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RESEARCH ARTICLE

SCREENING FOR MULTIPLE ENZYMES ACTIVITIES OF *TRICHODERMA* STRAINS ISOLATED FROM AGRICULTURE FIELD SOIL IN SENEGAL

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ABSTRACT

To A total of twenty *Trichoderma* strains were isolated from rhizospheric soils of tomato fields from different areas of Niaye zone, main area of horticulture production in Senegal. Identification of these *Trichoderma* isolates was based on morphological and cultural characters on potato dextrose agar medium. These strains were screened for the production of extracellular enzymes such as cellulase, L-Asparginase, chitinase and pectinase. The screening was done by following plate assay method on their respective solid media. Among the 20 strains, 14 strains showed the pectinase activity, 11 strains was positive for L- Asparginase production, 3 strains showed chitinase activity, 15 strains was positive for cellulase activity. The 2 strains (TS1 and NG4) was found to be positive for all the enzymes tested. The excretion of extracellular enzymes reveals their usefulness in the application of *Trichoderma* species as biocontrol in agricultural soils and in industrial and commercial applications.

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INTRODUCTION

Trichoderma species are imperfect filamentous fungi that can be found all over the World. Now it has gained importance in agriculture and industrial applications. In agriculture *Trichoderma* is used for their bio control ability against several soil-borne pathogens such as *Phytophthora palmivora* (Kebe *et al*., 2009*) Fusarium oxysporum f. sp. radicis-lycopersici*, *Pythium ultimum, Rhizoctonia solani, Sclerotinia sclerotiorum* and *Verticillium dahliae* (Caron *et al*., 2002), *Botryodiplodia palmarum* (Tapwal and Pandey, 2016). They are used for protection of many crops such as tomato, cucumber (Caron *et al*., 2002; Mouria *et al*., 2007) onion, eggplant (Caron *et al*., 2006). Antagonistic activity by *Trichoderma* is based on many mechanisms namely antibiosis, mycoparasitism, production of cell walldegrading enzymes and competition for space and nutrients (Zeilinger et Omann, 2007, Vinale *et al.,* 2008). *Trichoderma* species are able to use a wide range of compounds as carbon and nitrogen sources and secrete a variety of enzymes to break down compound plant polymers into simple sugars for energy and growth (Cherkupally *et al*., 2017). These lytic enzymes secreted by *Trichoderma* such as cellulase, chitinase, β-1, 3 glucanase and protease can attacks directly plant pathogen (Khushwaha and Verma, 2014) or they can hydrolyse the contents of fungal cell wall skeleton (chitin, proteine, glucan) one of the main mechanisms accounting for showing antagonistic activity against plant pathogenic fungi (Lunge and Patil, 2012). Enzymes secreted by *Trichoderma* are also known to have a broad industrial and commercial applications. Cellulase enzymes is extensively used in various fields and industries such as textile and food, bioconversion of lignocellulosic waste to alcohol, animal feed, genetic ingineering, pollution traitement paper and pulp industry (Aristidou and Penttilä, 2000). Pectinases are applied in juice processing (extraction and clarification), vegetable oil extraction, alcoholic beverage processing and other food industries (Bhat *et al.*, 2000; Kumar *et al*., 2011). L-asparaginase is one of the most widely studied therapeutic enzymes by researchers. It is known to be the most proper drug for the treatment of acute lymphoblastic leukemia and other cancer diseases. Its production has a broader prospectus in industrial area and even in pharmaceutical industries as the microbial production of L-asparaginase is inexpensive (Alzewari *et al.,* 2010; Kalyanasundaram *et al*., 2015. The objective of this study is for screening of fungal fungal strains for enzymatic activities of most important enzymes (cellulase, chitinase, pectinase, L-Asparginase) using fast and reliable plate methodologies that allowed to process and select antagonistic potential against fungal plant pathogens or which can be used in industrial applications.

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MATERIALS AND METHODS

Isolation of *Trichoderma*: The rhizosphere soil samples were collected from the tomato fields of 5 differnts areas of Niaye zone, main area of horiculture production in Senegal: UCAD, Sangalkam, Gorome, Notto Gouye Diama and Mboro. *Trichoderma* spp. were isolated on Potato Dextrose Agar (PDA) medium by soil dilution plate technique (Rapilly, 1968) using 10^{-3} to 10^{-5} dilutions. The plates were incubated at 28 ± 2°C for 5 days. *Trichoderma* colonies appeared in the plates were noted and sub cultured. After they were purified by single spore isolation method and maintained on potato dextrose agar (PDA) slants. Identification of *Trichoderma* isolates was based on culture characters as well as microscopic parameters (conidiophores branching, phialides shape and position, spore size and shape) (Nagamani *et al.,* 2006). The pure cultures were stored in the refrigerator at 4°C for further studies.

