Comparison of Multi-locus Enzyme and Protein Gel Electrophoresis in the Discrimination of Six *Trichoderma* Species Isolated from Andhra Pradesh

B. Pushpa Rajyam[1] and P. Murali Krishna[2]

[1]Student, M.Sc. Biotechnology, Department of Biotechnology, D.N.R college of Advanced Sciences and Research Centre (Affiliated to Andhra University), Bheemavaram, West Godavari District. A.P
[2]Department of Biotechnology, D.N.R college of Advanced Sciences and Research Centre (Affiliated to Andhra University), Bheemavaram, West Godavari District. A.P
E-mail: [1]pusparajyamb@gmail.com

Abstract—*Electrophoretic studies of multilocus-enzymes (MLEE) and whole-cell protein (SDS-PAGE) were carried out in order to evaluate the parity between different methods for the characterization of six Trichoderma species recovered from East Godavari district of Andhra pradesh by numerical taxonomy methods. Isoenzyme markers were used to study the genetic variability among the isolates of Trichoderma species collected from different regions. Isoenzymes of esterase, acid phosphatase, catalase, superoxide dismutase, polyphenol oxidase, malate dehydrogenase and soluble protein profiles were studied. The isolates included in the present study were T. viride, T. harzianum, T. hamatum, T. longibrachiatum,T. virens, K. koningii, from East Godavari district. On isozyme analysis two species of Trichoderma showed distinct cluster formation and remaining isolates showed species specific and isolate specific bands. Isozymes of esterase showed unique banding pattern for each isolate. SDS-PAGE of soluble protein profile showed a number of bands with very low variation among the isolates. Isolate specific isozyme markers can be utilized in monitoring the particular isolate incase of used as biocontrol agents.*

1. INTRODUCTION

Diseases are one of the major impeding factors for agriculture industry in India. Use of chemical control against plant diseases resulted in detrimental effects like toxicity to users, impairment of beneficial organisms, and development of resistance in plant pathogenic micro organisms to the active ingredient of synthetic fungicides causing great economic losses. The alternative choice is the use of biological control agents which are non-phyto toxic, systematic and easily biodegradable in nature. Keeping in view the importance of biological agents against plant pathogens.to find out the rhizospheremycorrhiza and *Trichoderma species* in geographically differently located regions in Andhra Pradesh The present study high lights the importance of *Trichoderma* biological control agent against plant pathogens.

2. MATERIALS AND METHODS

2.1 Trichoderma isolates

Pure isolates of *Trichoderma* were obtained from different regions of Andhra Pradesh the place / host of isolates were given in the table.

S. No.	Isolate code	Place of isolation
1	T. viride	Ambajipet
2	T. harzianum	Kakinada
3	T. hamatum	P.Gannavaram
4	T. longibrachiatum	Pulletikurru
5	T. virens	Ambajipet
6	T. koningii	Amalapuram

2.2 Isolation of *Trichoderma* species From Soil

Preparation of potato dextrose agar (PDA) medium was used for maintaining the fungi.*Trichoderma* were isolated from soil samples, as follows: 25 g of soil samples was suspended in 250 ml of 0.1% agar water. Samples were shaked for 20-30 minutes on a rotary shaker at 250 rpm. Serial dilutions 10-1, 10-2, 10- 3, and 10-4 were made for each soil sample and aliquot 0.1 ml of soil suspension dispensed onto *Trichoderma* - selective media (TSM) surface with a glass rod (1) (Elad*et al*., 1981). The plates were incubated at 25 0C for 5-7 days. After incubation, *Trichoderma* were identified from other fungi based on color, size, shape, and appearance of colony on surface of TSM and then it was transferred to a potato dextrose agar (PDA) medium for purification and further identification. For each soil sample and suspension concentration, 5 plates were considered as replicates.

2.3 Identification of *Trichoderma* Isolates:

Under microscopic observations were made throughout the coated cover slip and thin film of agar. The growing isolates were studied using fresh direct mounts in Lactephenol cotton blue under medium and high magnifications, x 20, and x40, respectively.

2.4 Isoenzyme studies

2.4.1 Extraction of enzymes

For extraction of enzyme, fungal mats of isolates grown on PDA were harvested and washed 3 to 4 times with sterile distilled water to remove the culture medium attached to mats and moisture was removed by pressing between tissue paper and (500mg) homogenized with 0.5 ml Tris-HCl buffer pH-8 containing sucrose (2% in Tris-HCl buffer, pH 8). The homogenate was centrifuged at 14,000 rpm for 10 min at 4^{o}C and the supernatantwas used as enzyme source.

