Taurine utilization by Purple non-sulfur bacteria

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Introduction

Taurine (2-aminoethanesulfonate) is an amino acid analogue of β-alanine and occurs in nature as a result of cysteine catabolism. In animal tissues and in urinary excretions taurine is present in both free and bound forms. Despite its presence in many animal tissues, animals are unable to metabolize taurine (Shimamoto and Berk, 1979). On the other hand, taurine belongs to the sulfonate compounds (which contain a sulfur atom covalently linked to a carbon), which are widely distributed in natural habitats and are byproducts of chemical syntheses for commerce (Chien et al, 1995).

Several studies have been done on taurine metabolism and only few microorganisms have been found that can grow utilizing this compound as the sole source of carbon, nitrogen, sulfur and energy. For example, *Agrobacterium* species can use taurine as the principal source of both carbon and nitrogen, with the production of ammonia and inorganic sulfate (Stapley and Starkey, 1970). The most recent studies noted that several enteric bacteria utilized sulfonate-sulfur for respiratory but not fermentative growth, perhaps reflecting the need for molecular oxygen in the attack C - S linkage (Uria-Nickelsen et al, 1993). In order to assess this possibility, purple non sulfur bacteria present an interesting type of microorganisms since they can growth anaerobically in the light and aerobically in the dark. The main goal of this research is try to test the capacity of the purple non-sulfur bacteria to utilize taurine as sole source of sulfur as well as electron donor for phototropic growth and as sole source of carbon, energy and sulfur for respiratory growth.

Materials and Methods

Strain Isolation. Bacteria were isolated from water samples of School Street Marsh using a membrane filter technique. We filtered 1.0, 5.0 and 10.0 ml samples through individual 0.45 μm membrane filters and placed them on PNSB agar plates (see below) at room temperature, under incandescent light in anaerobic conditions (GasPak jars). After pink, purple or red colonies became visible colonies were picked and restreaked in plates with the same agar medium and also in liquid cultures using screw capped tubes completely filled. The isolates were observed by phase contrast and electron microscopy.

Media. All the strains were growing on PNSB medium that contains: KH2PO4 1.0 g ; MgSO4 7H2O 0.4 g ; NH4Cl 0.5 g; CaCl2 2H2O 0.05 g ;

succinic acid 1.18 g ; SL12 (trace mineral solution) 1 ml and yeast extract 0.5 g. The same medium was used for testing the ability of differents sulfonates to serve as sole source of sulfur (phototrophic growth), except that MgCl2 6H2O and the corresponding sulfonate replaced in equimolar amounts, magnesium sulfate like magnesium and sulfur source, respectively. For testing the ability of taurine to serve as carbon source and electron donor, this compound was also used in other different medium, in an equimolar amount to replace the concentration of succinic acid. In the last two medium, yeast extract was replaced by six vitamin solution to avoid other sulfur sources. Other medium without sulfonate compound was used like negative control of growth (Appendix 1). Media were adjusted to pH 6.8 and sterilized before use.

Sulfonates utilization experiments. Five strains growing anaerobically on PNSB agar medium were used to inoculate screw capped tubes containing the same PNSB broth, magnesium chloride plus different sulfonates broth or only magnesium chloride broth.At all, six different sulfonates was used: Taurine, MOPSO, Isethionic acid, HEPES, Cysteic acid and Methanesulfonic acid. Experiments were replicated two times for phototropic or respiratory growth, during around 3 days at room temperature. Aerobic conditions were attained by shaking flasks with 10 ml of medium on dark. Similar procedure was conducted to determine taurine like only source of carbon and electron donor

Results

Strain isolation

Five strains were isolated from water samples of School Street Marsh under anaerobic conditions but none strain become to be pure culture like was showed by light microscopy (Figures 1, 2 and 3). In all cases can be apreciated two different morphologies: non-motile rods and motile gram negative spiral shape cells. The transmition electron microscopy show cells from PNSB*b* and PNSB*1* with a polar flagella (Figures 4 and 5).

