh.
The chemostat was inoculated with a mix of these inocula (``Supermix'').

Enrichment procedures.

Enrichments were started in liquid media and on agarplates with MSA as the sole carbon, energy and S-source as well as in liquid media with additional carbon and S-sources. All incubations were done at 30 C
Chemostat enrichments were started by inoculating a 1 l vessel, at a dilution rate of 0.1 per hour. The vessel was equipped with pH and temperature control. A constant temperature of 30 C and a pH of 7.1 were set throughout the experiment and air was supplied to the vessel (constant, but uncontrolled).
Growth in the chemostat was checked daily by measuring the OD 660nm and by microscopy. Samples were plated on MSA agar with MSA or MeOH as the carbon source as well on nutrient agar plates.

Analytical methods.

MSA and sulfate were measured by HPLC (Waters), equipped with an anionexchange (IC-PAK A HC) column eluted with gluconate/borate. The eluens was prepared as follows:
1 liter solution A contained: 16 g sodium gluconate, 18 g boric acid, 25 g sodium tetraborate decahydrate (Borax) and 250 ml glycerol. One liter of solution B (eluens) contained: 20 ml sole. A, 20 ml. 1-butanol and 120 ml acetonitrile. The eluens was filtered through a 0.22 micron filter and degassed before use. The flow rate of the HPLC was 2 ml/min. The detector was a 430 Waters conductivity meter running at 35 degrees.

RESULTS.

Chemostat enrichments.

After two days of incubation, some turbidity could be observed in the chemostat. The turbidity increased further in time (Table 1).

Table 1: Optical density, pH and MSA concentration in the chemostat.

Time (days)	OD 660 nm	pH	MSA mM
0	0.01	7.2	nd.
1	0.01	7.1	15.5
2	0.022	7.1	15.5
3	0.022	7.1	14.2
4	0.04	7.1	13.1
5	0.045	7.1	nd.
6	0.05	7.1	9.8
7	0.051	7.2	7.5
8	0.05	7.2	7.2

The chemostat had to be stopped after 8 days.

All samples taken from the chemostat showed growth on the three types of plates used. On MSA agar a shift in the colony types was observed in time: the first two days Streptomyces type cells were present besides short fat rods, but further in time the short fat rods were the only morphological types which could be observed on the MSA agar plates. On MeOH agar plates, similar colony types were found. On nutrient agar a wide variety of morphotypes could grow. None of the colonies growing on MSA agar plates were able to grow in liquid culture, even after the addition of 0.02% yeast extract, Na_2SO_4 and vitamins to the medium. However the colonies could grow in nutrient broth and medium with methanol.
Two pure cultures (msacp1 and msacp2) were isolated from the liquid cultures. Both strains were Gram negative, short fat rods. Msacp1 was motile, oxidase positive and katalase negative. Msacp2 was slightly motile on nutrient broth, but showed no motility on other media, and was slightly oxidase positive and katalase negative. Both strains (msacp1 and msacp2) could only grow under aerobic conditions. Growth was observed after 4 days of incubation with methanol, methylamine and formate as the substrate. No growth was observed after 4 days of incubation on MSA, methane, glucose , acetate and taurine. No growth was observed under anaerobic conditions (in the presence of nitrate) on methanol, methylamine, formate, MSA and methane.

Liquid culture enrichments.

In all liquid cultures some growth occurred, after two weeks of incubation, but this was not always correlated to substrate degradation. Growth was measured as optical density (table 2).

Table 2: Optical density of enrichments in liquid cultures with different carbon and sulfur sources after 2 weeks of incubation.

C and or S source	OD 660 nm.
10 msa	0.05
20 msa	0.05
msa + meoh	0.15
msa + sulfate	0.05
msa + meoh + sulfate	0.25
10 meoh	0.15
20 meoh	0.2
meoh + sulfate	0.25
blank without substrate	0.05

Samples from all cultures, except the blank, were taken and secondary enrichments were started on MSA, MeOH and nutrient aga . In all cases a wide variety of colony types grew on the nutrient agar. A few colony types grew on the MeOH agar and no growth was observed on the MSA agar plates, except for the MSA/MeOH enrichment. Several colonies from the methanol plates were restreaked and were obtained in pure culture. Three different morphological types could be distinguished: a pale white colony-forming short motile rod; a shiny white colony-forming fat motile rod and an irregular pink colony forming non-motile cocci. All were Gram-negative and oxidase positive
The colonies from the MSA agar were inoculated into liquid medium, but showed no growth after one week.