2.4.2 Isoenzyme analysis

Isoenzyme analysis carried on native polyacrylamide gel electrophoresis in anionic system (Laemmli*et.al*, 1970). Electrophoresis carried out at 13 mA for 12 to 16 hr at 4˚C. Bromophenol blue (BPB 1% in 30% sucrose solution) used as marker dye. After electrophoretic run, the gels were washed and incubated in staining solution.

2.5 SDS- PAGE

The soluble protein profiles of *Trichoderma* of isolates were carried out by SDS-PAGE by the method of (4) Laemmli. *et.al*(1970).

2.5.1 Protein extraction

Five hundred mg of *Trichodermal* mats of isolates were homogenized with 0.5 ml of Tris – HCl buffer pH - 8 and the homogenate was centrifuged at 13,000 rpm for 10 min at 4 ^{o}C . The supernatant wasused asprotein after adjusting the protein concentration 50 µg / 40µl .

3. RESULTS AND DISCUSSION

Isoenzymes

Isoenzyme markers were used to study the genetic variability among the isolates of *Trichoderma* species collected from different regions. Isoenzymes of peroxidase, esterase, acid phosphatase, catalase, superoxide dismutase, polyphenol oxidase, malate dehydrogenase and soluble protein profiles were studied. The isolates included in the present study were 1. *T. viride*, 2. *T. harzianum*, 3. *T. hamatum*, 4. *T. longibrachiatum*, 5. *T. virens*, 6. *K. koningii* (from different regions of Andhra Pradesh).

Isoenzymes of esterase resolved on 8% polyacrylamide gel electrophoresis showed a maximum of 19 loci (Fig. 1). Maximum loci (7) were present in the *T. viride*, *T. koningii*Bissett,J (8).whereas the minimum number of loci (2) were present in *T. harzianum*. The band with Rf value 0.65 was present in three isolates *T. viride*, and*T. virens* The six species of *Trichoderma*showed entirely different pattern of esterase isozymes.(Shalini*et.al.*,)(6).

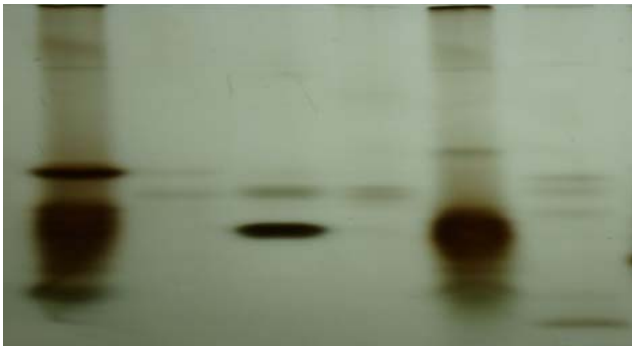

Fig. 1: PAGE of isozymes of esterase. Lanes

1. T. viride2. T. harzianum 3. T. hamatum4. T. longibrachiatum5. T. virens6. T. koningii,

Polyphenol oxidase showed 10 isozyme loci (Fig. 2). The isolates *T. harzianumT. hamatum*, and *T. lonibrachiatum* showed a maximum of two loci whereas other isolates showed single locus with different Rf values. The band with Rf value 0.06 was present in the isolate *T. longibrachiatum* whereas the band with Rf value 0.11 was present in the isolates *T. virens* and *T. Koningii*.

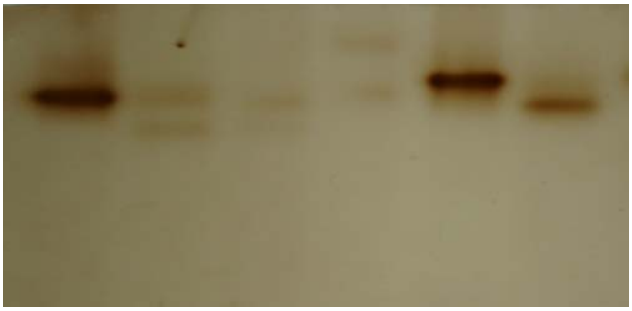

Fig. 2: PAGE ofisozymes of polyphenol oxidase. Lanes

1. T. viride 2.T. harzianum 3.T. hamatum 4. T. longibrachiatum 5. T. Virens 6. T. koningii

The isozymes of catalase showed 8 isozyme loci. The isolates *T. harzianum*, *T. longbrachiatum*, *T. virens* showed single locus with different Rf values. The band with Rf value 0.21 was present in T. *hamatum*, *T. longibrachiatum* whereas the band with Rf value 0.23 was present in *T. virens*, *T. koningii*. Among the six species, the band with Rf value 0.21 was present in *T. hamatum* and *T. longibrachiatum*.

Isozymes of malate dehydrogenase showed 14 loci (Fig. 3). The isolates *T. longibrachiatum*, *T. virens* and*T. koningii* showed 3 isozyme loci. The band with Rf value 0.38 was present in all isolates.except with Rf value 0.42 was shared between the *T. virens* and *T. koningii*.