Utilization of sulfonates as sulfur source

The different isolate strains show a wide range of capacity to utilize anaerobicly the sulfonates tested (Table 1). The strains PNSB*a* and PNSB*c* only couldn't grow on methanesulfonic acid mientras que PNSB*b* can grow in all the sulfonates, and it was the only that could used methanesulfonic acid under this condition. PNSB*a*, *b* and *c*, growth like the control in taurine (Figure 6) since like PNSB*1* and PNSB*2* couldn't grow on taurine and methanesulfonic acid. Under aerobic conditions (Table 2), all the strains grow less than the control on cysteic acid and showed a

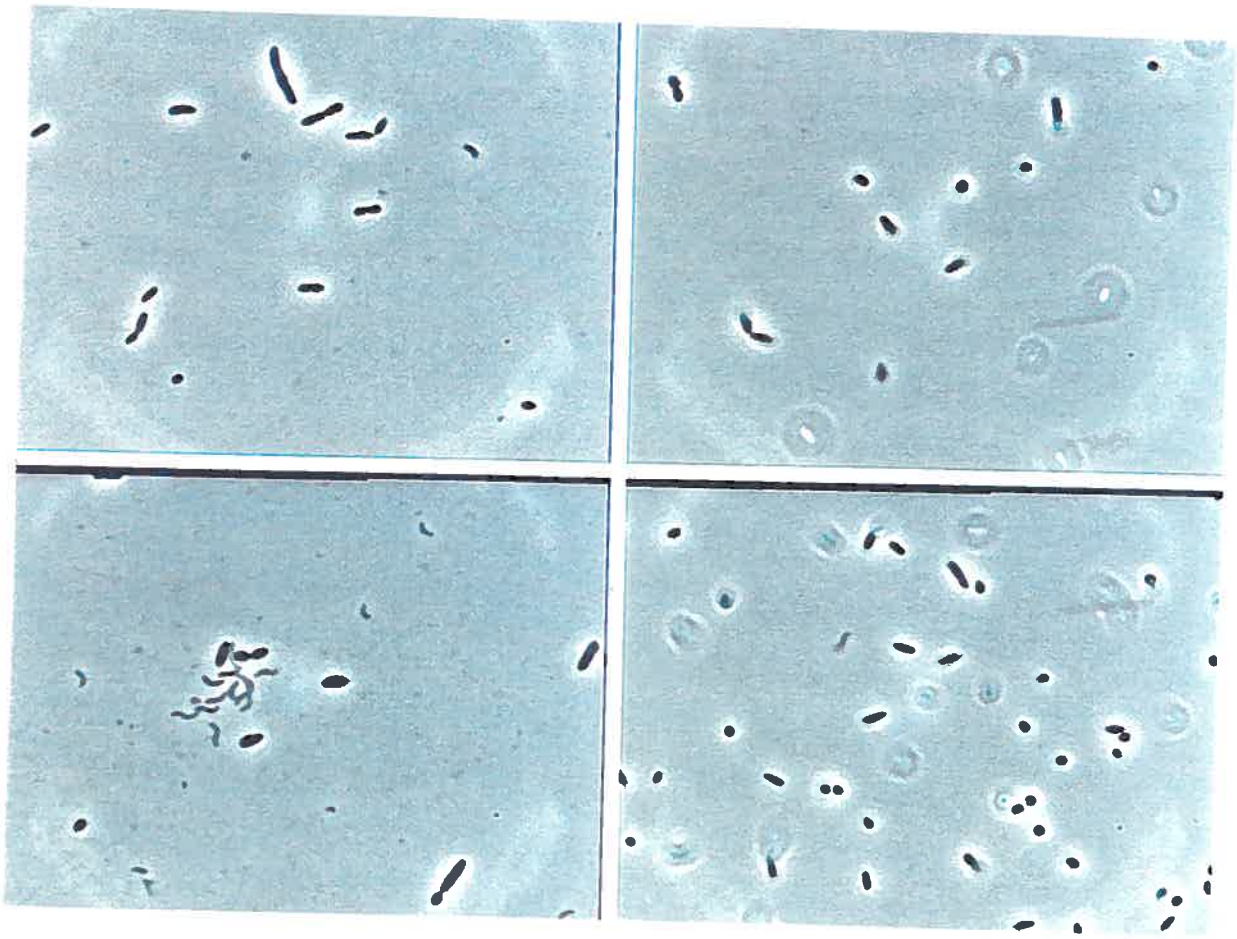

Figure 1: light microscopy of PNSB*a* (up) and PNSB*b* (down) growing on Taurine or PNSB broth (left or right, respectively).

