APPLIED AND ENVIRONMENTAL MICROBIOLOGY, June 1995, p. 2439–2441 Vol. 61, No. 6
0099-2240/95/$04.00+0

Oligotrophic Bacteria Enhance Algal Growth under Iron-Deficient Conditions

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Received 29 November 1994/Accepted 11 April 1995

A *Halomonas* sp., a marine halophilic and oligotrophic bacterium, was grown on exudates of *Dunaliella bardawil*. The bacteria increased the solubility of Fe, thereby enhancing its availability to the algae. As a result, the algal growth rate increased. Because of these syntrophic relations, growth of the marine alga *D. bardawil* was facilitated at Fe levels that would otherwise induce Fe deficiency and inhibit algal growth.

The role of Fe nutrition in marine phytoplankton has attracted much attention in recent years (10, 11, 16). The availability of Fe for microbial uptake appears to be an important variable in determining the stability and composition of aquatic ecosystems. Marine phytoplankton, like other living organisms, exhibits a specific nutritional requirement for Fe (14). Fe is essential for NO_3^- utilization, chlorophyll biosynthesis, and numerous other cellular functions in phytoplankton. In this paper we provide novel evidence for the involvement of Fe chelators produced by microorganisms (siderophores) in the uptake of Fe by algae via a syntrophic relationship between bacteria and algae. Microbial siderophores have been reported to be efficient Fe-chelating agents in soil solutions (7, 13), freshwater (12), and oceans (16). Siderophores are low-molecular-weight molecules with a high affinity for Fe^{3+}. A detailed review by Neilands (13) shows them to act as major components of the Fe uptake mechanism of bacteria, fungi, and blue-green algae (cyanobacteria). Although the role of siderophores produced by terrestrial bacteria in the Fe nutrition of higher plants has been demonstrated (3, 4, 7–9), the actual mechanism of Fe uptake by high marine algae has, to date, not been clarified. We report here, for the first time, how a *Halomonas* sp., a halophilic and oligotrophic bacterium, improves the availability of Fe to the benefit of the alga *Dunaliella bardawil* grown under Fe-deficient conditions. We hypothesize that siderophores produced by marine bacteria have the potential to increase the solubility of Fe, thereby making it more available to algae and facilitating algal growth under Fe-deficient conditions.

D. bardawil (5) (strain obtained from the Department of Biochemistry, Weizmann Institute of Science, Rehovot, Israel) was grown in an artificial seawater medium at pH 10 containing the following final concentrations in solution: 5 mM KNO_3, 5 mM $MgSO_4$, 0.3 mM $CaCl_2$, 0.1 mM KH_2PO_4, 1.5 M NaCl, 50 mM $NaHCO_3$, and 5×10^{-8} M Fe (total soluble and colloidal concentration). Under these conditions, the growth rate was limited by Fe deficiency. Fe was added at several concentrations (as $FeCl_3$) to axenic and xenic media. At the prevailing solution pH of 10, the expected calculated soluble Fe concentration is 10^{-9} M. The rest of the Fe precipitates as $Fe(OH)_3$ and as such is excluded from the solution. It was thus unavailable to the algal cells. In the xenic medium, a bacterial population which reached a level of 10^4 to 10^9 CFU/ml developed. Algae grown concomitantly with the bacteria were found to respond to lower Fe concentrations than those grown under axenic conditions (Fig. 1). The bacteria accompanying the algae could grow at the expense of dissolved organic carbon released by *D. bardawil* primary production. Relationships in which heterotrophic bacteria utilize extracellular dissolved organic carbon released from phytoplankton have been extensively studied (19–21). The algae, on the other hand, were able to utilize Fe solubilized by the bacteria but did not respond to the presence of bacteria at either the lowest or the sufficient Fe level (5×10^{-8} M Fe [Fig. 1a] or 2×10^{-6} M Fe [Fig. 1d], respectively). However, at intermediate Fe levels (5×10^{-7} to 1×10^{-6} M Fe), which still caused Fe deficiency (Fig. 1b and c), significant differences in both growth rate and final cell number were observed. We concluded therefore that the presence of bacteria does not interfere with the growth of *D. bardawil*. Moreover, at some critical Fe concentration, the bacteria solubilize $Fe(OH)_3$ precipitates to the benefit of the algae.

Bacteria were isolated from algal cultures grown on various solid media and were found to prefer halophilic and oligotrophic growth conditions (80 to 200 g of NaCl per liter plus algal spent growth medium as a sole carbon source or 1 g of yeast extract per liter) to the rich media containing wide ranges and high levels of nutrient supplies. Glucose, sucrose, malate, and succinate actually inhibited bacterial growth. The bacteria were identified as a new line in the *Halomonas* group (24). The isolated bacterial strain was characterized by basic physiological tests, fatty acid analysis, and 16S rRNA sequencing (German Collection of Microorganisms and Cell Cultures, DSM, Braunschweig, Germany).

