Distribution of *Euglena mutabilis* Schmitz and its Function on the Formation of Chemical Environment in Acid Mine Drainage (AMD)

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Abstract

Euglena mutabilis Schmitz is an index species of acid mine drainage (AMD). We found the new distribution sites of *E. mutabilis* in northern part of Kyushu; Insen, spring of Bougatsuru mire and Tadewara mire, Minamidagawa river of AMD. The water chemistry at the distribution sites were not acidic and *E. mutabilis* does not necessarily requires acidic habitats. The significant difference was not observed in water chemistry between the habitats of with and without *E. mulabilis*. We carried out incubation experiment with AMD and *E. mutabilis* growing in Minamidagawa river to analyze function in the water chemistry formation of *E. mutabilis*. The oxidative bacteria produce $Fe(OH)_3$ and decreases pH and concentration of dissolved iron in AMD. Under dark condition with increasing concentration of dissolved iron, the morphology of the organisms changed and mobility was lost. *E. mutabilis* inhibit pH decrease under exposure to light.

Introduction

The flagellate, *Euglena mutabilis* Schmitz is an index species of acid mine drainage (AMD) and the species distributes usually in acidic water stream of mining sites[1]. AMD is usually characterized by low pH and concentration of heavy metals and sulfate ion. Thus, *E. mutabilis* has tolerance to highly acidic condition and high contamination of heavy metals[2]. However, the environmental condition of the habitat of *E. mutabilis* and the requirement for the growth has not been reported. In this study, we surveyed the distribution sites of E. mutabilis in the northern part of Kyushu and estimated the water chemical condition for the growth of the species in natural condition. And then we determined the chemical requirements of the species by the experiments of incubation under different chemical condition.

Material and methods

We survey the 14 sites of acidic spring including AMD in the northern part of Kyushu and recorded the existence or nonexistence of E. mutabilis as well as water chemistry at the surveyed sites. We measured temperature, pH, EC、Cu、SO_4^{2-}、Fe、Ca、Mg、Si of the water. The existence of *E. mutabilis* was first observed at the field and then determined with a microscope in the laboratory.

Sampling site of materials used for incubation experiments was Minamidagawa river in Sensui, Kurate, Fukuoka. We used AMD, biofilm including *E. mutabilis*, *E. mutabilis* biofilm with sediment material, and sediment material without biofilm for incubation media. Five types of incubation media were prepared; (1) AMD (60ml) + *E. mutabilis* (7 mL) + sediment material (6g), (2) AMD (60ml) + *E. mutabilis* (7 mL), (3) AMD + sediment material (6g), (4) AMD (60ml), (5) AMD (60ml) + chloroform (1mL). Chemical change of AMD was measured with test tube (30mm x 200mm) containing each of the media and incubated in growth chamber at 20°C, photon flux density of 160μmol m^{-2} sec^{-1}, with 12hours light-dark cycle condition. Incubation was also made under constantly dark conditions and under constantly light conditions. We measured pH, ORP and concentration of dissolved iron at every day.

Results and discussion

Existence of *E. mutabilis* was confirmed in Insen, spring of Bougatsuru mire and Tadewara mire, and Minamidagawa river. We show data of pH、Ca、Mg、Si 、Fe、SO_4^{2-} at each of the surveyed sites in Fig.1-4. Despite *E. mutabilis* is reported to distribute highly acidic habitats, we found the species even under neutral condition of pH=7.04. Thus we found that *E. mutabilis* is acid tolerant species, however low pH is not a required condition that the species grow. The significant differences of water chemistry were not observed between sites with and without *E. mutabilis* in the surveyed sites. The Minamidagawa river was the most abundant site of *E. mutabilis* showed high concentration of SO_4^{2-} (2055 ppm), Ca (477.4 ppm) and Fe (100.4 ppm). *E. mutabilis* has high tolerance to sulfate and metal ions typical for AMD.

