Isolation of bacteria that reduce As (V) to As (III)

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INTRODUCTION

The arsenic compounds are present in the atmosphere, water and soils. The main chemical forms of these compounds in aqueous solutions at pH 7.0 are $HAsO_4^{-2}$, $H_2AsO_4^-$, H_3AsO_3, As and AsH_3 . Under oxidizing conditions both anionic arsenate species occur in appreciable proportions, whereas the uncharged species dominates in reducing environments, developing in both cases an environmental problem of contamination. The microorganisms play an important role in the transformation of arsenic, some bacteria can reduce As(V) and precipitate As(III). The biomineralization process according with the As(V) reduction form a bright yellow solid of arsenic trisulfide. For example, some As (V) reducing bacteria such as *Desulfotomaculum auripigmentum* precipitate As_2S_3 both intra and extracellularly.

The toxicity of the arsenic compound and its mobility in the environment due to microorganisms makes it very interesting to study how bacteria are able to transform arsenic participating in the arsenic cycle.

We tried to isolate bacteria that reduce As(V) to As(III) from sea water and determinate its physiology and molecular characterization.

MATERIALS AND METHODS

Samples. A wood piece from the dock of the Eel Pond in Woods Hole, MA. was taken as a sample to start the enrichments.

Medium. Enrichment cultures were grown in salt water minimal medium , supplemented with 10 mM lactate, and 50 mM sodium arsenate. The medium was buffered at pH 6.8 with bicarbonate and reduced with sulfide. We used LB agar plates too.

Analyses. As (V) was measured by the molybdenum blue spectrophotometric assay. The use of lactate was determined by HPLC.

PCR. A single colony from the agar sakes was used directly to amplify the 16S rDNA using universal primers and thermal cycling regime as is usual at the lab. The PCR product was analyzed in 1% agarose gels, stained with GelStar (FMC Products) and visualized in UV lamp.

Cloning and sequencing. We tried to clone the 16S rDNA amplification, but no recombinant plasmid was obtain, so we sequenced the PCR product directly. The sequence was determined in an automatic sequencer.

Data analysis. The sequence was analysed and compared with the databases. The homology with others species was done by a Blast search.

RESULTS

1. How to get a pure culture?

1.1 The wood piece was suspended in the supplied medium and incubated at 30°C.After a week, the precipitation of yellow arsenic trisulfide indicated the presence of some marine bacteria that could grown in the presence of arsenate.

1.2 Afterwards, we transferred the culture and additionally made agar shakes to isolate single colonies. Five days later, singles yellow colonies grew which we took to continue the experiments.

1.3 A single colony was removed from the agar, suspended in buffer and used as template to do 16S rDNA PCR directly. Only one band was obtained and the product was sequenced.

1.4 Another colony was taken from the agar tubes, transfer to a new medium and after 3-4 days the culture was grown. After that, was inoculated in LB plates and single colonies obtained as pure culture. The organisms were visualized to the microscope showing motile rods.

2. Is the As_2S_3 precipitation due to a biological process?

From an anaerobic culture we inoculated at the same time in three different conditions:

2.1. The bacteria in the prepared medium as is usual. The bacteria grew as usual, with the precipitation of the As_2S_3.

2.2. The bacteria in the medium were autoclaved. There was neither growth nor As (V) reduction.

2.3. The bacteria in the medium with 4% formaldehyde and neither growth nor arsenite precipitation.

So the appearance of As_2S_3 precipitate is due to the presence of actively growing bacteria isolated from the sea water in the medium.

3. Is a facultative anaerobe?

The position of the piece of wood in the surface of the water suggested the idea that these bacteria could grow in aerobic conditions, something previously not described . Those experiments consisted in:

3.1 The inoculation of the bacteria from an anaerobic culture to an aerobic conditions and shaking. After a few days, the bacteria was grew with the correspond precipitation of As_2S_3.