DISCUSSION.

Chemostat enrichments.

The organisms enriched in the chemostat were able to convert MSA (see table 1). Within one week the amount of MSA decreased from 15.5 mM to 9.8 mM. Since no additional carbon and/or sulfur sources were added to the medium, this degradation was done by microorganisms, which are able to use MSA as the sole carbon, energy and sulfur source.
The secondary enrichments on plates showed some growth, but after transferring them into liquid medium with MSA as the sole carbon, energy and sulfur source no growth was observed within one week. There are some speculations to make about this phenomena. It could be that the bacteria which could grow on the MSA agar plates were growing on traces of carbon in the agar, but couldn't use MSA as a substrate. Another speculation might be that MSA was degraded by two organisms, where one converts the MSA into an intermediate plus sulfate and the other converts the intermediate into CO2. Possible intermediates are methanol, formate or formaldehyde.

Reactions which could be involved in the conversion of MSA:

1a) MSA + 2 O2 -----> CO2 +H2O + H2SO4
1b) MSA + O2 -----> formaldehyde/methanol/formate +H2SO4
2) methanol + 1.5 O2 -----> CO2 + 2 H2O
3) formaldehyde + O2 -----> CO2 + H2O
4) formate + 0.5 O2 -----> CO2 + H2O

It might be that the second organism could grow faster and was isolated instead of the MSA degrader, which might not grow on agar or very poorly. Thompson et al. also mention that the MSA degrading bacteria TR-3 and PSCH4 that they isolated were only able to grow on less than 0.5% agar noble. Since we used 2% normal agar this may be an explanation for the lack of growth of our MSA degrader. The inability of methylotrophs to grow on agar (Lees et al; 1991) seems to be relatively common. In commercial preparations of agar there may low concentrations of inhibitory compounds.
The two isolated strains (msacp1 and msacp2) are methylotrophic strains, since they can grow on all the C-1 compounds tested (except for MSA).It is not sure whether they are obligate methylotrophs, since only a few other carbon sources were tested for growth They are not responsible for the MSA degradation in the chemostat, but probably could grow on an intermediate from the MSA degradation.

Liquid culture enrichments.

The enrichments in liquid cultures showed that methanol was degraded very easily and therefor that it is easy to enrich for methylotrophs from soil or sediment.
But after 2-3 weeks of incubation no growth could be observed in the enrichments with MSA. So it can be concluded that within the time of the experiments MSA is not degraded in batch cultures, and none of the other enrichments was able to convert Msa in secondary enrichments.

CONCLUSIONS.

-A chemostat is a useful tool for the enrichment of MSA degrading micro-organisms.
-Methanol is an easily degradable substrate for methylotrophs and it is relatively easy to obtain isolates from these enrichments.
-Liquid culture enrichments maybe are useful tools to enrich for MSA degrading organisms, but not a longer incubation time is needed for them to develop.
-Several pure cultures could be isolated during the time period of 3.5 weeks.

RECOMMENDATIONS
for future students/research

-It would be useful to have a chemostat running during the whole period of the project, especially because the doubling time of the MSA degrading organisms is around 10 hours. It takes a while to get a steady state in the reaction vessel and to obtain enough biomass to do further enrichments.
-Several agartypes should be tried out in order to get the MSA degrading bacteria growing (agar noble, phytagel plates, lower concentrations of agar).

LITERATURE.

CC Chien, ER Leadbetter and W Godchaux III (1995); Sulfonate sulfur can be assimilated for fermentative growth. FEMS Microbiol. Letters 129:189-194.

