Factors affecting the chitinase activity of *Trichoderma asperellum* isolated from agriculture field soils

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**ABSTRACT**

In the present study, 20 fungal strains were isolated from tomato rhizosphere of Senegal. Of 20 strains, five showed the chitinolytic activity on chitin agar medium. Of the five strains, NG4 showed the maximum solubilization zone. This strain was identified by preliminary biochemical and 18S rRNA sequencing analysis. Enzyme production started after 3 days of incubation and maximum was observed after 5 days of incubation. Culture filtrate amended with 0.1% colloidal chitin was used in the production medium. The optimum conditions for maximum chitinase activity are – 6 days of growth and temperature of 30°C at pH 6.0. The chitinase activity was also influenced by the addition of carbon and nitrogen sources in the production medium.

**1. INTRODUCTION**

Chitin, the second most abundant polysaccharide in nature after cellulose, is a linear polymer of β-1, 4-N-acetylglucosamine. Bacteria, fungi, yeasts, actinomycetes, and plants are the main sources of chitin [1] Complete hydrolysis of chitin can be carried out by a group of chitinolytic enzymes including exochitinase, endochitinase, and chitobiase and release N-acetyl-d-glucosamine subunits [2]. Chitinases have a wide range of applications such as biocontrol agents and biopesticides [3]. They were considered as the best biological control agents of plant pathogens due to their ability to degrade fungal cell walls [4,5]. *Trichoderma* species were reported to be act as mycoparasites against soil-borne pathogens such as *Rhizoctonia solani*, *Sclerotinia rolfsii*, and *Fusarium* sp. [6-8]. In the present study, the optimal culture conditions for maximum production of chitinase production by *Trichoderma* strains under solid-state fermentation were studied and results were discussed.

**2. MATERIALS AND METHODS**

**2.1. Isolation of Trichoderma**

For this study, *Trichoderma* strains isolated from rhizosphere soil samples collected from tomato fields of five different areas of Niaye zone, the main area of horticulture production in Senegal: UCAD, Sangalkam, Gorome, Notto Gouye Diama, and Mboro was used. Identification of *Trichoderma* isolates was based on culture characters as well as microscopic parameters (conidiophores branching, phialides shape and position, spore size, and shape) [9]. The pure cultures maintained at 4°C were used for further studies. The best producer of enzyme was identified by 18S rRNA sequencing (Macrogen, South Korea) as *Trichoderma asperellum*.

**2.2. Screening of Isolates for Chitinase Production**

The chitinase activities were determined using chitinase detection medium [10] with slight modifications (colloidal chitin 10 g, MgSO₄ 0.3 g, NH₄SO₄ 3.00 g, KH₂PO₄ 2.00 g, agar 20.00 g, and distilled water 1000 ml). Colloidal chitin prepared from commercial chitin was used as a carbon source in the above medium. Four days after incubation, a clear zone surrounding the colony indicates the positive chitinase activity. For positives strains, diameter of hyaline zone around the colony (mm) at different incubation periods (days) was measured.
Table 1: Screening for chitinase production by fungi isolated from Senegal.

| Fungal isolates | Diameter of zone around the colony (mm) at different incubation periods (in days) |
|-----------------|---------------------------------------------------------------------------------|
|                 | 3      | 4      | 5      | 6      | 7      |
| TS1             | 6      | 8      | 10     | 10     | 10     |
| TN1             | 4      | 6      | 6      | 6      | 6      |
| TG4             | 8      | 12     | 16     | 16     | 16     |
| TG3             | 6      | 8      | 12     | 12     | 12     |
| TM1             | 4      | 6      | 6      | 6      | 6      |

Table 2: Effect of incubation period on chitinase production by Trichoderma asperellum.

| Incubation period (days) | Biomass production (mg/100 ml) | Enzyme activity (U/ml) |
|-------------------------|-------------------------------|------------------------|
| 3                       | 148.6±0.24                   | 1.80±0.01              |
| 4                       | 227.3±0.49                   | 1.92±0.02              |
| 5                       | 353.3±0.6                    | 2.81±0.02              |
| 6                       | 250±0.33                     | 1.50±0.03              |
| 7                       | 113.3±0.31                   | 0.85±0.02              |
| 8                       | 86.6±0.58                    | 0.83±0.01              |

Table 3: Effect of pH on chitinase activity by Trichoderma asperellum.

| pH   | Biomass production (mg/100 ml) | Enzyme activity (U/ml) |
|------|-------------------------------|------------------------|
| 4.0  | 136±0.47                      | 0.66±0.05              |
| 5.0  | 143.6±0.42                    | 1.88±0.41              |
| 6.0  | 453.3±0.38                    | 2.81±0.29              |
| 7.0  | 353.3±0.53                    | 2.53±0.40              |
| 8.0  | 290±0.73                      | 2.01±0.07              |
| 9.0  | 130±1.04                      | 1.30±0.04              |
| 10.0 | 85.3±0.49                     | 0.35±0.03              |
for enzyme activity and 411.6 mg/100 ml for biomass production). The galactose showed the lowest chitinase production (0.94 U/ml) compared to control.

