Molecular Identification of Fungal Isolates from Textile Wastewater by ITS-RFLP Method

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Abstract

Eight strains isolated from textile post-finishing wastewaters were subjected to molecular identification by ITS-RFLP (Internal Transcriber Spacer-Restriction Fragment Length Polymorphism) method, using ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') DNA amplification primers for ITS region of the nuclear small-subunit rRNA gene. Restriction fragments analysis was conducted post PCR amplification, and cleaved with *Hae*III, *Hinf*I, *Hha*I, *Mse*I and *Sdu*I restriction enzymes. Differences in the lengths of the obtained amplicons were detected after gel electrophoresis, and restriction analyses of the PCR fragments led to identification of six out of eight isolates as belonging to fungi *T. parceramosum*/ *T. reesei*/ *T. longi*, *Polyporus squamosus*, *Fusarium oxysporum* and *Trichoderma atroviride* groups. The PCR ITS-RFLP molecular identification technique can be used successfully for routine screening of fungal isolates from various environments.

Keywords: *fungi, ITS-RFLP, textile, wastewater.*

INTRODUCTION

Textile industry generates large amounts of wastewater with high chemical content, such as acids and alkali, levelling agents, dispersant agents, dyes (Olikanni *et al.*, 2006), which are discharged in public water effluents without prior treatment and have become a real environmental concern. Due to great microbial biodiversity, textile wastewaters have the potential of providing microbial strains that can be used successfully in bioremediation techniques. Fungal strains have shown great potential in treatment of textile wastewaters (Dayaram and Dasgupta, 2008; Andleeb *et al.*, 2010), waste treatment and textile dye microbial degradation requiring suitable strains for extended bioremediation potential.

AIMS AND OBJECTIVES

The study aims to identify previously isolated fungal strains (Iordache and Cornea, 2014)

from textile wastewaters that have the ability to degrade and decolorize textile dyes produced in the final effluents of textile dye processing. Microbial isolates from samples of textile industry wastewaters were preferred instead of collection strains, due to their already existent adaptive profiles to wastewater present agents.

MATERIALS AND METHODS

DNA extraction was performed according to the method of Raeder and Broda (Raeder and Broda, 1985), slightly modified (cell lysis was optimized by using glass beads and a Mini-Beadbeater BioSpec), using DNA amplification for ITS region by ITS1 and ITS4 primers (White *et al.*, 1990). Five restriction enzymes were used for PCR product restriction, with corresponding cleaving sites: *Hae*III (5'...GG[^]CC...3' and 3'...CC[^]GG...5'), *Hinf*I (5'...G[^]ANTC...3' and 3'...CTNA[^]G...5'), *Hha*I (5'...GCG[^]C...3' and 3'...C[^]GCG...5'), *Mse*I (5'...T[^]TAA...3'

Fig. 1. Electrophoretic profiles of fragments obtained with restriction enzymes

Tab. 1. Restriction profiles for isolated fungal strains

Isolate	Restriction fragments length (bp)				
	<i>HaeIII</i>	<i>HinfI</i>	<i>HhaI</i>	<i>MseI</i>	<i>SduI</i>
<i>T.parceramosum/T.reesei/T.longi</i>	70+90+480	-	110+150+250	-	630
<i>T.parceramosum/T.reesei/T.longi</i>	70+90+480	-	110+150+250	-	630
<i>T.parceramosum/T.reesei/T.longi</i>	70+90+480	-	110+150+250	-	630
<i>Fusarium oxysporum</i>	90+110+340	-	-	540	540
<i>Polyporus squamosus</i>	90+110+340	-	-	50+90+370	540
Unidentified	210+400	80+210+340	100+230+300	-	-
Unidentified	50+190+320	70+140+190+340	70+100+160+200+220	-	-
<i>Trichoderma atroviride</i>	170+450	-	110+160+210	-	190+430

and 3'...AAT[^]T...5') and *SduI* (5'...GdGCh[^]C...3' and 3'...C[^]hCGdG...5'). Differences in the lengths of the amplicons, post-PCR method, were detected after agarose gel electrophoresis, after staining with ethidium bromide and UV visualization.

RESULTS AND DISCUSSION

Eight fungal strains were subjected to molecular identification by ITS-RFLP method (White *et al.*, 1990). Post analysis of electrophoretic profiles (Fig. 1) of the restrictions fragments allowed identification of fungal isolates, when compared to *T.asperellum*, *T.hamatum*, *T.viride*, *T.atroviride*, *T.harzianum*, *T.reesei*, *T.longi*, and *T.parceramosum* as belonging to *T.parceramosum/T.reesei/T.longi* (isolates 11-13), *Fusarium oxysporum* (isolate 4), *Polyporus squamosus* (isolate 15) and *Trichoderma atroviride* (isolate 18) strains. Strains 6 and 7 were the only yeast strains isolated and their restriction profiles were compared to *Sacharomyces cerevisiae*, *Yarrowia lipolytica*, *Metschnikowia pulcherima*, *Rhodotorula spp.*, which did not allow the identification of the species (Tab. 1).

CONCLUSION

In the present study, molecular techniques by ITS-RFLP analysis were successfully applied for the identification of some fungal isolates. Textile industry wastewater can develop a great diversity of bio-burden content, which can be used in reducing the residual concentration of textile dyes in post-finishing solutions.

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