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Isoenzyme analysis of *Arthrobotrys*, a nematode-trapping fungus

J.V. Araújo[1], T.G. Junghans[2], A.C. Alfenas[2] and A.P.S. Gomes[1]

[1]Departamento de Veterinária and [2]Departamento de Fitopatologia, Bioagro, Universidade Federal de Viçosa, Viçosa, MG, Brasil

Abstract

Extraction and isoenzyme analysis of four isolates of *Arthrobotrys* including *A. musiformis*, *A. robusta* and *A. conoides* were conducted. Among the 14 enzymes studied by starch gel electrophoresis, using morpholine-citrate as gel/electrode buffer, the following nine enzymes showed interpretable banding patterns: α-esterase, fumarase, hexokinase, isocitrate dehydrogenase, leucine aminopeptidase, malate dehydrogenase, 6-phosphogluconate dehydrogenase, phosphoglucomutase and phosphoglucoisomerase. All isolates studied displayed typical isoenzyme phenotypes for each species. Two isolates of *A. conoides* differed in their α-isoesterase banding patterns, but no differences were observed for the other enzymes. The assay was satisfactory for enzyme extraction and resolution of *Arthrobotrys* and could be used in future taxonomic and genetic studies of this organism.

Correspondence
J.V. Araújo
Departamento de Veterinária
Universidade Federal de Viçosa
36571-000 Viçosa, MG
Brasil
Fax: 55 (031) 891-2317
E-mail: jvictor@mail.ufv.br

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The fungi antagonistic to nematodes consist of a wide variety of organisms including nematode-trapping or predacious fungi, endoparasitic fungi, parasites of nematode eggs and cysts, and those producing nematotoxic metabolites. It is remarkable that fungi belonging to highly divergent orders and families occur in each of the above groups. Predacious, parasitic and biochemical relationships with nematodes have evolved among almost all major groups of soil fungi, ranging from Phycomycetes to Basidiomycetes (1). The genus *Arthrobotrys* currently presents 27 species and belongs to the group of nematode-predacious fungi (2). These fungi are relatively easy to culture on artificial media and numerous isolates are currently maintained in various laboratories worldwide. The identification of *Arthrobotrys* spp is based on morphological characteristics mainly following the keys of Cooke and Godfrey (3) and Van Oorschot (2). The teleomorph of most *Arthrobotrys* species is presently unknown and identification is mainly based on conidial size, morphology of conidiophores and trapping organs of the anamorph. However, in some cases the morphological features of the anamorph alone do not allow correct species identification (4). In this case, one may use molecular markers to establish taxonomic relationships among isolates. Isoenzyme analysis has proved to be a powerful tool in taxonomy and genetics of fungi (5,6). In the present study we describe the techniques for protein extraction and starch gel isoenzyme electrophoresis for three *Arthrobotrys* species used in previous studies (7-9) to investigate their

predacious ability on animal and plant parasite nematodes.

The following four isolates of *Arthrobotrys* grown on nematode larvae collected in different regions of Brazil were isolated according to Santos et al. (10) and stored on 2% potato-dextrose-agar at 4ºC: *A. musiformis* (Figure 1, lane A), *A. conoides* (Figure 1, lanes B and D), and *A. robusta* (Figure 1, lane C). The cultures were identified according to the keys of Cooke and Godfrey (3) and Van Oorschot (2). Two single-conidial cultures of each isolate were obtained and compared to the respective original culture. Enzyme extraction was performed from mycelium grown in liquid medium (11). After 7 days of incubation at 25ºC in the dark, the cultures were filtered through a Buchner funnel containing Whatman No. 1 filter paper. The mycelium cake was washed three times with distilled water, excess moisture was removed by blotting the mycelium on filter paper and 400 mg of each sample was crushed with an ice-cold mortar and pestle containing 1 ml ice-cold extraction buffer of the following composition: 0.34 M dibasic sodium phosphate, 0.2 M sucrose, 2.56% polyvinylpyrrolidone-40, 5.7 mM L-ascorbic acid, 5.8 mM sodium diethyldithiocarbamate, 2.6 mM sodium bisulfate, 2.5 mM sodium borate, 0.2% ß-mercaptoethanol, and 1% polyethylene glycol 6000 (6). During homogenization, small quantities of polyvinylpolypyrrolidone were added to the sample. The homogenate was adsorbed onto 12 x 5 mm chromatographic Whatman No. 3 paper wicks and stored in microcentrifuge tubes at -85ºC until the time for electrophoresis.

Electrophoresis was performed on 13% hydrolyzed starch gels containing 3% sucrose and 2 mM morpholine-citrate, pH 7.1, diluted 1:20, as gel buffer and 40 mM morpholine-citrate, pH 6.1, in the electrode compartments. After electrophoresis, the gels