3.6. Effect of Colloidal Chitin Concentration on Chitinase Production

Different concentrations of colloidal chitin (0.1%, 0.5%, 1.0%, 1.5%, and 2.0%) were selected to study the effect on biomass and chitinase production [Table 6]. Our results showed a gradual increase of the two parameters with the increase of colloidal chitin concentration up to 1% that showed the maximum biomass production (353.3 mg/100 ml) and enzyme activity (2.81 U/ml). After that, a reduction of biomass and chitinase activity was observed.

3.7. Effect of Nitrogen Sources on Chitinase Production

Effect of nitrogen sources on IAA production by Trichoderma strains was studied by the addition of various nitrogenous compounds (NaNO₃, (NH₄)₂SO₄, yeast extract, beef extract, and peptone). These different nitrogen sources have a significant effect on biomass production and enzyme activity compared to control [Table 7]. Among all the nitrogen sources used, yeast extract was found to be the best nitrogen source for biomass production (456.6 mg/100 ml) and enzyme activity (2.89 U/ml). It is followed for enzyme activity, respectively, by peptone (2.72 U/ml) and beef extract (2.35 U/ml). Between the nitrogen sources used, (NH₄)₂SO₄ showed the lowest biomass production (180 mg/100 ml) and chitinase activity (1.04 U/ml).

Several Trichoderma species are able to produce many enzymes like chitinase involved on biocontrol of many soil-borne pathogenic fungi. However, the production of these enzymes depends on many factors such as incubation periods, pH, temperature, carbon sources, and nitrogen sources. Among the 20 strains screened, the results of this study showed that the strains TG4 identify by 18S rRNA sequencing T. asperellum showed the maximum zone of inhibition after 5 days of incubation. Optimization of chitinolytic activity of TG4 showed a maximum chitinase and biomass production at 5 days of incubation at 30°C, pH 6. The decrease of chitinase yield after the optimum period of incubation was probably due to the reduced nutrient level of the medium affecting the enzyme synthesis by the fungus [12,13]. Variation of chitinase production of pH and temperature was observed by many authors. Similarly, to our results Mallikharjuna Rao et al. [14], Ulhoo and Peberdy [15] found maximum chitinase production at 30°C and pH 6. Sandhya et al. [13] found maximum chitinase production after 96 h of incubation at pH 4 using Trichoderma harzianum in submerged fermentation. Nampoothiri et al. [12] also observed maximum chitinase after 96 h of incubation at 30°C, pH 4.5 with T. harzianum in solid-state fermentation. Aida et al. [16] showed high level of chitinase production in culture medium with pH 5 at 30°C for 5 days at shaking condition using Aspergillus terreus species. Increase in chitinase production at acidic pH in the present study was in coincidence with the results of El-Katatny et al. [17], in T. harzianum Rifai. Among the various carbon sources tested, colloidal chitin showed the maximum biomass and chitinase production. Same results were observed by Sandhya et al. [13] and Nampoothiri et al. [12]. Rao et al. [16] also showed that maximum chitinase production was observed when chitin or dried cell walls of S. rolfsii were incorporated in the media. The production of extracellular enzymes like chitinase increases significantly when Trichoderma spp. are grown in media supplemented with either autoclaved mycelium or isolated purified host fungal cell walls [18,19]. Our results showed that yeast extract was the best nitrogen source for biomass and chitinase production by Trichoderma strains. Similar results was reported by Nampoothiri et al. [12], while Sandhya et al. [13] reported that peptone and tryptone showed maximum enzyme production by T. harzianum.

4. SUMMARY

The present study reports the factors affecting chitinase production by Trichoderma strains. Of the 20 Trichoderma strains isolated, five showed chitinolytic activity. Among the five strains, TG4...
(T. asperellum) showed maximum chitinase activity. Optimization studies on T. asperellum showed maximum chitinase activity at 5 days of incubation, pH 6.0, and 30°C temperature. Addition of colloidal chitin and yeast extract as carbon and nitrogen sources, respectively, greatly influenced the chitinolytic activity of Trichoderma strains. These observations, thus, confirmed that T. asperellum (TG4) could be a potential fungus for the production of extracellular chitinase.

5. ACKNOWLEDGMENTS

The authors are thankful to CV Raman International Fellowship for African Researcher of the Department of Science and Technology, Government of India, for the financial assistance under Fellowship Project (DO NO. DST/INT/CVRF/2016). We are also thankful to the Department of Botany and Microbiology, Acharya Nagarjuna University, for providing the facilities to do this work.

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How to cite this article:
Gueye, N, Kumar GK, Ndiaye M, Sall SYD, Ndiaye MAF, Diop TA, Ram MR. Factors affecting the chitinase activity of Trichoderma asperellum isolated from agriculture field soils. J App Biol Biotechn. 2020;8(02):41-44. DOI: 10.7324/JABB.2020.80207