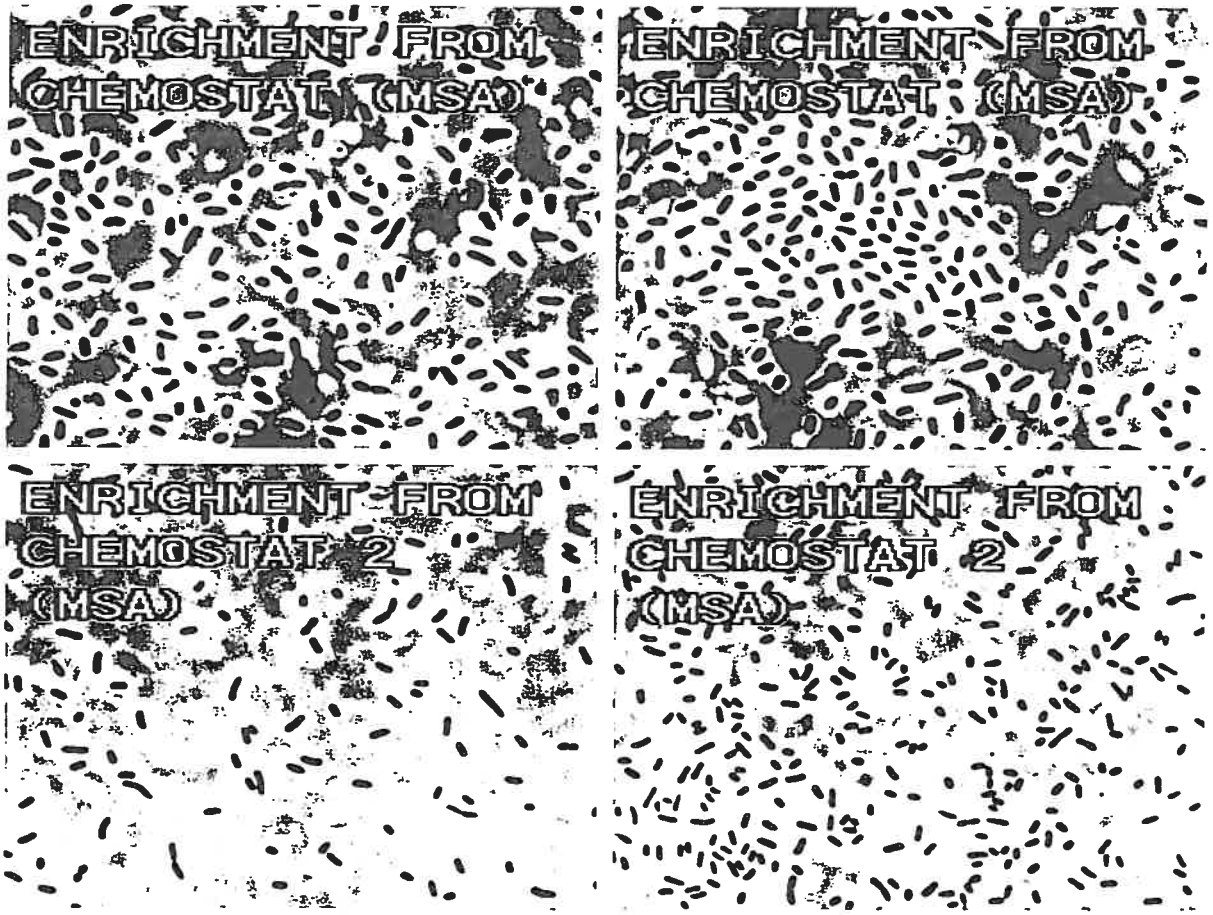
DP Kelly, SC Baker, J Trickett, M Davey and JC Murrell (1994); Methanesulfonate utilization by a novel methylotrophic bacterium involves an unusual monoxygenase. Microbiol 140:1419-1426.

RP Kiene and ME Hines (1995); Microbial formation of dimethylsulfide in anoxic Sphagnum peat. Appl.Environm. Microbiol. 61:2720-2726.

AS Thompson, NJP Owens and JC Murrell (1995); Isolation and characterization of methanesulfonic acid degrading bacteria from the marine environment. Appl.Environm.Microbiol 61 :2388-2393.

V Lees, NJP Owens and JC Murrell (1991); Nitrogen metabolism in marine methanotrophs. Arch.Microbiol. 157:60-65.

Appendix



Photographs (phase contrast) of secondary enrichments from the chemostat grown on methanol-agar plates

CP