Qualitative screening of enzymes: Enzyme assay of *Trichoderma* isolates was carried out by plate assay on respective solid media for extracellular enzymes. Screening was based on the formation of clear zones for production of cellulase, pectinase, protease, and chitinase enzymes or change of colour and intensity around the fungal colonies for production of L- asparginase. The independent experiments were performed for this screening step with three replicates for each strain. For each Petri plate the *Trichoderma* strain was growing by inoculation aseptically a disc of 6mm of agar cut from 7 days old pure fungal culture. Diameters of the colony and the clear zone were measured.

Cellulase screening: For cellulase screening (Hankin and Anagnostakis, 1975), *Trichoderma* strains were grown on the Czapek-Mineral Salt Agar Medium (KH_2PO_4 1.00 g, $MgSO_4.7H_2O$ 0.50 g, $NaNO_3$ 2.00 g, KCl 0.50 g, Peptone 2.00 g, and Agar 20.00g, Distilled water 1000ml) supplemented with Carboxy Methyl Cellulose (CMC) 5.00g. After 4 days of incubation the plates were flooded with iodine potassium – iodine solution (Iodine 1,00g, Potassium iodine 5,00g in 330 ml distilled water) for 15 min. Observed inhibtion zone is an indication of cellulase activity.

Pectinase screening: For pectinase activity screening (Hankin and Anagnostakis, 1975), Pectinase Agar Medium was used (Citrus Pectin 10.00g, $(NH_4)_2HPO_4$ 3.00g, $MgSO_4$ 0,1g, Agar 20.00g, Distilled water 1000 ml, pH 5,5). After four days of incubation the plates were flooded with iodine potassium – iodine solution (Iodine 1,00g, Potassium iodine 5,00g in 330 ml distilled water). A zone of inhibition indicates pectinase activity.

Chitinase screening: The chitinase activities was determinated by using Chitinase Detection Medium (Agrawal and Kotasthane, 2012) with slight modifications (colloidal chitin 10, 00g, $MgSO_4$ 0,3g, NH_4SO_4 3.00g, KH_2PO_4 2.00g, Agar 20.00 g, Distilled water 1000ml). Colloidal chitin was prepared from commercial chitin and was amended in the chitinase assay medium as a sole carbon source. Four days after incubation a clear zone in the opaque agar around colonie show positive chitinase activity.

L-Asparginase screening: Asparginase medium was used for L-Asparginase screnning (KH_2PO_4, 3g, L-Aspargine 5g, $MgSO_4$, $7H_20$ 0,5g, $CaCl_2$ $0,014_g$, glucose 3g, phenol acide 2,5%, NaCl 0,5g, Agar 20g, distilled water 1000ml, pH 6, 8). L-aspargine was added to the media after sterilisation and cooling. Four days after incubation the appearance of a pink zone around the fungal colony on a yellow color media is an indication of the L-Asparaginase activity.

RESULTS AND DISCUSSION

In present study, a total of 20 *Trichoderma* spp. were obtained from rhizosphere soil samples (Table-1) after macroscopic and microscopic observations. *Trichoderma* isolates show fast growing and are initially white and became slightly green after two days of incubation in PDA medium. Microscopic observations of the conidiophores showed typically formed paired branches and displayed pyramidals arranged along the length of the primary axis (Figure-1). Main branches of the conidiophores produce lateral side branches that may be paired or not, the longest branches distant from the tip and sometimes phialides arising directly from the first axis to the tip.

Table 1. Number of strains isolated from different sites

Site	Number of isolate strains
UCAD (Botanique garden)	3strains : TU1, TU2, TU3
Sangalkam	4 strains : TS1, TS2, TS3, TS4
Gorome	7 strains : TG1, TG2, TG3, TG4, TG5, TG6, TG7
Notto Gouye Diama	3 strains : TN1, TN2, TN3
Mboro	3 strains : TM1, TM2, TM3

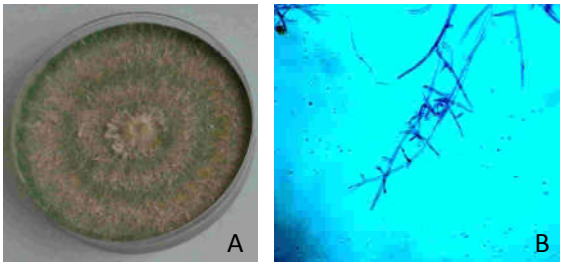

Figure 1. Morphological aspect of *Trichoderma* isolates
A : mycelium, B: Conidiophore

