Original Research Paper

Evaluation of hydrolytic enzymes and antifungal activity of extracellular bioactive compounds of *Desmonostoc alborizicum* and *Neowestiellopsis persica* against plant pathogenic fungi

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Abstract

Agriculture requires the extensive use of chemical pesticides to protect crops against pests and diseases. An important mechanism for the biological control of pathogenic fungi is the breakdown of their cell walls. Cyanobacteria are found commonly growing as blooms which provides a competitive advantage to these organisms. This is one of the critical factors responsible for the production of several hydrolytic enzymes with antifungal activity. However, the role of the hydrolytic enzymes of *Neowestiellopsis* and *Desmonostoc*, which are implicated in the fungicidal activity of several biocontrol strains, has not been explored. Therefore in this study, hydrolytic enzymes (chitosanase, protease, FPase, carboxymethyl cellulose, xylanase, cellobiohydrolases and cellobiase) of two cyanobacteria strains were evaluated against a set of phytopathogenic fungi (*Alternaria alternata, Fusarium solani, Fusarium oxysporum, Macrophomina phaseolina, Verticillium dahliae* and *Phytophthora*). The results of statistical analysis showed that the level of protease, FPase and xylanase activity in *Desmonostoc alborizicum* cyanobacterial extract has been significantly higher than in *Neowestiellopsis*. Moreover, IAA hormone activity and soluble protein content were significantly higher in *Desmonostoc alborizicum* cyanobacterial extract. While CMCase, cellobiohydrolases, cellobiase, and chitosanase activity was significantly higher in *Neowestiellopsis persica* A1387 cyanobacterial extract in comparison to *Desmonostoc alborizicum*. Moreover, *Neowestiellopsis*

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persica was observed to be highly potent in terms of its fungicidal activity. Comparative evaluation of the activity of hydrolytic enzymes and antifungal activity revealed that such enzymes might contribute to the fungicidal activity of the cyanobacterial strains, besides other bioactive compounds, including IAA, which are established promising traits for biocontrol agents. This study is a first-time report on the production of hydrolytic enzymes by these two cyanobacteria strains, which can be potential candidates for the development of biocontrol agent(s) against selected phytopathogenic fungi.

Keywords

hydrolytic enzyme, cyanobacteria, plant pathogenic fungi, *Neowestiellopsis persica*, *Desmonostoc alborizicum*

Analiza aktivnosti hidrolitičnih encimov in protiglivne aktivnosti zunajceličnih bioaktivnih snovi cianobakterij *Desmonostoc alborizicum* in *Neowestiellopsis persica* proti fitopatogenim glivam

Izvleček

Kmetijstvo zahteva obsežno uporabo kemičnih pesticidov za zaščito pridelkov pred škodljivci in boleznimi. Pomemben mehanizem biološkega nadzora patogenih gliv je razgradnja njihovih celičnih sten. Cianobakterije pogosto rastejo večjih sestojih na vodni površini, kar je eden od kritičnih dejavnikov, odgovornih za proizvodnjo več hidrolitičnih encimov s protiglivičnim delovanjem. Vendar pa vloga hidrolitskih encimov vrst *Neowestiellopsis* in *Desmonostoc*, ki sta vpletena v fungicidno aktivnost več biokontrolnih sevov, ni bila raziskana. Zato smo v tej študiji ovrednotili delovanje hidrolitskih encimov (hitozanazo, proteazo, FPazo, karboksimetil celulazo, ksilanazo, celobiohidrolaze in celobiazo) dveh sevov cianobakterij proti nizu fitopatogenih gliv (*Alternaria alternata, Fusarium solani, Fusarium oxysporum, Macrophomina phaseolina, Verticillium dahliae* in *Phytophthora*). Rezultati statistične analize so pokazali, da je bila stopnja aktivnosti proteaze, FPaze in ksilanaze v ekstraktu cianobakterije *Desmonostoc alborizicum* bistveno višja kot pri vrsti *Neowestiellopsis persica* A1387. Poleg tega sta bila aktivnost hormona IAA in vsebnost topnih beljakovin znatno višja v ekstraktu cianobakterije *D. alborizicum.* Nasprotno je bila aktivnost CMCase, celobiohidrolaz, celobiaze in hitozanaze bistveno višja v ekstraktu cianobakterije *N. persica* v primerjavi z *D. alborizicum*. Poleg tega je bilo ugotovljeno, da ima vrsta *N. persica* zelo močno fungicidno delovanje. Primerjava aktivnosti hidrolitskih encimov in protiglivične aktivnosti je pokazala, da lahko takšni encimi, poleg drugih bioaktivnih spojin, vključno z IAA, prispevajo k fungicidni aktivnosti cianobakterijskih sevov in imajo obetavne lastnosti za biokontrolo. Ta študija je prvo poročilo o proizvodnji hidrolitskih encimov pri teh dveh sevih cianobakterij, ki sta lahko potencialna kandidata za razvoj biokontrolnih sredstev proti izbranim fitopatogenim glivam.

Ključne besede

hidrolitični encimi, cianobakterije, fitopatogene glive, *Neowestiellopsis persica*, *Desmonostoc alborizicum*

Introduction

Cyanobacteria represent a small taxonomic group of prokaryotes, which are equipped with the power to harm and help plants, animals and humankind and possess tremendous potential for producing a wide range of secondary metabolites (Verma et al., 2022). A preponderance of cyanobacteria was responsible for the inherent and sustained fertility of rice fields, which led to the cyanobacterial populations in soil being evaluated in terms of their diversity and utility as biofertilizers, not only for rice but for other crops, including wheat (Prasanna et al., 2008).