3.2 At the same time, we inoculated in aerobic and anaerobic conditions, and measured during the growth the concentration of As(V) by the color molybdenum assay, and the concentration of lactate by the HPLC. Duplicate cultures were made and samples was taken every 10 hours approximately.

Fig 1: Model pattern at A_{865} nm for As(V) concentrations.

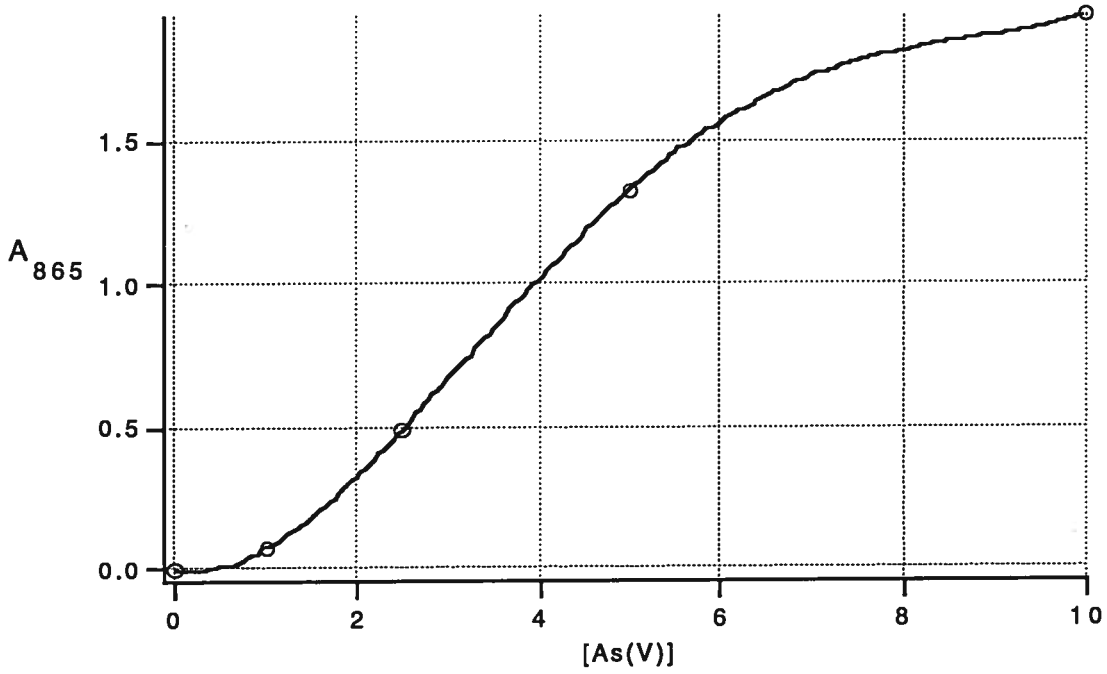
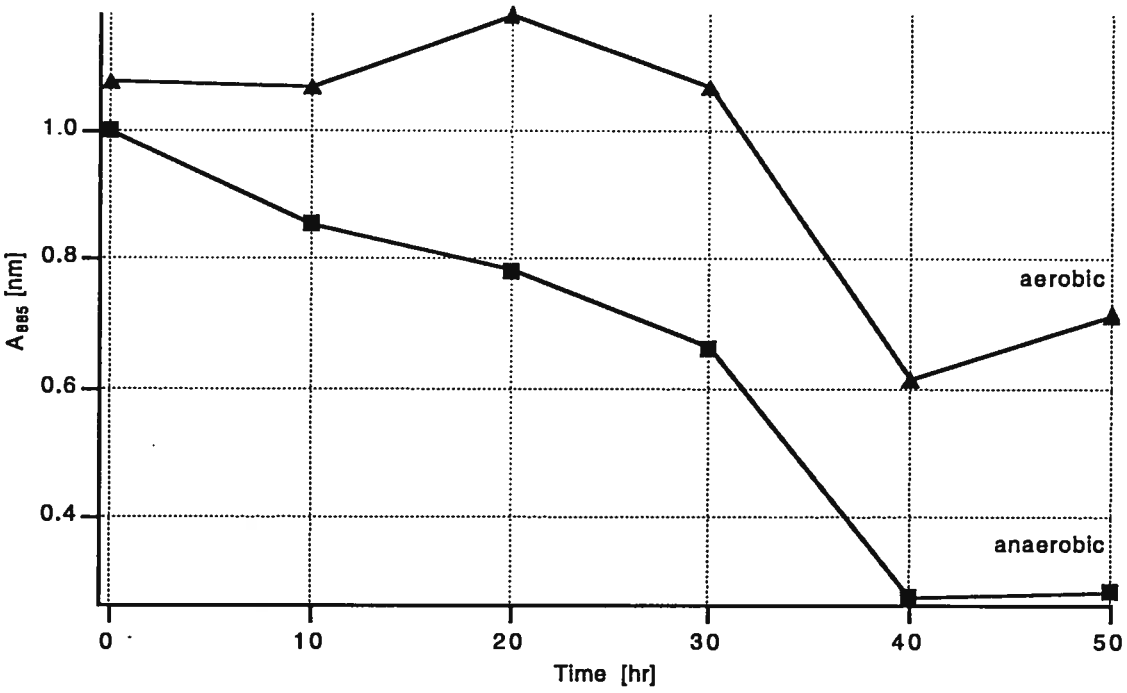