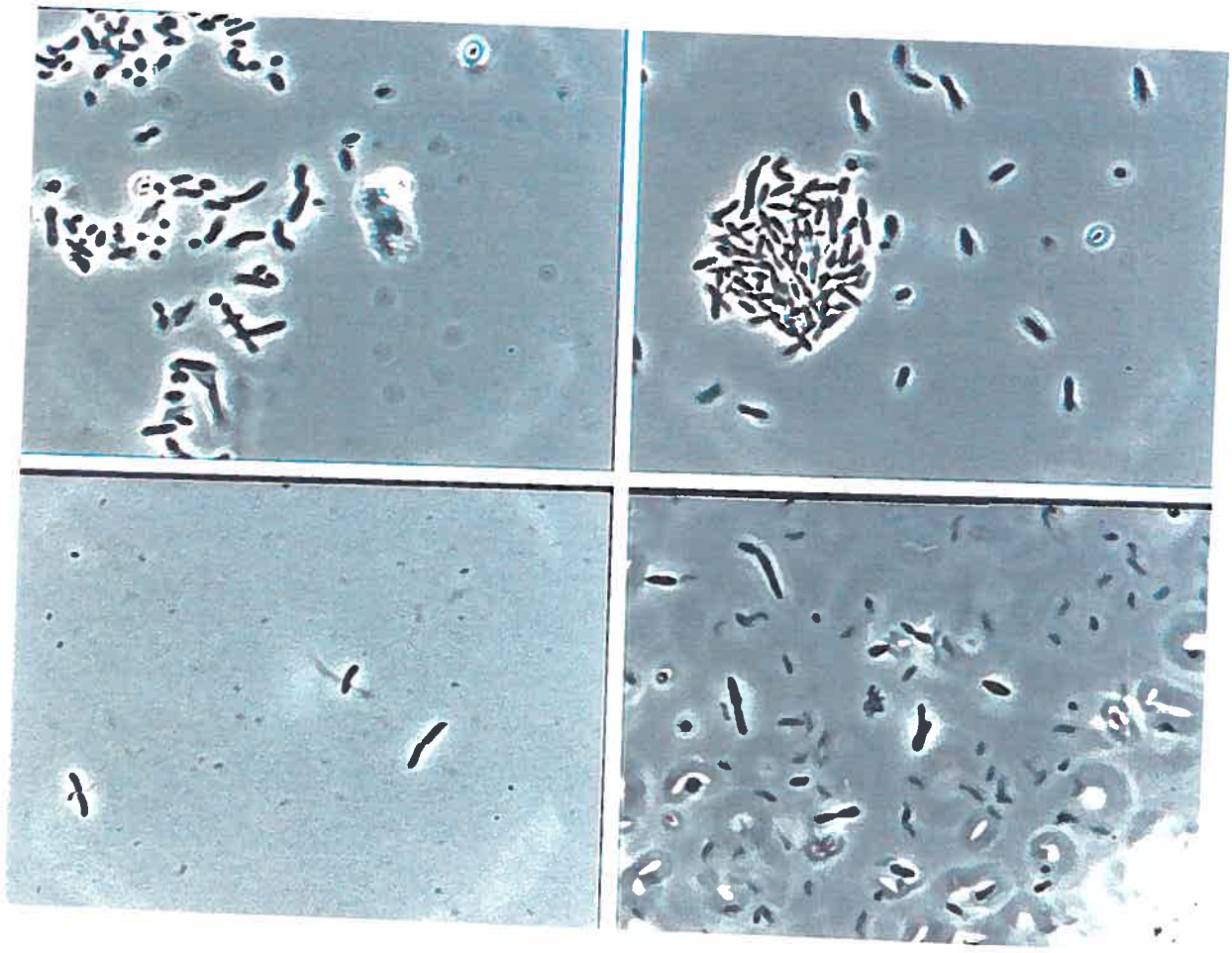

Figure 2: light microscopy of PNSB*c* (up) and PNSB*l* (down) growing on Taurine or PNSB broth (left or right, respectively).