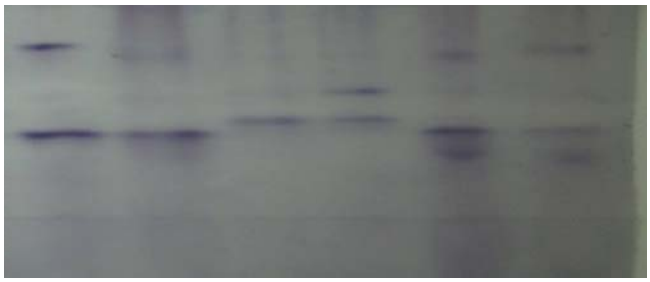

Fig. 3: PAGE ofisozymes of malate dehydrogenase. Lanes

1T. viride 2. T. harzianum 3.T. hamatum 4. T. longibrachiatum 5.T. virens, 6. T. koningii,

The isoenzyme of superoxide dismutase showed 6 loci. (fig4)The band with Rf value 0.27 was present in *T.viride*, *T.harzianum* and *T.hamantum* whereas the band with Rf value 0.28 was present in *T.hamantum*, *T.longibrachiatum*a narrow distribution and relatively low population densities (cf.Macrozamiariedlei)(3).

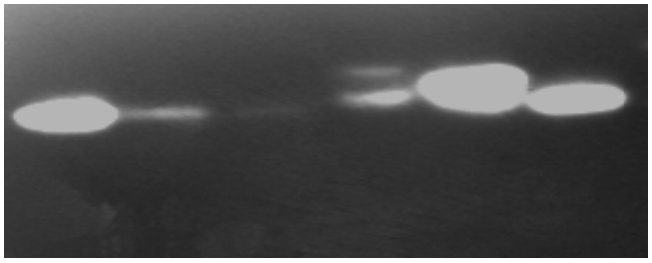

Fig. 4: PAGE ofisozymes of superoxide dismutase. Lanes

1. T. viride 2. Tharzianum 3. T.hamatum 4. Tlongibrachiatum 5. T. virens6. T. koningii,

On isozyme analysis two species of *Trichoderma* showed distinct cluster formation. Many isolates showed species specific and isolate specific bands. Isozymes of esterase showed unique banding pattern for each isolate.

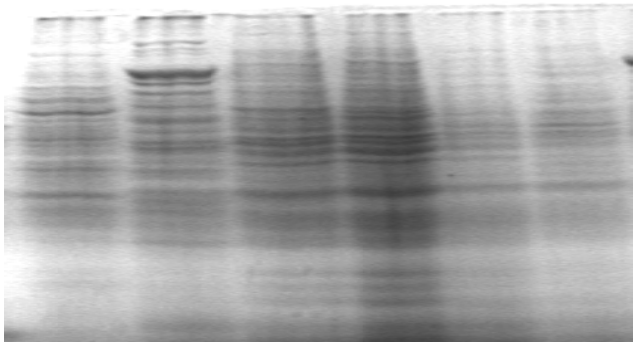

Fig. 6: SDS-PAGE of soluble proteins. Lanes

1T. viride 2. T. harzianum 3.T. hamatum 4. T. longibrachiatum 5. T. virens6. T. koningii.

SDS-PAGE of soluble protein profile (Fig. 6) showed a number of bands with very low variation among the isolates. Isolate specific isozyme markers can be utilized in monitoring the particular isolate incase of used as biocontrol agents.

REFERENCES

[1] Elad, Y, Chet, I. and Katan, J. (1980) Trichodermaharzianum: A biocontrol agent of sclerotiumrolfsii and Rhizoctoniasolani. Phytopathology 70:119-121.4.

[2] Elad, Y., Katan, J. and Chet, I.(1980) Physical, biological and chemical control integrated

[3] BymeM.andS.H.james 1991. Genetic diversity in the cycad. *Macrozamiariedlei.heridity* 67.

[4] Laemmli, U. K., Molbert, E., Showe, M., and Kellenberger, E., *J. Mol. Biol.*, 49, 99 (1970).

[5] Laemmli, U. K., Beguin, F., and Gujer-Kellenberger, G., *J. Mol. Biol.*, 47, 69 (1970).

[6] For soil-borne diseases in potatoes. *Phytopathology* 70:418-422. 5.

[7] Shalini, K.P.Narayan. lata, and A.S. Kotasthane. 2006.genetic related ness among trichoderma isolates inhibiting a pathogenic fungi Rhizoctoniasolani*Afr.J.Biotechnol*.5:580-584.

[8] Bissett,J.1991 A revisiuon of genus trichoderma. II infragenericclassification.Can*J.Bot*.69:2357-2372.