Azotobacter Siderophore as a Source of Nitrogen from Azotobacter vinelandii to Support the Growth of the Green Algae Scenedesmus sp. BA032

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Abstract

Microalgae are viewed as a potential future agricultural and biofuel feedstock and also provide an ideal biological means of carbon sequestration based on rapid growth rates. Any potential improved biological route to fix carbon will require addition fertilizer inputs to provide the necessary nitrogen required for protein and nucleotide biosynthesis. Conventional crops such as soybeans and clover are able to meet their nitrogen requirements by forming symbiotic relationships with diazotrophic soil bacteria. The free-living diazotroph Azotobacter vinelandii can fix nitrogen under aerobic conditions in the presence of reduced carbon sources such as sucrose or glycerol, and is also known to produce a variety of siderophores to scavenge for different metals. In this study, we have identified a strain of the green algae Scenedesmus that is capable of utilizing the A. vinelandii siderophore azotobactin as a source of nitrogen. When grown in a co-culture, the algae obtained all nitrogen required for growth through the association with A. vinelandii. These results provide a proof of concept for the first step toward developing a mutualistic or symbiotic relationship between these two species.

Materials and methods

Bacterial strains and growth conditions

Cultures of Azotobacter vinelandii DJ were grown aerobically for 48 h in minimal medium, containing 5.5 mM succinate, 0.14 mM CaCl2, 0.4 mM MgSO4, 5.0 mM NaCl, 2.0 mM KH2PO4, and 2.32 mM K2HPO4, buffer at 30 °C with agitation at 200 rpm (Page and Sadoff, 1975), but no source of iron. Each gene deletion strain (SIDK3 and SID9) was cultured in the same way as the parental A. vinelandii strain. The cultures of Scenedesmus sp. BA032 were aerobically grown in MB medium for inducing siderophore production after 3 days of growth with A. vinelandii modified with 0.42 mM of NaCl and 0.11 g/L of CuCl.

Protochelin, Azotochelin and Aminochelin are nitrogen and their percentage.

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Discussion

The carbon sequestration from microorganisms, plants, or other feedstock for the production of sugars or biofuels may demand unsustainable inputs of nitrogen fertilizers. It is of interest in the biotechnological, biomedical and pharmaceutical fields and is one of the best candidates for the production of biological products.

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References


Vyn, D., Yamanaka, K., Yamanaka, M., Shibutani, W., and Misaka, K. (2009). Structure of the siderophores produced by Azotobacter that contain nitrogen and their percentage. Azotobacter is a pyruvate-like siderophore; Protochelin, Azotorchel and Aminochelin are catechol siderophores.

Figure 1. Structure of the siderophores produced by Azotobacter that contain nitrogen and their percentage. Azotobacter is a pyruvate-like siderophore; Protochelin, Azotochel and Aminochel are catechol siderophores.

Figure 2. Production of siderophores in Azotobacter vinelandii wild type and knock out construction of the gene for the siderophore synthetase. The absorbance of the siderophores was measured using a Varian 50 Bio visible spectrophotometer. Catechols quantitation was determined using the extinction coefficient at 310 nm (Page et al, 1988) and azotobactin was determined using the extinction coefficient at 380 nm for culture supernatants.

Figure 3. Growth of different consortiums using only atmospheric nitrogen as a nitrogen source and sucrose as a carbon source for A. vinelandii. A. vinelandii strains were grown with S. sp. BA032 in co-culture (panel A). Superoxidant from growths of A. vinelandii were filtered through a 0.2 µm filter and used to grow S. sp. BA032 (panel B). The microalgae grow in modified Burk’s medium lacking any source of nitrogen other than air (black squares), with 0.7 mM NaNO3 (open squares) with A. vinelandii WT strain (black triangles). A. vinelandii: PCRSIDK3 (grey circles) and A. vinelandii: PCRSIDK9 (open diamonds). Each data point represents the average and standard deviation from three independent samples.

Figure 4. Light microscopy images of cultures of A. vinelandii WT (White arrow) and Scenedesmus sp. BA032 (Black arrow). A. vinelandii grows around the cells of BA032 forming aggregates.