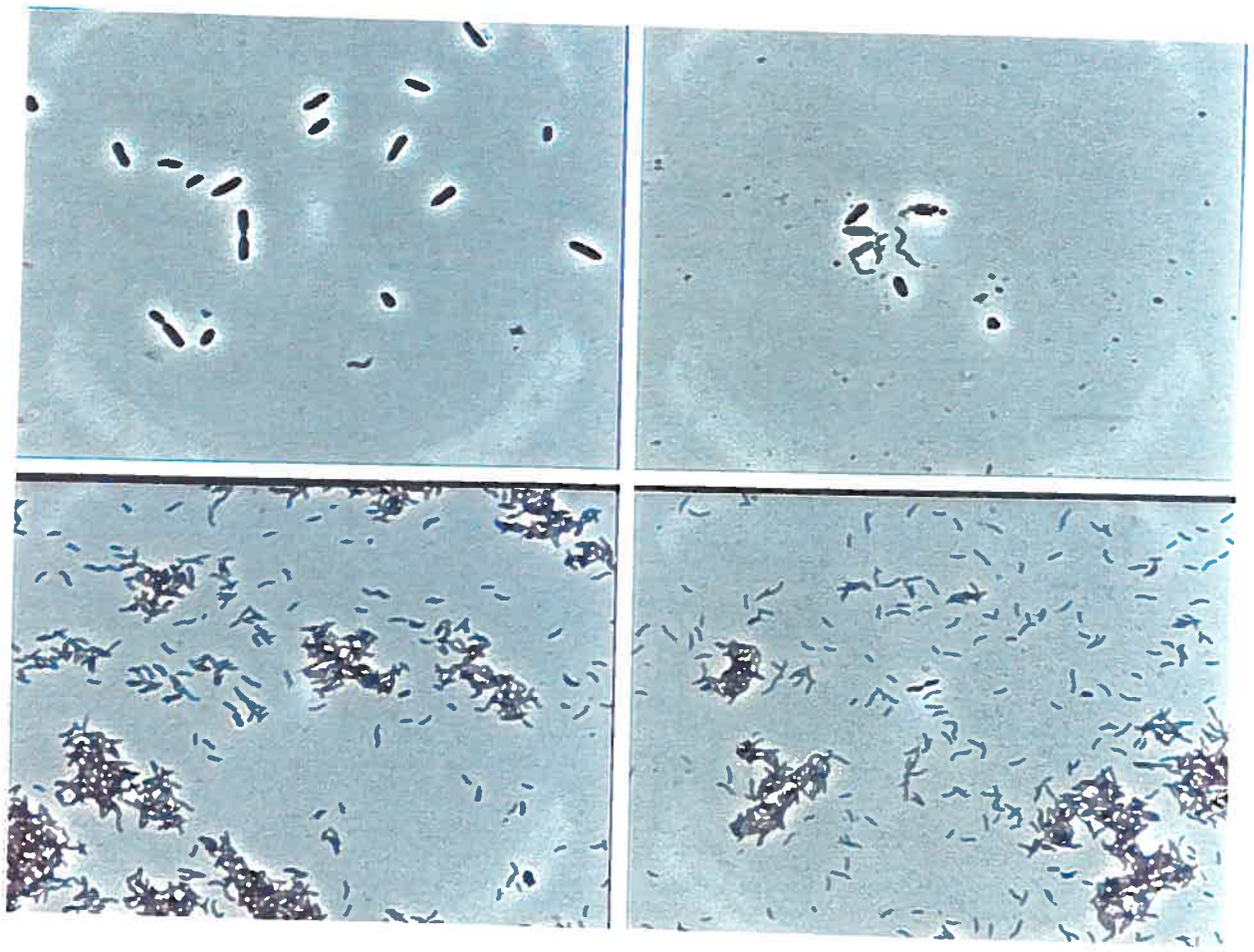

Figure 3: light microscopy of PNSB*2* (up) and PNSB*2* on gran stain (down) growing on Taurine or PNSB broth (left or right, respectively).