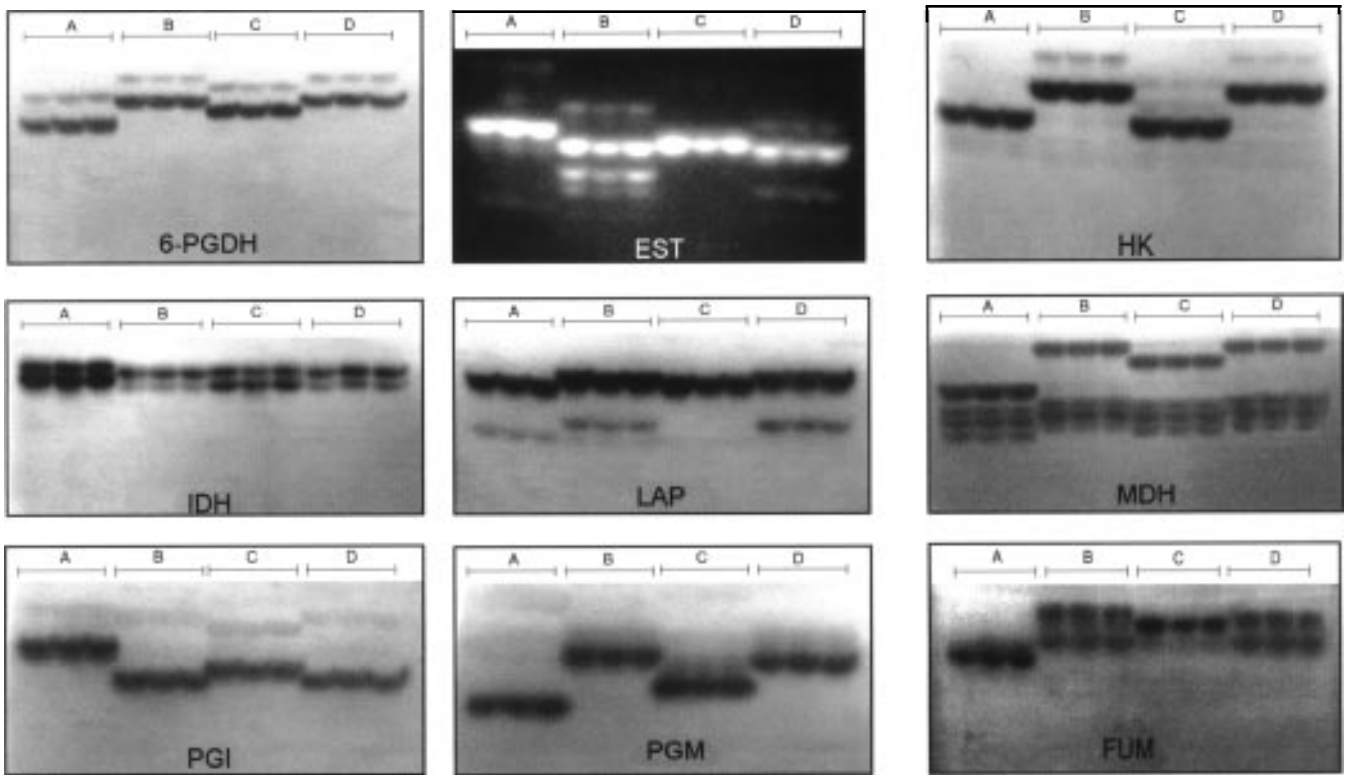

Figure 1 - Isoenzyme phenotypes of *Arthrobotrys* isolates. *Lane A, A. musiformis*; *Lane B, A. conoides*; *Lane C, A. robusta*; *Lane D, A. conoides*. The original and two monoconidial cultures are shown from left to right for each isolate. For enzyme abbreviations, see text.

were stained for the following enzyme activities (12): acid phosphatase (ACP, EC 3.1.3.2), aconitase (ACO, EC 4.2.1.3), diaphorase (DIA, EC 1.8.1.4), α-esterase (α-EST, EC 3.1.1.1), fumarase (FUM, EC 4.2.1.2), glutamate dehydrogenase (GDH, EC 1.4.1.3), hexokinase (HK, EC 2.7.1.1), isocitrate dehydrogenase (IDH, EC 1.1.1.42), leucine aminopeptidase (LAP, EC 3.4.11.1), malate dehydrogenase (MDH, EC 1.1.1.37), 6-phosphogluconate dehydrogenase (6-PGDH, EC 1.1.1.44), phosphoglucoisomerase (PGI, EC 5.3.1.9), phosphoglucomutase (PGM, EC 2.7.5.1) and superoxide dismutase (SOD, EC 1.15.1.1).

The extraction method and buffer systems used for electrophoresis were suitable for 9 (α-EST, FUM, HK, IDH, LAP, MDH, 6-PGDH, PGM and PGI) of the 14 enzymes tested. These enzymes exhibited good activity and resolution and were all polymorphic, displaying typical isoenzyme phenotypes for each species, with the exception of IDH which was monomorphic for all isolates studied (Figure 1). Fumarase exhibited low enzyme activity, but scorable banding patterns. MDH, IDH, LAP and 6-PGDH showed up two zones of activity whereas α-EST, FUM, HK, PGI and PGM showed a single locus. Enzyme activity was not detected for ACP, ACO, DIA, GDH or SOD. The two isolates of *A. conoides* studied differed in their isoesterase profiles but no differences were observed for the other enzymes. Mankau (1) collected approximately 50 isolates of *A. conoides* and most of these isolates were unique and could be separated from the others on the basis of some physiological, morphological, ecological or biochemical characteristics. The isolates exhibited different patterns of sporulation and chlamydospore production, different responses and growth rates on a variety of comparative media, and different degrees of predaciousness or response to trap-stimulatory factors. In the present study, the original cultures displayed a pattern similar to that of the monoconidial culture of each isolate for all enzymes tested. Although we only studied two single spore cultures, we did not expect variability between them, since the original culture was obtained from the same conidiophore. However, analysis of several monoconidial isolates from different sources and geographic origins would be valuable to estimate the genetic variability of the fungal population.

The extraction buffer seems to affect enzyme stability. R.D. Lima (unpublished results), working in our laboratory with several species of *Arthrobotrys*, including *A. conoides*, *A. musiformis*, and *A. robusta*, did not obtain satisfactory results on starch or polyacrylamide gels when Tris-HCl (0.62 M, pH 6.8) was used as extraction buffer. Later, Lima (13) obtained satisfactory results using our technique and 29 isolates of *Arthrobotrys* spp exhibited 52 isoenzyme phenotypes.

The isoenzyme systems studied are potentially important for future studies involving the taxonomy and genetics of *Arthrobotrys* spp.

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