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Growth inhibition of the cyanobacterium *Microcystis aeruginosa* and degradation of its microcystin toxins by the fungus *Trichoderma citrinoviride*

Zakaria A. Mohamed [a, *], Mohamed Hashem [b, c], Saad A. Alamri [c]

[a] *Botany Department, Faculty of Science, Sohag University, Sohag 82524, Egypt*
[b] *King Khalid University, Faculty of Science, Biology Department, P.O. Box 10255, Abha 61321, Saudi Arabia*
[c] *Assiut University, Faculty of Science, Botany Department, Assiut 71516, Egypt*

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ABSTRACT

Harmful cyanobacterial blooms are recognized as a rapidly expanding global problem that threatens human and ecosystem health. Many bacterial strains have been reported as possible agents for inhibiting and controlling these blooms. However, such algicidal activity is largely unexplored for fungi. In this study, a fungal strain kkuf-0955, isolated from decayed cyanobacterial bloom was tested for its capability to inhibit phytoplankton species in batch cultures. The strain was identified as *Trichoderma citrinoviride* Based on its morphological characteristics and DNA sequence. *Microcystis aeruginosa* co-cultivated with living fungal mycelia rapidly decreased after one day of incubation, and all cells completely died and lysed after 2 days. The fungal filtrate of 5-day culture also exhibited an inhibitory effect on *M. aeruginosa*, and this inhibition increased with the amount of filtrate and incubation time. Conversely, green algae and diatoms have not been influenced by either living fungal mycelia or culture filtrate. Interestingly, the fungus was not only able to inhibit *Microcystis* growth but also degraded microcystin produced by this cyanobacterium. The toxins were completely degraded within 5 days of incubation with living fungal mycelia, but not significantly changed with fungal filtrate. This fungus could be a potential bioagent to selectively control *Microcystis* blooms and degrade microcystin toxins.

1. Introduction

Occurrence of cyanobacterial blooms in many large freshwater lakes has become more frequent worldwide. In Particular, *Microcystis aeruginosa* proliferates rapidly and accumulates in water bodies causing significant adverse impacts on aquatic environments and public health (Hua et al., 2009). This is due to the ability of this species to produce microcystin toxins (Codd et al., 2005) which are associated with allergies, irritation reactions, gastroenteritis, liver diseases, and tumors (Bell and Codd, 1994; Dawson, 1998). Many strategies including physical, chemical and biological methods have been adopted to reduce and remove these nuisance blooms and their toxins in water supplies all over the world. However, high treatment costs (Hua et al., 2009) and secondary pollutants formed as a result of using chemical algicides (Jeong et al., 2000; Mason, 2002) make these methods undesirable.

Biological methods, on the other hand, are considered to be a more economical and environment-friendly way to control cyanobacterial blooms (Jia et al., 2010a) and degrade their cyanotoxins (Mohamed and Alamri, 2012). Biological control agents such as viruses, bacteria, fungi and heterotrophic flagellates were found to play a major role in

* Corresponding author. Tel.: +20 932320667, +20 1141705691 (mobile).
E-mail address: mzakaria_99@yahoo.com (Z.A. Mohamed).

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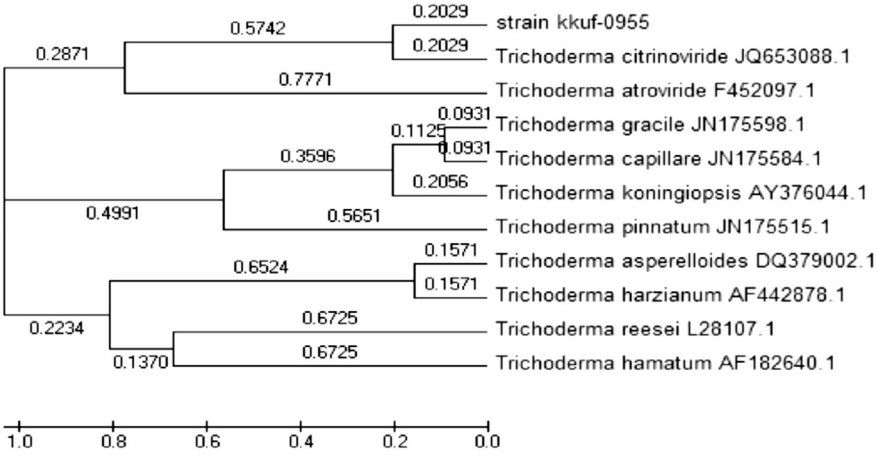