Table 2. Primary screening of *Trichoderma* spp. for enzymes production

Trichoderma strain	Enzymes			
	Pectinase	Cellulase	L-Asparginase	Chitinase
TS1	+	+	+	+
TS4	+	+	-	-
TG6	-	-	-	-
TU2	+	-	-	-
TN2	+	+	+	-
TM1	+	+	+	-
TG3	+	+	-	+
TN3	+	+	-	-
TM3	+	+	+	-
TU1	+	+	+	-
TN1	+	+	+	-
TG4	+	+	+	+
TS2	+	+	+	-
TU3	+	+	-	-
TS3	-	+	-	-
TM2	-	-	-	-
TG1	+	+	+	-
TG2	-	-	-	-
TG5	-	+	+	-
TG7	-	-	+	-

+ : strain showing enzyme activity; - : strain showing no enzyme activity

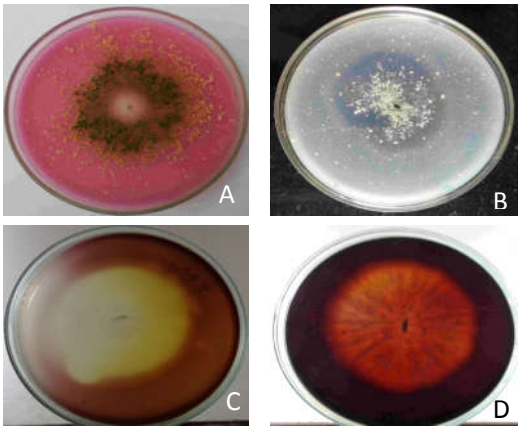

Figure 2. Aspect of positives strains: A: positive for L-Asparginase activity, B: positive for chitinase activity, C: positive for cellulase activity, D: positive for pectinase activity

Phialides are long, enlarged in the middle like bottle shaped, inflated at the base, and some were cylindrical. Conidia are wooly, filled up and were compact at the midpoint of a Petri dish with mostly dark green colour. The macroscopic and microscopic caracteristics observed correspond to the descriptions of Nagamani *et al.,* (2006) about *Trichoderma* genus identification.

Enzyme screening: All the isolates of *Trichoderma* spp. were screened for secretion of lytic enzymes cellulase, pectinase, chitinase and L-Asparginase (Figure-2 and Table-2). Among the 20 strains, 14 strains showed the pectinase activity and 15 strains are positive for cellulase activity. The fungi *Trichoderma* is reported to degrade cellulose by the production of cellulase enzyme (Ju and Afolabi, 1999, Kumar *et al.*, 2012; Ranga Rani *et al*., 2017). Pectinase is one of the extracellular enzymes produced by *Trichoderma* involved on the decomposition of organic matter and can extract nutrients from the plant tissues and helps in the entry of fungal hyphae. The secretion of pectinase by many species of *Trichoderma* was also discribed by Cherkupally *et al.,* **(**2017**)** and Nazia *et al*., (2003)**.** For L-asparginase screening, 11 strains show positive activity. Production of asparaginase by *Trichoderma* was also reported by Karthikeyan (2014) using the species *Trichoderma viride* in wheat bran, coffee husk and urea containing like substrate in asparaginase medium. Chitinolytic activity was showed by 3 *Trichoderma* strains. Chitinolytic enzymes have been detected and purified from various *Trichoderma* sp. (Lorito et *al.,* 1998). Chitinases are found in several organisms in which fullfill different functions. In fungi specifically they have autolytic, nutritional and morphogenetic roles (Patil *et al*., 2000). It has been demonstrated that chitinase produce by *Trichoderma* spp. can be effective biocontrol (Kubicek *et al*., 2001). Many studies have proved the potential of *Trichoderma* sp. as biological agents antagonistic to several soil borne plant pathogens. Bruce *et al.,* (1995), reported that the production of lytic enzymes influenced by the composition of the culture media.

Conclusion

This work was conducted to screen the *Trichoderma* isolation and for their capacity to secrete multiple enzymes, usefull in agriculture and in industrial applications. Based on morphological characteristics, 20 *Trichoderma* strains were isolated from different areas in main zone of horticultural production in Senegal. This study show the capacity of *Trichoderma* strains to produce multiple enzymes. Among these 20 strains 14 were positive for pectinase, 3 showing positive activity for Chitinase, 11 positives for Asparginase and 15 showing for cellulase activity. In this 20 strains, 2 have capacity to produce all the four enzymes screened (pectinase, L-Asparginase, chitinase and cellulase). These strains, TG4 and TS1 must have many interest in pharmaceuticals, agrochemicals and industrial applications.

Conflict of interest: The authors declare that there is no conflict of interest.

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