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# *Biochemical and Molecular characterisation of Trichoderma species.*

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# Abstract

The growing importance of many *Trichoderma* strains as biological control agents and producers of valuable metabolites and enzymes has made their distinction from other *Trichoderma* isolates essential. However, the use of morphological and cultural characters alone to differentiate individuals within the genus *Trichoderma* to a level that is most informative has proved difficult due to a lack of reliable characters.

In this study, alternative biochemical and molecular techniques were assessed for their ability to differentiate between isolates of the genus *Trichoderma*. Fifty isolates representing the *Trichoderma* species *T. atroviride*, *T. hamatum*, *T. inhamatum*, *T. koningii*, *T. virens*, *T. viride* and five morphological sub-groupings of the *T. harzianum* species were examined. ITS sequence data, RAPD PCR and the ability of an isolate to produce the metabolite 6-pentyl-α-pyrone (PAP) were all used to differentiate between morphologically indistinguishable isolates. Altogether four levels of variation were recognised. The greatest level of resolution was achieved with the RAPD PCR technique, followed by both morphological characters and sequence data from the ITS1 region of the ribosomal gene complex. Sequence data from the ITS2 region provided the third level of resolution. The fourth level of resolution was achieved with both sequence data from the second variable region (D2) of the 28S-like ribosomal gene and determination of an isolate's ability to produce the metabolite PAP. Based on these results, it was proposed that a new taxonomic system be established in which individuals of the genus *Trichoderma* are distinguished by a combination of morphological, cultural, biochemical and molecular characters.

Sequence data and RAPD PCR data were also tested for their reliability in estimating the phylogeny of *Trichoderma*. Sequence data from the ITS1 region proved to be the most reliable for predicting the phylogeny of morphologically defined species, whereas RAPD data was most useful for predicting the unrooted phylogeny of strains of morphologically identical isolates (i.e. isolates with less than 10% nucleotide divergence). None of the data employed in the present study were able to resolve all the species tested. It was concluded that additional sequence from a more variable region would be required to achieve this.

In addition to the characterisation and phylogenetic studies, two approaches were undertaken in an attempt to isolate a gene(s) vital to the production of the antifungal metabolite PAP, a metabolite believed to be important in the biological control activity of a number of the isolates under

investigation. Both attempts were unsuccessful and additional studies undertaken to determine how important PAP is in the biological control activity of *Trichoderma* isolates were inconclusive. Nevertheless, a natural PAP deficient mutant was identified among the 50 isolates under investigation. Furthermore, synthetic PAP was found to inhibit the infection of lentil seedlings by *Sclerotium rolfsii* when 10 mg was added to a pot containing six seedlings and three viable sclerotia of the pathogen. The metabolite did not appear to have any detrimental effects on the growth and development of the seedlings.

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# Abbreviations

| | |
|---|---|
| AAP | 6-amyl-α-pyrone |
| bp | base pairs |
| °C | degrees Celcius |
| CI | Consistency Index |
| cm | centimetre |
| D2 | second variable domain within the 28S rRNA gene |
| DAPI | diamidino-2-phenylindole |
| diam | diameter |
| DNA | deoxyribonucleic acid |
| EDTA | ethylene diamine tetra-acetic acid |
| eV | electron volts |
| dATP | 2′-deoxyadenosine 5′-triphosphate |
| dCTP | 2′-deoxycytidine 5′-triphosphate |
| dGTP | 2′-deoxyguanosine 5′-triphosphate |
| dTTP | 2′-deoxythymidine 5′-triphosphate |
| dNTP(s) | 2′-deoxynucleotide 5′-triphosphate(s) |
| *g* | acceleration of gravity |
| g | grams |
| GC | gas chromatography |
| GCG | Genetics Computer Group Inc. |
| h | hour(s) |
| HCl | hydrochloric acid |
| HortResearch | The Horticultural and Food Research Institute of New Zealand |
| HPLC | high pressure liquid chromatography |
| Hz | Hertz |
| ICMP | International Collection of Microorganisms from Plants |
| ITS | internally transcribed spacer |
| Kb | Kilobase(s) |
| $KCl_2$ | potassium chloride |
| λ | Lambda bacteriophage |
| M | molar (moles/litre) |
| m | metre |
| MEA | malt extract agar |
| MEGA | molecular evolutionary genetics analysis |
| $MgCl_2$ | magnesium chloride |
| min | minute(s) |
| mL | millilitre |
| mm | millimetre |
| μL | microlitre |
| MP | maximum parsimony |
| MPW | millipore filter purified water |
| mRNA | messenger RNA |
| MS | mass spectrometry |
| mtDNA | mitochondrial RNA |
| Mw | molecular weight |
| ng | nanograms |
| nm | nanometre |
| NZ | New Zealand |
| PAP | 6-pentyl-α-pyrone |

| | |
|---|---|
| PAUP | phylogenetic analysis using parsimony |
| PCR | polymerase chain reaction |
| pmol | picomole |
| p.s.i. | pounds per square inch |
| PTP | permutation tail probability |
| RAPD | random amplified polymorphic DNA |
| rDNA | ribosomal DNA |
| RFLP | restriction fragment length polymorphism |
| RNA | ribonucleic acid |
| rpm | revolutions per minute |
| rRNA | ribosomal RNA |
| s | second(s) |
| SDS | sodium dodecyl sulphate |
| SDW | sterile distilled water |
| SEM | scanning electron microscopy |
| TBE | Tris-borate acid-EDTA buffer |
| TLC | thin layer chromatography |
| Tris | Tris(hydroxymethyl)-aminomethane |
| UPGMA | unweighted pair group method using arithmetic averages |
| UV | ultra violet |
| v | volume |
| w | weight |
| WA | water agar |

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