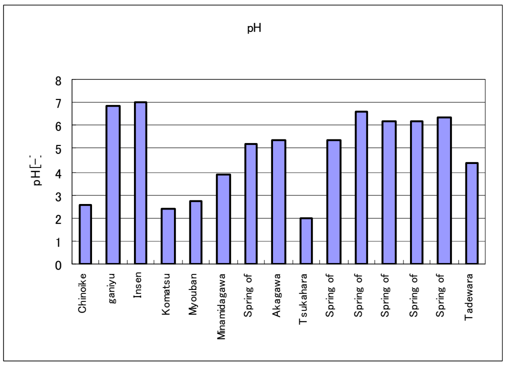


Fig.1 Value of pH

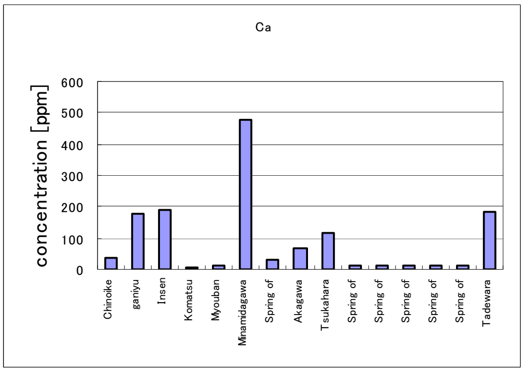


Fig.2 Concentration of Ca

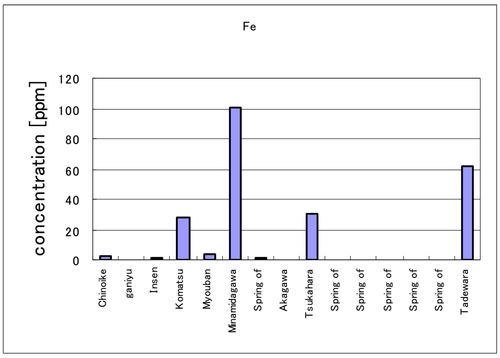


Fig.3 concentration of Fe

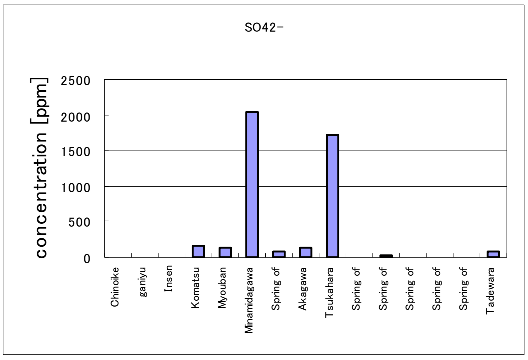
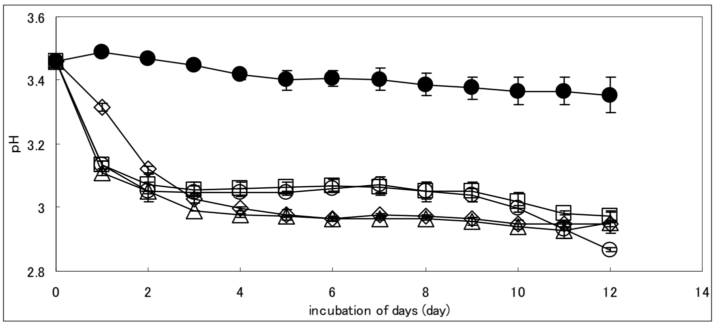


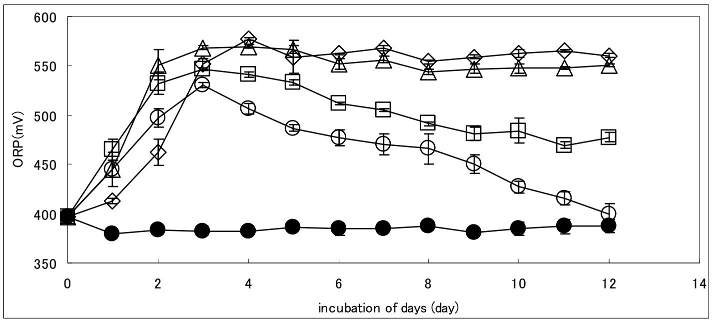
Fig.4 concentration of SO_4^{2-}

We showed the result of incubation experiments in Fig. 5-7. During the incubation, pH and concentration of dissolved iron decreased and ORP increased, except for AMD containing chloroform. This shows that the microorganisms in AMD works for changing water chemistry. And the sample including sediment material showed rapid ORP increase as well as rapid decrease of pH and dissolved iron concentration compared as medium without sediment. Therefore, there were oxidative bacteria in the sediment material and AMD. Under the light condition, AMD with chloroform showed slow decrease of pH and dissolved iron concentration during 12 days of incubation, whereas medium with *E. mutabilis* and AMD showed high rate of decrease of these chemical parameters. Under the dark condition, pH and dissolved iron concentration decreased to a lower value within one day of incubation except for AMD and AMD with chloroform. The incubation medium including *E. mutabilis* and sediment material showed decrease of pH and increase of dissolved iron concentration from the 10th day of incubation. At the moment, morphology of *E. mutabilis* changed and mobility was lost. Under the constantly light condition as well as light-dark condition, the incubation medium with only *E. mutabilis* showed the little change of pH.



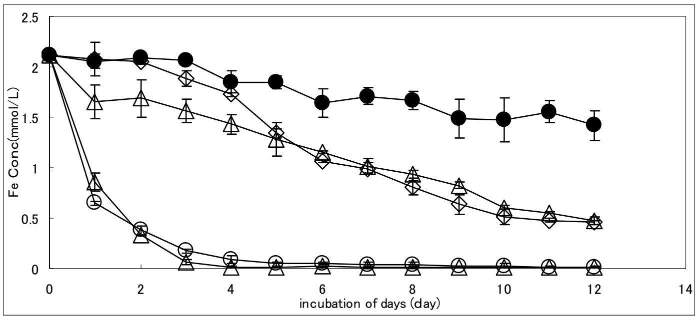
○ :*E.mutabilis*+bottom of mateiral △: *E.mutabilis* □:bottom of material
◇:AMD ●:sterile AMD

Fig.5 Value of pH under the dark conditions



○ :*E.mutabilis*+bottom of mateiral △: *E.mutabilis* □:bottom of material
◇:AMD ●:sterile AMD

Fig.6 Value of ORP under the dark conditions



○ :*E.mutabilis*+bottom of mateiral △: *E.mutabilis* □:bottom of material
◇:AMD ●:sterile AMD

Fig.7 Fe concentration under the dark concitions

Therefore, *E. mutabilis* inhibit pH decrease by exposure to light. One of the possible reasons why *E. mutabilis* inhibit pH decrease by exposure to light is the consumption of CO_2 by photosynthesis of *E. mutabilis*. In addition, dissolved iron concentration in incubation media with *E. mutabilis* increased from the 10th day in AMD under the dark conditions. The increase of iron concentration just corresponded to the morphological change of organisms. Thus we found that *E. mutabilis* release accumulated iron after the death of organisms.

Unfortunately, we were not successful to determine the cultivation condition of *E. mutabilis*. If we are successful to culture *E. mutabilis*, it can be used for the accumulation of irons as well as heavy metals, and reduce acidity of acidic waste water. *E. mutabilis* could be the new useful microorganisms for the water treatment. It will be necessary to carry out incubation experiment to detect required condition for the growth of *E. mutabilis*.

Acknowledgement

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