To verify the existence of syntrophic relations between *D. bardawil* and the *Halomonas* sp., a controlled inoculation experiment using a pure culture of bacteria was conducted. Algal cells were grown at the Fe concentration which had shown the most significant difference in growth caused by bacterial coculturing (5×10^{-7} M as $FeCl_3$), and growth was compared with that in a culture medium with no added Fe (5×10^{-8} M Fe). These treatments were applied to both axenic and inoculated cultures. Under these conditions, algae grown in the axenic culture did not respond to the Fe amendment, whereas inoculation with the *Halomonas* sp. significantly stimulated algal growth (Fig. 2). The growth of *D. bardawil* was stimulated only in the presence of a reservoir of precipitated $Fe(OH)_3$ in the

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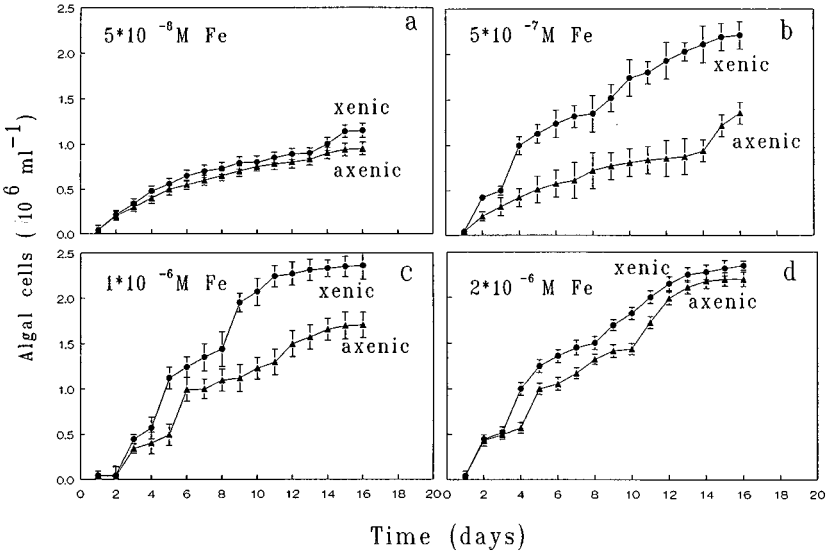

FIG. 1. Effects of Fe concentration and bacterial coculture on growth of *D. bardawil* under axenic and xenic conditions.

culture. This reservoir was solubilized by siderophores excreted by the *Halomonas* sp., and Fe uptake by the algae followed. The bacteria, on the other hand, responded well to the lowest Fe concentration of 5×10^{-8} M, and their cell number increased 4 orders of magnitude (from 10^5 to 10^9 CFU/ml). Since these results provided indirect evidence for siderophore production, the siderophore concentration was measured directly. The chrome azural S (CAS) test (Fluka, Buchs, Switzerland) (17), which is based on a color reaction of the reagent solution in accord with the complexing capacity of the tested solution, was performed on the culture solution. The results deduced from changes in absorbance between free CAS and the Fe-CAS complex indicated significant secretion, exhibiting high Fe-chelating capacity in the *Halomonas-Dunaliella* coculture (Fig. 2c). These observations comply with earlier reports showing that terrestrial (7) as well as marine (15, 16, 22, 23) bacteria use a siderophore-mediated ferric uptake system in response to low Fe environments.

The results shown in this paper provide an explanation for the growth enhancement of *D. bardawil* in coinoculated media under Fe stress conditions. Allnutt and Bonner (1, 2) have shown earlier that green algae are able to utilize Fe chelated by microbial siderophores. We obtained a similar response with *D. bardawil* to the microbial siderophores ferrioxamine B and ferrichrome, in addition to the data described in this paper. The mechanism of Fe uptake under Fe stress of *D. bardawil* probably involves reduction of the Fe^{3+} chelated to the siderophore released by the bacteria. Following the reduction, Fe^{2+} is transported to the cell and the free ligand is released to the medium. The mechanism for reduction of Fe by Fe-deficient plants has been proposed to be a trans-plasma-membrane electron transport system (6, 18).

We investigated the involvement of the halophilic and oligotrophic *Halomonas* sp. bacterium in the improvement of Fe utilization by the marine alga *D. bardawil* under Fe stress conditions. We then demonstrated the existence of syntrophic relations between bacteria and algae. This relationship enhances the availability of Fe to the benefit of algae grown under Fe deficiency, by releasing siderophores that act as Fe carriers to the algal cell. The algae are thus able to grow at Fe levels that would otherwise induce Fe deficiency.

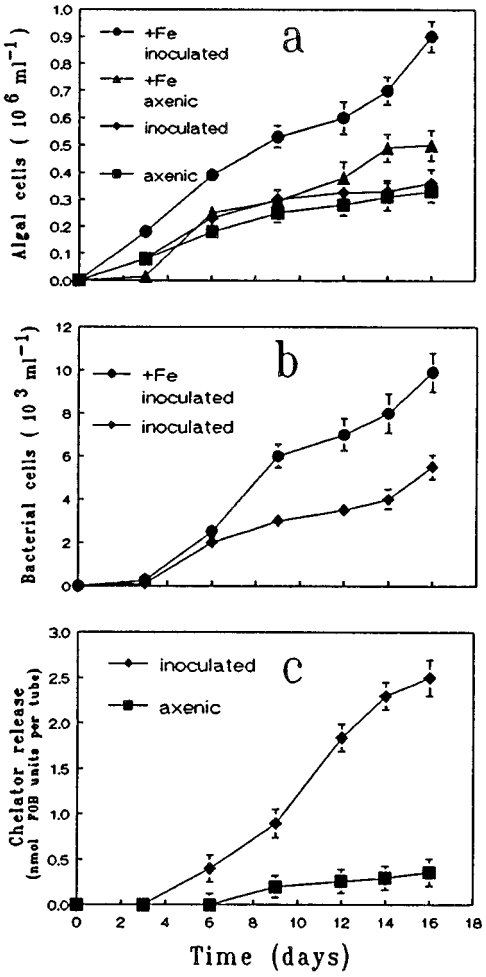

FIG. 2. Effects of inoculation of *D. bardawil* culture with a *Halomonas* sp. at two Fe levels: no added Fe (5×10^{-8} M, as measured with an inductively coupled plasma-atomic emission spectrometer) and 5×10^{-7} M as $FeCl_3$. In the Fe-amended treatments, the CAS test is not effective (c). FOB, ferrioxamine B.

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