Fig 2: Graphic of the As(V) concentrations in anaerobic and aerobic samples .

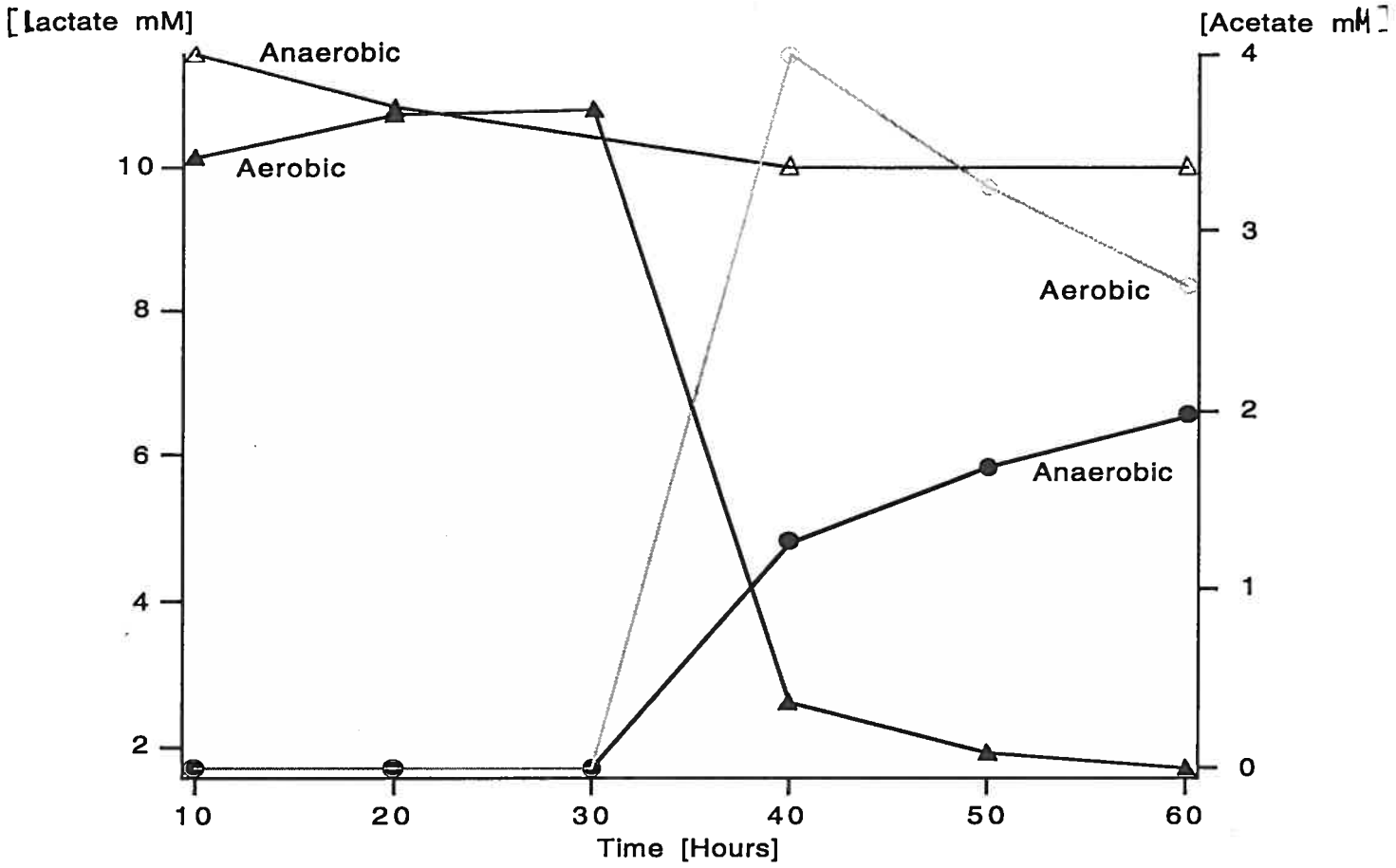


In anaerobic conditions, we observed a great decrease in the concentration of arsenate after 40 h of growth, in the moment when the yellow precipitate formed.

The concentration of arsenate in aerobic conditions takes longer to be reduced. So although in aerobic conditions this bacterium is able to grow, the rate of transformation from arsenate to arsenite is lower in aerobic conditions. Possible explanations are that bacteria can live in aerobic or anaerobic conditions, but the use of the oxygen is preferred as electron acceptor. (Newman D, 1998). On the other hand, the reduction of arsenate in sea water occur to slow rate due to the reoxidation of As (III) to As (V) (Johnson D.L,1972).

4. Is the use of lactate related to the transformation of arsenite?

The measurements of the concentration of lactate showed a high decrease in the lactate concentration after 40 h, when As_2S_3 formed and at this point acetate appeared in the medium. The rates are according with the stoichiometry of the reaction :