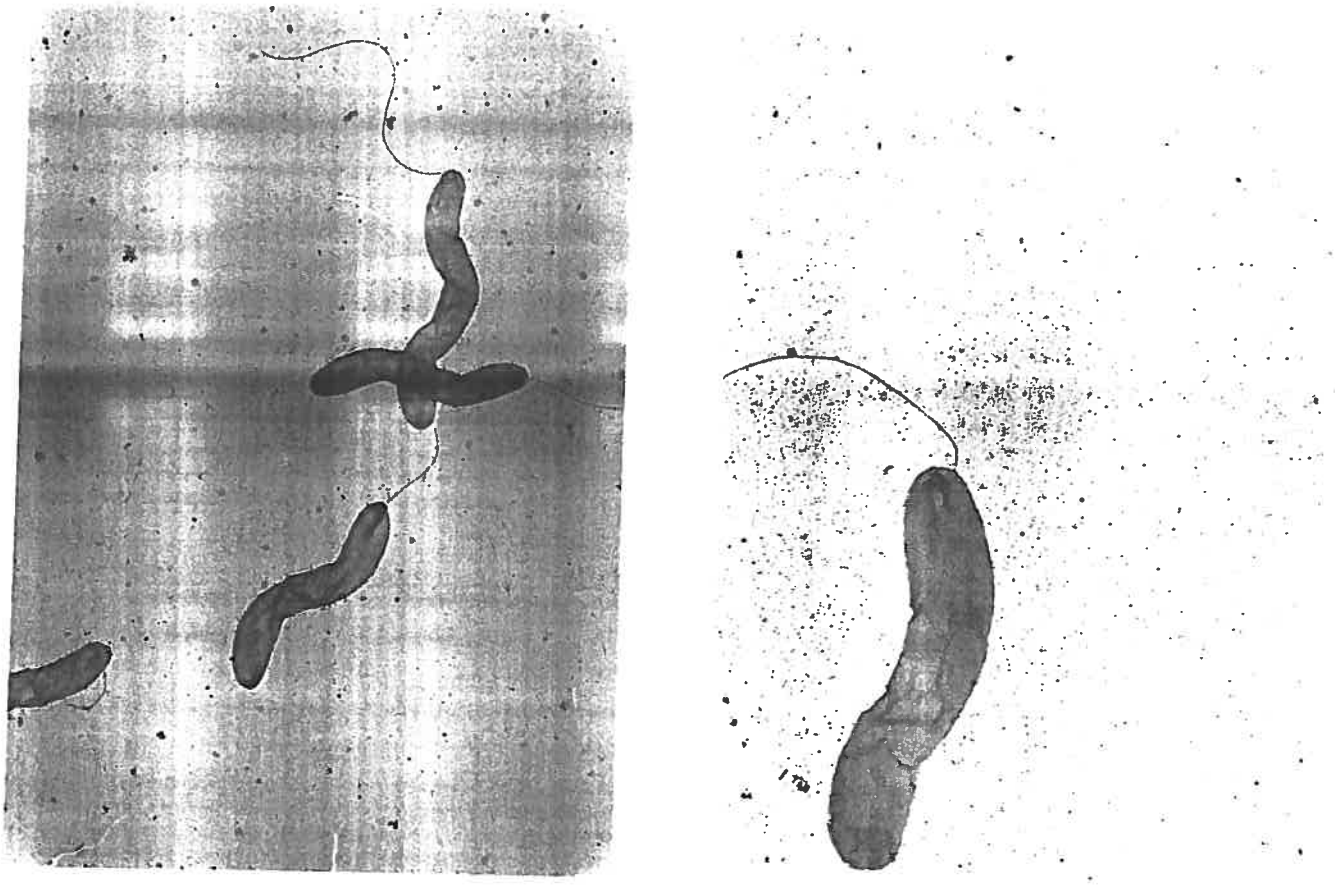

Figure 4: transmition electron microscopy of PNSB*b* growing on PNSB broth. A polar flagella can be observed.

Figure 5: transmition electron microscopy of PNSB*1* growing on PNSB broth. flagella can be observed in all the field.

PNSB strains	Taurine (e-donor)	MOPSO buffer	Isethionic acid	HEPES buffer	Cysteic acid	Methanesulfonic acid	Magnesium Sulfate	Magnesium chloride
a	-	$+^{+}$	$+^{+}$	+	d^{+}	-	++	-
b	-	++	$+^{+}$	++	d^{+}	+	++	-
c	-	$+^{+}$	$+^{+}$	$+^{+}$	d^{+}	-	++	-
1	-	$+^{+}$	$+^{+}$	$+^{+}$	d^{+}	-	++	-
2	-	+	$+^{+}$	$+^{+}$	-	-	++	-

Table 1: Anaerobic utilization of sulfonate compounds in some strains of purple non-sulfur bacteria (PNSB). Magnesium sulfate is the usual source of sulfate used to prepare PNSB medium (positive control). Magnesium chloride was used in the medium like negative control.

