Microbiological Research 214 (2018) 83–90

Contents lists available at ScienceDirect

Microbiological Research

journal homepage: www.elsevier.com/locate/micres

Novel *Trichoderma* strains. isolated from tree barks as potential biocontrol agents and biofertilizers for direct seeded rice

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ARTICLE INFO

Keywords:
Trichoderma erinaceum
Biocontrol
Biofertilizer
Stress enzymes
Sheath blight
Nutrient uptake
Direct seeded rice

ABSTRACT

This study is the first time report of utilization of *Trichoderma* spp. isolated from different tree barks from Odisha state of India for rice crop health management and higher productivity. Six isolates of *Trichoderma* spp. were identified based on the morphological characteristics and species determination was performed by molecular assays. One of the isolated strains determined as *Trichoderma erinaceum* outperformed others. *Trichoderma erinaceum* controlled three soil borne plant pathogens i.e. *Rhizoctonia solani, Sclerotium rolfsii* and *Sclerotium oryzae* effectively under controlled condition and *R. solani* and *Helminthosporium oryzae* under filed condition. Seed treatments with the formulated isolates improved the germination rate of rice and enhanced vigour. These parameters along with higher chlorophyll content could be related to higher yield observed in two rice varieties; *Karuna and Shabhagidhan*. Among the six isolates tested, *Trichoderma erinaceum* treatment recorded highest yield. Significantly higher expression of some stress related enzymes was observed in *Trichoderma* treated plants which helped in better crop growth both under biotic and abiotic stresses. These isolates helped both the varieties to accumulate more nutrients This study proves that *Trichoderma erinaceum* obtained from tree bark may be incorporated in integrated rice crop management both as biocontrol agent and biofertilizer.

1. Introduction

Trichoderma spp., are the most widely used microorganisms with disease biocontrol and plant growth promoting activity (Druzhinina et al., 2011; Druzhinina and Kubicek, 2013). These fungi are mycoparasites and produce a plethora of antimicrobial secondary metabolites including phytohormones (Harman et al., 2004; Harman, 2006; Howell, 2006; Shoresh et al., 2010). Phytohormones produced by *Trichoderma* spp. promote plant (root and shoot) growth (Harman et al., 2004; Shoresh et al., 2010) and these fungi mobilize plant nutrients for better crop yield (Mastouri et al., 2010, 2012). They are presently marketed as biopesticides, biofertilizers, growth and yield enhancers as well as nutrient solubilizers and organic matter decomposers (Woo et al., 2014). An interesting aspect of *Trichoderma* mediated biocontrol is their ability to colonize roots and induce systemic resistance against invading fungi, bacteria, viruses and even insects, at a site away from *Trichoderma* inoculation (Segarra et al., 2007; Contreras-Cornejo et al., 2011; Salas-Marina et al., 2011). *Trichoderma* spp. are generally isolated from soil or rhizosphere for their use in biocontrol, and there are only a few reports on the evaluation of *Trichoderma* as biocontrol agents isolated from above-ground habitats (*e.g.* Mukherjee et al., 2014). Rice provides food security to more than half of the global population and to about 85% population of India (Ghose et al., 2013). The major constrain of rice production is different biotic stresses which reduces yield considerably. Rice suffers from several fungal diseases like brown spot, sheath blight, blast, seedling blight, false smut *etc*. Of these, brown spot and blast are major foliar diseases and sheath blight and seedling blight being major soil borne diseases. To manage these diseases huge amount of fungicides are being used which are not only hazardous for the farmers but also for the environment. In order to manage these diseases in an ecofriendly way it is needed to search for alternative management practices. Use of biocontrol agents (BCA) especially *Trichoderma* based BCA have been good commercial success in different crops other than rice. But unfortunately the most of the BCA present in the Indian market

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https://doi.org/10.1016/j.micres.2018.05.015
Received 19 January 2018; Received in revised form 20 April 2018; Accepted 17 May 2018
Available online 23 May 2018
0944-5013/

Table 1
Details of *Trichoderma* isolates used in the present study.

Strain Designation	Source of collection	Place of collection	GPS Location	Species identified	NCBI Accession Numbers.
CRRI-T1	bark of a *Litchi chinensis*	NRRI, Cuttack	85°92′E, 20°45′N	*Trichoderma harzianum*	KX853519.1
CRRI-T2	bark of a *Cassia tora*	42-Mouza(Barala), Cuttack	86°92′E, 20°44′N	*Trichoderma erinaceum*	KR014407.1
CRRI-T3	bark of a *Cassia tora*	42-Mouza(Barala), Cuttack	86°58′E, 20°45′N	*Trichoderma atroviride*	KR014408.1
CRRI-T5	bark of a *Mangifera indica*	Manitri, Jagatasinghapur	88°12′E, 20°45′N	*Trichoderma atroviride*	KX853518.1
CRRI-T9	bark of a *Mangifera indica*	Barala, Cuttack	86°02′E, 20°44′N	*Trichoderma atroviride*	KX863696.1
CRRI-T13	bark of a *Mangifera indica*	Barada (Kishan Nagar) Cuttack	86°92′E, 20°45′N	*Trichoderma atroviride*	KX863695.1