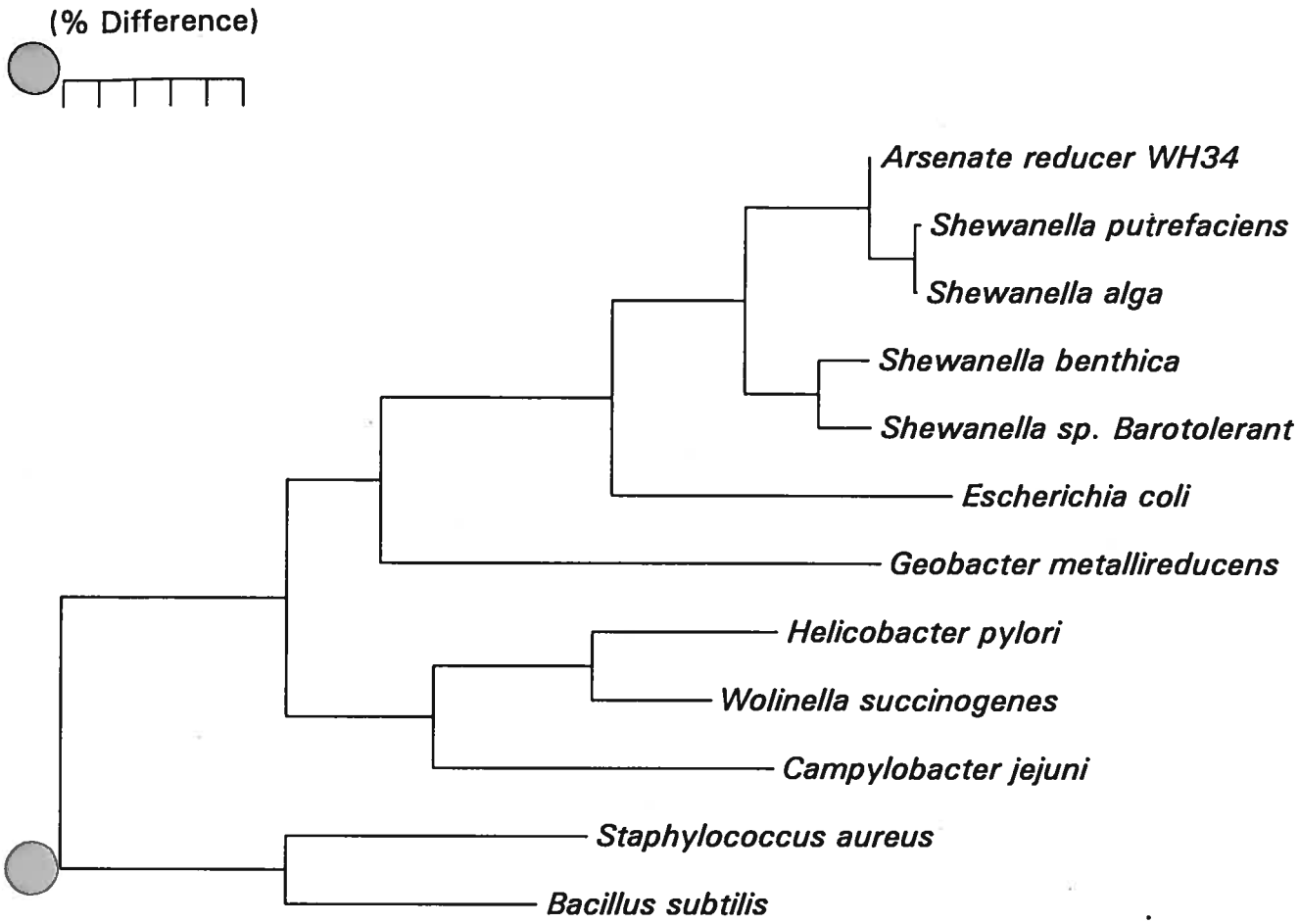


The ability of this organism to grow and reduce arsenate completely to arsenite with the addition of lactate as a carbon source, suggest that such arsenate reduction is respiratory.

5. Which bacteria is it?

The comparison with the data bases shown a highly homology (97%) with *Shewanella* sp.

To compare the physiology we inoculated at the same time, the strain *Shewanella* in the presence of arsenate in aerobic and anaerobic conditions, but it didn't grown.


(% Difference)
Arsenate reducer WH34
Shewanella putrefaciens
Shewanella alga
Shewanella benthica
Shewanella sp. Barotolerant
Escherichia coli
Geobacter metallireducens
Helicobacter pylori
Wolinella succinogenes
Campylobacter jejuni
Staphylococcus aureus
Bacillus subtilis

CONCLUSIONS

The results shown that an organism able to reduce arsenate to arsenate, living in the seawater has been isolated. The possibility to be an anaerobe facultative is a new approach for the As reducing bacteria. Surprisely, our isolate is able to precipitate the arsenic trisulfide with the presence of oxygen. These characteristics suggests that a new specie of *Shewanella* could be isolated, but further experiments are required. Some interesting approaches could be:

- to experiment which is the highest level of As(V) in which these bacterium can survive.
- to determinate if the ability of transform the arsenate to arsenite is due to the presence of some specific plasmid.
- to calculate the most probably number of *Shewanella* sp. in the environment that respire arsenic.
- to detect the most effective rates of transformation of arsenate to arsenite
- to determinate the highest growth at differents concentrations with differents carbon sources.
- SEM

Acknowledgments

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