Legend:

+ ; grow
- ; no grow
++ ; good grow (like in positive control)
d^{+} ; light grow
$+^{+}$; almost like the positive control

Figure 6: PNSB*b* growing on taurine broth can be observed. In the first 24 hours this strain grow better in taurine than in the positive control (PNSB broth with magnesium sulfate).

PNSB strains	Taurine	MOPSO buffer	Isethionic acid	HEPES buffer	Cysteic acid	Methanesulfonic acid	Magnesium Sulfate	Magnesium chloride
a	-	++	++	++	+	++	++	-
b	-	++	++	++	+	++	++	-
c	d^+	++	++	++	$+^+$	++	++	-
1	d^+	++	++	++	+	++	++	-
2	d^+	++	++	++	+	++	++	-

Table 2 : Aerobic utilization of sulfonate compounds in some strains of purple non-sulfur bacteria (PNSB). Magnesium sulfate is the usual source of sulfate used to prepare PNSB medium (positive control). Magnesium chloride was used in the medium like negative control.

Legend:

+ ; grow
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d^+ ; light grow
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litle utilization of taurine, but grow very well on the rest of sulfonates tested. MOPSO, Isethionic acid and HEPES can be used under phototropic and respiratory conditions, but cysteic acid was better used under aerobic than anaerobic conditions.

Utilization of Taurine like carbon source and electron donor

The isolated strains didn't grow when taurine was added like unique source of carbon and electron donor, don't matter the grow conditions.

Discussion

A pure culture is defined as the progeny (clone) of a single cell. To establish a pure culture, demonstrate its purity beyond doubt, and maintain it free from contaminants is one of the most important tasks for microbiologists. By careful separation of single colonies, their suspension in suitable liquid, and repeated streaking out it is possible to obtain pure cultures of the majority of microorganisms (Schlegel, 1993). Althougt, many times result necesary repeat this procedure a lot of times and you don't have success, like in our case. It probably happen that the bacteria grow together like a 'consortia' and is more dificult to obtain a pure culture.In our experiments, we don't take care of the fact that we don't get pure cultures and we tested the five isolate strains.

Although the strains aren't pure cultures, different sulfonates were test like sulfur source for the purple non-sulfur isolates, and was clear the utilization of MOPSO, Isethionic acid and Hepes for all the strains under aerobic and anaerobic conditions. Taurine utilization was most notorius under anaerobic (phototropic) conditions.On the other hand, the fact that methanesulfonic acid can be used as a source of sulfur by purple non-sulfur bacteria, enhance the recent finding of that enteric bacteria also can utilize this compound (Uria-Nickelsen et al, 1993). The utilization was clear only under aerobic conditions and these reveal that molecular oxygen is probably neccesary to use this compound (Leadbetter, E. com. pers).

The purple non-sulfur bacteria tested weren't able to utilize taurine like sole source of sulfur, carbon and electron donor and this was also observed in *E. coli* by Uria-Nickelsen et al (1993). These researchers didn't found evidences for taurine toxicity and yet is unknown why can't be used like carbon source and electron donor. It seem that the chemical structure (carbon long-chain) of the sulfonate compounds don't affect the utilization of the compound like sulfur source, but probably does it when is used like sole source of carbon and energy. More studies about sulfonates utilization in purple non-sulfur bacteria must be done in the next future.

Conclusions

Purple non-sulfur bacteria have great capacity to utilize sulfonates compounds, at least for what we can expect in terms of the literature.

Purple non-sulfur bacteria can use different sulfonates like sulfur source, don't matter how long is the carbon chain of the compound

More research is necesary to know more about why taurine can't be easily utilized

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PNSB medium	Sulfonates as sulfur source	Taurine as sulfur and carbon source and e^- donor
$MgSO_4 \cdot 7H_2O$	6 different sulfonates + $MgCl_2 \cdot 6H_2O$	Taurine + $MgCl_2 \cdot 6H_2O$
Succinic acid	Succinic acid	Taurine (equivalent to amount of succinic acid)
Yeast extract	Six vitamin solution	Six vitamin solution

Appendix 1: Differences on medium composition to studie utilization of sulfonate compounds like sulfur source, or Taurine like sulfur and carbon source and electron donor.

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