Protein Patterns in Coastal Saline Tolerant Strains of Arbuscular Mycorrhizal Fungi (AMF) Spores

C. Karthikeyan and N. Thajuddin

Department of Biotechnology, J.J. College of Arts and Science, Pudukkottai-622 422, Tamilnadu, India

Department of Microbiology, Bharathidasan University, Tiruchirappalli-24, Tamilnadu, India

Abstract: Protein profiles obtained from one dimensional Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (ID SDS-PAGE) have been used for distinguishing various species of AM fungi. This technique was used to assess the differences in the total spore protein profiles of eight different saline tolerant strains of AM fungi. The results show that a greater number of molecular mass polypeptides was noted in Glomus geosporum, G.aggregatum, G.deserticola, G.microcarpum and Sclerocystis pakistanika, compared to the polypeptide profiles of other AMF species with fewer high molecular mass polypeptide like Gigaspora margarita, Acaulospora Scrobiculata and Scutellospora heterogama and a greater number of low molecular polypeptide (Gigaspora margarita and A.Scrobiculata). SDS-PAGE of AMF spores is a simple, highly reproducible and sensitive technique capable of distinguishing between AMF species that could be used for the identification of unknown AMF isolates.

Key words: SDS-PAGE • AMF Spores • Molecular mass Polypeptides

INTRODUCTION

Arbuscular mycorrhizal Fungi (AMF) form associations with most plants. The interaction between the fungus and the plant cell takes place at both an extracellular and intracellular level [1]. Identification of AMF has been carried out using spore morphological characteristics [2] anatomy of infection [3] biochemical characteristics [4-6] and nucleic acid based techniques [7-8]. However microscopic analysis of spores is time-consuming, labor-intensive and can be intimidating to the inexperienced taxonomist.

Differences in qualitative and quantitative expression of proteins have been shown both in ectomycorrhizas [9-10] and in arbuscular mycorrhizas [11,12,11,12]. It seems that the mycorrhiza formation increases the expression of low molecular weight proteins as suggested by the results obtained in tobacco [13] and Soyabeen [14] although in red clover some high molecular weight polypeptides were expressed in mycorrhizal roots but not in non-mycorrhizal ones [1]. The first difficulty of this research is to know the role that the differential proteins play in the symbiosis. One possibility is the study of some enzymatic activities, as done by Pacovsky et al. [15] and Palma et al. [16].

Total cellular protein profiles obtained from one dimensional Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (ID SDS-PAGE) have been used for distinguishing various species of microorganisms including bacteria, ecto and endomycorrhizal fungi [17-19]. We describe a simple, reliable and reproducible method for ID SDS-PAGE of saline tolerant strains of AMF spores. This technique was used to assess differences in the total spore protein profiles of eight different AMF species.

MATERIALS AND METHODS

Sampling: The AMF species used in this study were Glomus aggregatum, G.deserticola, G.geosporum, G.microcarpum, Sclerocystis pakistanika, Gigaspora margarita, Acaulospora Scrobiculata and Scutellospora heterogama. Eight different saline tolerant strains of AMF species were originally isolated from coastal saline soil localities of the west coast of Kerala and monospecific cultures of the AMF species maintained in the laboratory in an autoclaved saline soil condition on Setaria italica host plant for 45 days and were stored at 4°C for up to one month before analysis.
Protein Extraction: AMF spores were extracted from monospecific cultures by wet sieving and decanting technique [20] as modified by floatation - adhesion technique [21]. 500 numbers of intact spores from all AMF species were manually removed from the filter using Pasteur pipette and placed in a sterile 500 µl. Eppendorf tube containing 50 µl of sterile deionized water. The tubes were stored at 4°C for upto 24 hrs.

The AMF spores in the Ependorf tube were centrifuged at 8160 g for 10 min and resuspended in 100 µl extraction buffer (2% SDS, 2mM dithiothreitol, 1mM EDTA and 2mM phenyl methyl sulphonyl fluoride in 50mM Tris-HCL, pH 8.0). The eppendorf tubes containing the AMF spores were placed on ice and the spores were crushed using a glass pestle and centrifuged at 4°C for 15 min at 11750g. The supernatant was transferred to a clean, sterile eppendorf tube and stored at 80°C.

Electrophoretic Separation of Proteins: Separation of proteins was made using Sodium Dodecyl Sulphate - Polyacrylamide Gel Electrophoresis (SDS-PAGE) [22] at different acrylamide / bisacrylamide concentrations (usually 12% resolving / 4% stacking gels) using a vertical slab gel electrophoresis apparatus (Bangalore Genei). Each lane received 50 µl of protein. Electrophoresis was conducted at 50-100v. Experiments were repeated at least four times and the results were reproducible.

Staining: Polypeptides were detected in the gels by staining with coomassie brilliant blue R250 for 2 to 4 hours [1]. Then the gel was detained in destaining solution for 1 to 2 hours until clear background was obtained. The protein bands were analyzed by using a Gel Documentation System (GDS) linked to a computerized Image Analyzer (Lark Innovative Fine Teknowledge, Chennai).

RESULTS AND DISCUSSION

Symbiosis established with mycorrhizal fungi in some higher plant roots enables the plant to uptake the non-available phosphorous from soils [23]. Important changes in the plant metabolism occur after the symbiosis has taken place [16,1] although very little is still known of the biochemical mechanisms responsible for this association.

The AM fungal species had a unique, distinct protein banding pattern, that could be differentiated visually (Fig.1). A greater number of molecular mass polypeptides was noted in *Glomus geosporaum*, *G. aggregatum*, *G. deserticola*, *G. microcarpum* and *S. pakistanika*, compared to the polypeptide profile of other AMF species with fewer high molecular mass polypeptide (*Giaspora margarita, A.scrobiculata* and *S.heterogama*) and a greater number of low molecular polypeptides (*Gigaspora margarita* and *A.scrobiculata*). The same studies can also be employed to study relationships among species or subspecies [18,24].

In the present study, it has been found that coastal saline soils containing few AMF spores can present difficulties in the assessment of mycorrhizal biodiversity of the ecosystem. This problem is usually managed by enhancing spore production using a mycorrhizal hosts under controlled conditions. The use of this SDS-PAGE technique eliminates the need for large number of spores required for identification methods using biomolecules other than DNA [24].
**Fig. 2:** Zymogram depicting extracellular protein profile from spores and sporocarps of different saline tolerant strains of AM fungi

Zymogram depiction protein profiles (Fig 2) of different AMF species was found to produce a total of 7 to 15 bands. All the strains were found to be variable in terms of production of proteins. The banding patterns showed that *G. aggregatum*, *G. geosporum*, *G. deserticola*, *G. microcarpum* and *S. Pakistanika* formed one group, while *A. scrobiculata*, *G. margarita* and *S. heterogama* formed another separate group. Among these strains, Glomales resembles one another whereas the strains of *A. scrobiculata*, *G. margarita* and *S. heterogama* do not resemble any other strains.

**CONCLUSIONS**

In the present study, total proteins of eight saline tolerant strains of AMF were differentiated on SDS PAGE. A greater number of polypeptides were used in Glomales, compared to the polypeptide profile of other AMF species with fewer high molecular mass polypeptides. Protein banding pattern showed by AMF species revealed polymorphism among the AMF species. This technique could be used to know the genetic variability among the species. Protein analysis on polyacrylamide gel electrophoresis provides a well-established and efficient tool for revealing genetic variability in AM fungal populations. When polymorphism is detected, it reflects directly the genetic background of the AM fungus.

**REFERENCES**


