Bulletin of Environment, Pharmacology and Life Sciences
Volume 1, Issue 5, April 2012: 40 - 42
Online-ISSN 2277- 1808

BEPLS
www.bepls.com

Original Article

Isolation of Sulfide Oxidizing Bacteria from Sewage - *Thiobacillus* Species

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ABSTRACT
In the course of pioneering studies, it is examined that the properties of the very large rod-shaped immotile bacteria of the Thiobacillus group, which occur characteristically in sulfide rich environments, can oxidize sulfur. These bacteria can be purified and grown without difficulty, as a result of their ability to oxidize chemically reduced forms of sulfur, notably Thiosulfate and elemental sulfur. These bacteria can be cultured and grown without difficulty from the sewage samples taken.
Key words: Sulfide Oxidation, Thiobacillus, Bacteria

INTRODUCTION

The polarly flagellated rods, placed in the genus Thiobacillus occur widely in both marine and terrestrial environments. The range of Thiobacillus DNA base composition is extremely wide, indicating substantial genetic heterogeneity. The chemoautotrophic growth of these organisms are rapid, some having generation time as short as 2 hours when grown at the expense of Thiosulfate. They have the ability to oxidize chemically stable reduced forms of sulfur, [1-5] notably Thiosulfate and elemental sulfur.

MATERIALS AND METHODS

1. T_2 Medium for *Thiobacillus* (ATCC Medium 1193)

Composition per liter:
Solution A- 250.0 ml
Solution B- 250.0 ml
Solution C- 250.0 ml
Solution D- 250.0 ml
pH 7.0 $\pm$ 0.2 at 25º C

1.1 Solution – A

Composition per 250.0 ml:
$Na_2S_2O_3.5H_2O$ - 5.0 g
KNO_3 - 2.0g
NH_4Cl - 1.0g

1.2 Preparation of Solution – A

Add components to distilled/ deionized water and make the volume to 250.0 ml mix thoroughly. Filter, sterilize.

1.3 Solution – B

Composition per 250.0 ml:
KH_2PO_4 - 2.0g

1.4 Preparation of Solution - B

Add KH_2PO_4 to distilled /de-ionized water and make the volume to 250.0 ml, mix thoroughly. Filter and sterilize.

1.5 Solution – C

Composition per 250.0 ml
$NaHCO_3$ - 2.0 g

1.6 Preparation of Solution – C

Add $NaHCO_3$ to distilled/ de-ionized water and make the volume to 250.0 ml, mix thoroughly. Filter and sterilize.

1.7 Solution – D

Composition per 250.0ml

$MgSO_4.7H_2O$ - 0.8 g

$FeSO_4.7H_2O$ (2% w/v in HCl) -100.0 ml

Trace metal solution -1ml

1.8 Preparation of Solution – D

Add the components to distilled / deionized water and make the volume to 250.0 ml, mix thoroughly. Filter and sterilize.

1.9 $FeSO_4.7H_2O$ Solution

Composition per 100.0 ml:

$FeSO_4.7H_2O$ - 2g

HCl 1N Solution - 100.0 ml

1.10 Preparation of $FeSO_4.7H_2O$ Solution

Add $FeSO_4.7H_2O$ to HCl Solution. Mix thoroughly.

2. Trace Metal Solution

Composition per liter:

EDTA - 50.0 g

$ZnSO_4$ - 22.0 g

$CaCl_2$ - 5.54 g

$MnCl_2$ - 5.06 g

$FeSO_4.7H_2O$ - 4.99 g

$CoCl_2$ - 1.11 g

$CuSO_4$ - 1.57 g

$(NH_4)_2MoO_4$ - 1.1g

2.2 Preparation of Trace Metal Solution

Add components to distilled / deionized water and make the volume to 1 litre. Mix thoroughly. Adjust pH to 6 KOH solutions.

2.3 Preparation of Medium

Aseptically combine the four sterile solutions: Solution-A, Solution-B, Solution-C and Solution-D. Adjust pH to 7.0. Aseptically distribute the combined solution in to sterile tubes or flasks.