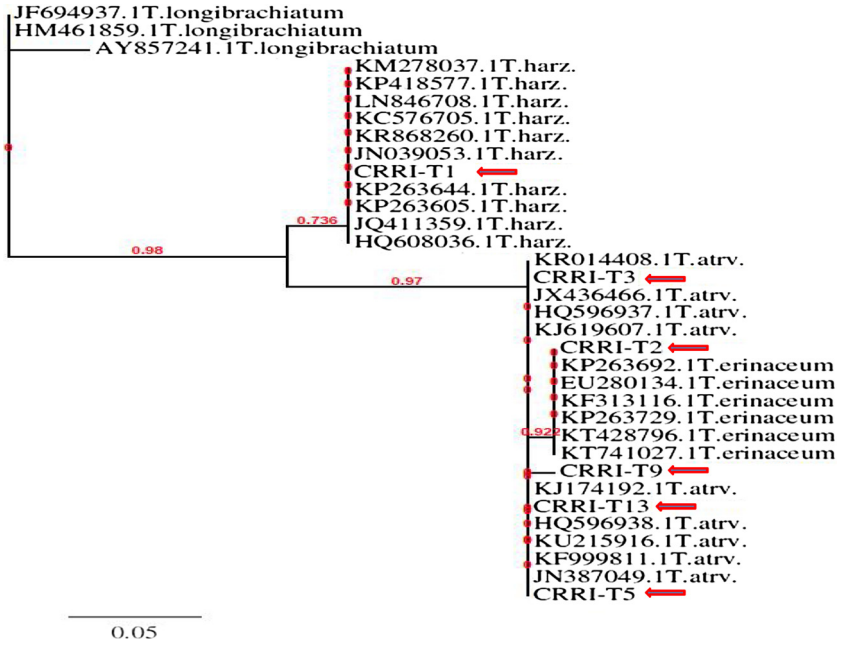

Fig. 1. Phylogeny of the isolated *Trichoderma* spp. (CRRI-T1 to CRRI-T13) used for the present study.

Table 2
Confrontation assay showing the inhibition of pathogen growth by different *Trichoderma* isolates in PDA medium.

Treatment Name	Percentage of inhibition		
	Rhizoctonia solani	*Sclerotium oryzae*	*Sclerotium rolfsii*
CRRIT1	99.57^{A}	58.57^{B}	22.83^{B}
CRRIT2	100.00^{A}	67.38^{A}	35.48^{A}
CRRIT3	100.00^{A}	62.75^{AB}	32.98^{A}
CRRIT5	90.77^{B}	46.24^{C}	4.35^{D}
CRRIT9	80.25^{C}	30.61^{D}	15.75^{C}
CRRIT13	80.26^{C}	30.08^{D}	16.30^{C}
CV (%)	1.46	4.08	6.07
Tukey's HSD at 5%	3.801	5.705	3.6646

Means with same letter are not significantly different at $p \leq 0.05$.

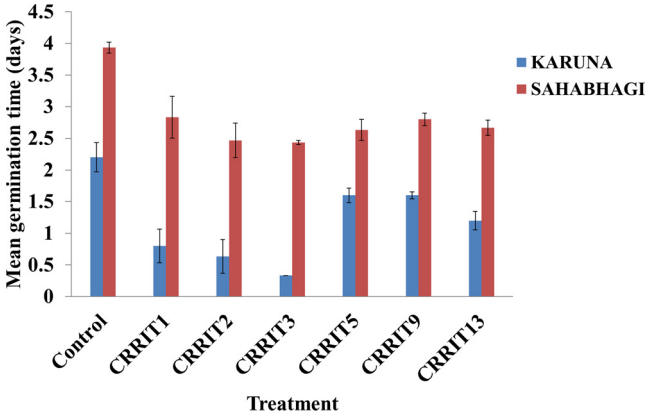

Fig. 2. Mean germination time in rice varieties treated with *Trichoderma* and control condition. (± = standard error).

originated from the same source *i.e.* with *Trichoderma viride* collected from soil (Mukherjee et al., 2013). In a recent study it has been reported that native *Trichoderma* isolates exhibit better performance (Mukherjee et al., 2014). Even though *Trichoderma* spp. has proved effective in management of diseases in a wide variety of crops, their applications in rice have been limited to laboratory condition only. It is generally thought that *Trichoderma* spp., being strictly aerobic, may not perform in paddy field, especially in flooded rice fields.

One of the best approaches to find effective BCA for application to soil or seed material is to seek them from those locations where the pathogen is supposed to cause disease but isn't (Cook, 1985). The main habitat of *Trichoderma* is classically viewed as soil or plant rhizosphere, even though the maximum diversity of these species occurs aboveground *e.g.*, on tree bark and wild mushrooms (Druzhinina et al., 2011). *Trichoderma* spp. isolated from tree bark mainly is being used for the production of enzymes for decomposition of agro-wastes. This is the first attempt to study the utilization of *Trichoderma* spp. isolated from bark of trees in Odisha state of India, as a potential biocontrol and biofertilization agent in rice. In this study, in addition to biocontrol properties we investigated changes in some stress related enzymes, plant nutrient uptake and crop growth parameters on use of *Trichoderma* spp. formulation as seed treatment under field condition.