Fig. 1. Phylogenetic relationship between the *T. citrinoviride* strain kku-0955 and other ITS sequences of published strains. In the phylogenetic tree, kku-0955 and *T. citrinoviride* were clustered together as one clade Segments corresponding to an evolutionary distance of 0.005 are shown with bars. Accession numbers for sequences are as shown in the phylogenetic tree.

the regulation and termination of harmful algal blooms (Sigee, 2005; Mohamed and Al-Shehri, 2013). Most biological studies have focused mainly on screening cyanophages and bacteria for their ability to control and degrade harmful cyanobacteria in aquatic environments (Ahn et al., 2003; Mayali and Azam, 2004; Kang et al., 2005; Mu et al., 2007; Dillon and Parry, 2008; Alamri and Mohamed, 2013). However, little attention has been paid to fungi with algicidal activity on cyanobacteria and other harmful algae. Some studies have reported an antagonistic activity of some antibiotic producing fungi toward cyanobacteria (Redhead and Wright, 1980). Other studies demonstrated that fungal strains that produce no antibiotics, particularly white rot fungi, can inhibit the growth of cyanobacteria species (Jia et al., 2010a,b 2011; Wang et al., 2010). Moreover, a recent study by Jia et al. (2012) revealed the ability of *Trichaptum abietinum* 1302BG, a white rot fungus, to degrade microcystin-LR in the harmful algal culture of *M. aeruginosa* PCC7806. It is, therefore, necessary to screen more fungal species of different groups for their algicidal activity to differentiate the strong aligicidal species to be used as an efficient bioagents against harmful algal and cyanobacterial blooms. In this study, we investigated the ability of other fungal species, *Trichoderma citrinoviride* to inhibit the growth of the toxic cyanonabacterium *M. aeruginosa* and to degrade its microcystin toxins as a contribution to the knowledge of algicidal fungi.

2. Materials and methods

2.1. Fungal isolation and identification

The fungus *T. citrinoviride* was isolated from decayed *Microcystis* bloom collected from a Saudi eutrophic lake on yeast extract malt extract agar medium (YMA) containing; 3 g L^{-1} yeast extract, 3 g L^{-1} malt extract, 5 g L^{-1} peptone, 10 g L^{-1} glucose, 0.033 g L^{-1} rose bengal and 0.1 g L^{-1} chloramphenicol. The fungal mycelia were transferred into 50 mL glass flask containing 25 mL liquid medium. After 5 d of cultivation, the mycelial pellicles were used as inocula for algicidal experiments. The fungus was identified preliminarily based on the morphological and cultural characteristic. The identification was confirmed by molecular identification. The fungal genomic DNA extraction was

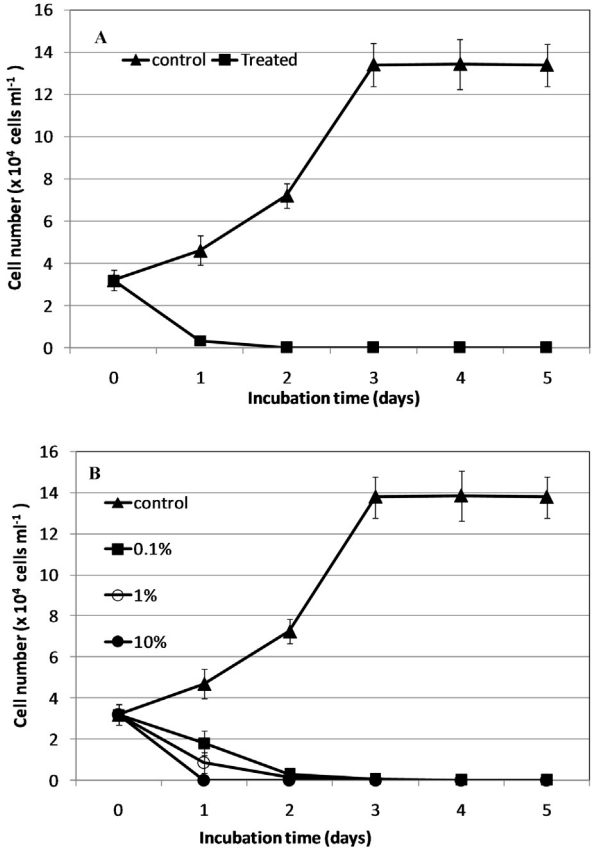

Fig. 2. Changes in the growth (cell density) of *Microcystis aeruginosa* treated with the mycelia (A) and extract (B) of *T. citrinoviride*.