2.4 Preparation of Culture Strains

The above medium was prepared and sterilized. About 1 gm of sewage sample was taken and made in to 10 ml. Simple streaking was done by taking sample from that diluted sewage. All the works were done in laminar flow chamber to prevent contamination. Streaked Petri dishes were incubated at a temperature of 27º C for one week. Colonies of the organisms were found in the Petri dishes. This confirmed the presence of Thiobacillus in the sewage.

3. Experimental Work Done

The grown colonies of Thiobacillus species which were found in the Petri dishes were selected for its development. These strains were inoculated in broth, for its further development. The selected broth was taken in sterilized conical flasks along with a control broth. Both the inoculated broth and the control broth were kept on the shaker. Change in turbidity of the broths due to culture growth was monitored for Thiobacillus strains with the help of Nephelometric Turbidity Meter. The turbidity was monitored for every 24 hours.

RESULTS AND DISCUSSION

The turbidity values of the inoculated and control broth is given in the Table 1. The turbidity values were found to increase in the inoculated broth as the time progresses. This shows the growth of Thiobacillus species in the inoculated broth (in which selective medium was used). Whereas, the turbidity of the control broth was found to be more or less stable. That indicates that there is nothing in the control broth. The growth of bacteria in inoculated broth and control broth is shown in Figure1.

Table 1: Growth of Thiobacillus Culture

Time	Turbidity Value for Inoculated Broth (NTU)	Turbidity Value for Control Broth (NTU)
After 24 hours	12.0	11.3
After 48 hours	12.5	11.3
After 72 hours	15.1	11.3
After 96 hours	17.5	11.3
After 120 hours	20.0	11.4
After 144 hours	22.5	11.6
After 168 hours	27.0	11.7
After 192 hours	32.0	11.9
After 336 hours	34.0	12.0
After 384 hours	42.0	12.0

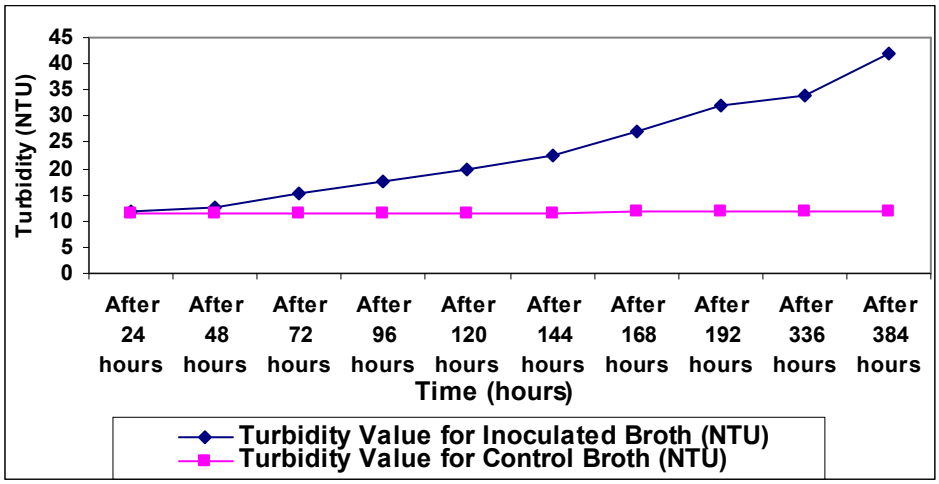

Figure 1. Growth of Bacterial Culture in Broth

CONCLUSION

From the experimental study conducted, it is evident that the sulfide oxidizing bacterial species like Thiobacillus are present in sewage. These bacterial species can be identified, cultured and grown from the sewage samples also. These bacterial species can be employed to oxidize the sulfide present in the anaerobic reactor when wastewater containing sulfate is subjected to anaerobic treatment. This could improve the performance of the anaerobic reactor.

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