Biochemical and Molecular Characterization of an Azotobacter vinelandii Strain with Respect to its Ability to Grow and Fix Nitrogen in Olive Mill Wastewater

M. Papadelli,* A. Roussis,* K. Papadopoulou, A. Venieraki,* I. Chatzipavlidis, P. Katinakis* & K. Ballis*

*Agricultural University of Athens, Department of Agricultural Biology and Biotechnology, Laboratory of Molecular Biology, Iera Odos 75, 11855 Athens, Greece

INTRODUCTION

The bacterial strain A belongs to a collection of nitrogen fixing bacteria isolated from soil treated with olive mill wastewater (OMW). This strain can grow in OMW showing significant nitrogen fixing capacity. The study of growth and nitrogenase activity of the above strain during its growth into the waste showed that the maximum value of total acetylene reduction activity (expressed in nmol Ethyl/24 h/ml of culture) was obtained after 24 h of incubation as well as the maximum value of bacterial population. When the above nitrogen fixing capacity was expressed in reference to the bacterial population (nmol Ethyl/24 h/ug bacterial protein) its maximum value was observed earlier, since the first 7 h of incubation. Western blot analysis of total bacterial proteins, extracted at specific time intervals showed that nitrogenase activity was induced 30 mins after the inoculation of the waste with the strain A. The respective time of the enzyme’s induction in chemical media (N-free) was 1 h. Southern blot analysis of total genomic DNA of strain A using as probes the three structural genes (nifH, nifD, nifK) encoding nitrogenase-1 in Azotobacter vinelandii gave hybridization patterns which are conserved between the above two bacteria. These results strongly support parallel biochemical taxonomy data indicating that strain A may belong to Azotobacter vinelandii species. © 1997 Published by Elsevier Science Limited. All rights reserved.

RESULTS

OMW was inoculated with an overnight liquid culture of A. vinelandii strain A and samples for the measurements of acetylene reduction capacity
Fig. 3. Southern blot analysis of total genomic DNA from strains *Areniventer simulans* and *A*. *titillator* (a) and (b). (c) and (d) were used as probes.
Characterization of an A. Vinelandii strain with respect to OMW

and bacterial population as well as for bacterial protein extraction were obtained after 7, 15, 24, 48 and 72 h of incubation at 30°C. Maximum values of total acetylene reduction activity as well as of bacterial population were obtained after 24 h of growth in OMW (Fig. 1). When the acetylene reduction capacity was expressed in reference to the bacterial population (nmol of ethylene per µg of bacterial protein per 24 h) its maximum value was observed after 7 h and then decreased to almost zero 72 h after inoculation (Fig. 2).

Western blot analysis of bacterial proteins obtained at specific time intervals between 0.5 and 72 h of growth of strain A in OMW, was performed using the antibody against Component-I of nitrogenase from Rhizobium. We observed that nitrogenase activity was induced 30 min after inoculation of the OMW with strain A (data not shown). The respective time of enzyme induction in chemical media without a nitrogen source was 1 h.

The next stage of this study was to examine whether the nitrogenase genes of strain A exhibit structural and sequence similarities with the nitrogenase of the reference strain of Azotobacter vinelandii. For this purpose, total genomic DNA was isolated from strain A and from the reference strain A. vinelandii, digested with EcoRI restriction endonuclease and analysed by agarose gel electrophoresis. The resulting gel was Southern blotted and hybridized with the three structural genes encoding for nitrogenase-1 of A. vinelandii (Jacobson et al., 1989): nifH encodes for subunit γ of Component II, nifD encodes for subunit α of Component I and nifK encoding for subunit β of Component I of nitrogenase-1. In each case positive hybridization signals were obtained, forming patterns conserved between the above two strains (Fig. 3). These results strongly support parallel biochemical taxonomy data indicating that strain A belongs to Azotobacter vinelandii species.

CONCLUSIONS

Strain A grows in OMW and fixes nitrogen.

The nitrogenase structural genes are arranged in a similar manner both in strain A and Azotobacter vinelandii (reference strain).

The induction of nitrogenase of strain A is observed earlier when the bacteria grows in OMW than in chemically defined media.

REFERENCES