Cyanobacteria are also found commonly growing as blooms in eutrophic lakes, reservoirs and as floating assemblages in marine ecosystems. Such blooms have been notorious and associated with the production of toxins/allelopathic compounds, which provide a competitive advantage to these organisms (Prasanna et al., 2010). This is one of the critical factors responsible for their abundance in diverse environments, especially eutrophic water bodies. However, the chemical potential of these ubiquitous prokaryotes, widely distributed in diverse soil types, aquatic environments and ecologies, in terms of the production of metabolites has not been much investigated. Agriculture requires the extensive use of chemical pesticides to protect crops against pests and diseases. Several of these chemicals pollute our groundwater and drinking water, and therefore some governments have decided to reduce these chemical inputs substantially (Schweitzer and Noblet, 2018). This urges the need for alternative crop protectants. One of these alternatives is the use of biological control agents, among which are microorganisms that can protect plants against diseases (Bonaterra et al., 2022). The colonization and defensive retention of the rhizosphere niche by microorganisms are enabled by the production of allelochemicals, antibiotics, biocidal volatiles and lytic/detoxification enzymes.

Genus *Neowestiellopsis* was originally described by Kabirnataj et al. (Kabirnataj et al., 2018) from Mazandaran (Iran) and belongs to the order Nostocales and family Hapalosiphonaceae. Strains of this genus can be found in both paddy fields and agricultural zones and due to their ability to fix nitrogen, some strains have an important role in agriculture. After that, Nowruzi et al., 2022, found evidence of the poisoning of humans from feeding/due to the ingestion of crop of *Crataegus* plant contaminated with cyanobacterial toxins of *Neowestiellopsis* ca. *persica*, which is most abundant in the agricultural zones of Kermanshah province of Iran using a polyphasic approach (Nowruzi et al., 2022). They recorded the presence of a gene cluster coding for the biosynthesis of a bioactive compound (Nostopeptolides) that is very rare in this family and present toxic compounds (*microcystin*) which might account for the poisoning of humans. Moreover, our previous study revealed that *Desmonostoc alborizicum* strain 1387 is a potentially toxic species isolated from a water supply system in Iran since the *mcy*D and *mcy*G genes of the microcystin synthetase (*mcy*) cluster were successfully sequenced (Nowruzi and Becerra-Absalón, 2022). Using mass spectrometry, detectable amounts of the hepatotoxin microcystin-LR were present in cell extracts of the *Desmonostoc* strain (Nowruzi and Becerra-Absalón, 2022).

Casamatta and Wickstrom (Casamatta and Wickstrom, 2000) reported that the exudates of *Microcystis aeruginosa* were inhibitory towards bacterial plankton communities. A number of nucleosides – tubercidin, toyocamycin and their corresponding derivatives isolated from Scytonemataceae members were observed to be toxic towards *Aspergillus oryzae, Candida albicans, Penicillium notatum* and *Saccharomyces cerevisiae* (Prasanna et al., 2010).

A diverse range of compounds is also known to exhibit a bioregulatory role as a result of their cytotoxic, immunosuppressive and enzyme-inhibiting activities, which are of tremendous pharmaceutical significance (Indumathi, 2016). However, the role of the hydrolytic enzymes of *Neowestiellopsis* and *Desmonostoc*, which are implicated in the fungicidal activity of several biocontrol strains, has not been explored. It is worth noting that the above-mentioned features will specify the furthermore convincing evidence to introduce two toxic species of cyanobacteria as biocontrol agent(s) in agriculture.

Materials and Methods

Cyanobacteria culture and extraction

Desmonostoc alborizicum and *Neowestiellopsis persica* strains were taken from the Cyanobacteria Culture Collection (CCC) affiliated with the Science and Research Branch of the Islamic Azad University, Tehran, Iran. The isolates were maintained in a 250 mL cotton-stoppered Erlenmeyer flask containing liquid Z8 medium at 28±2 °C

with periodic shaking (twice a day), illumination of ca. 50-55 µmoL photons $m^{-2}s^{-1}$, and a regime of 14:10 h light: dark cycle (Oudra et al., 2002).

Preparation of cyanobacterial extracellular products

Extracellular products from 1000 ml of two cyanobacteria cultures were prepared (Kwon and Kim, 2008). Biomass was separated by centrifugation (CRU-5000 Centrifuge, Damon/IEC Division, Needham, Massachusetts, USA) for 20 min at 6000 rpm and 4°C. The culture filtrates were concentrated ten times using a rotary evaporator (Heidolph, Kehlheim, Germany) and then sterilized by filtration through 0.2 µm Seitz filters. They were kept refrigerated at four °C until use.

Estimation of fungicidal activity of cyanobacterial extracellular products

Various concentrations of 1, 5, 10, 15 and 20 mg/ml of cyanobacterial extracellular products of studied cyanobacteria strain were tested for their antifungal activity using the paper disk diffusion method against the growth of *Alternaria alternata, Fusarium solani, Fusarium oxysporum, Macrophomina phaseolina, Verticillium dahliae* and *Phytophthora*.

A loop of each slant was mixed in 5 mL of saboraud dextrose agar (SDA) and incubated at 28 °C until 0.5 McFarland standards 2.3×10^3 (AL-Abedi et al.). 100 mL of each concentration was dissolved in 1 mL of 5% dimethyl sulfoxide (DMSO). Sterilized paper discs (6 mm) were prepared. The previously saturated discs (6 mm) were placed on the sides of the SDA plates. SDA plates were incubated at 28 °C for five days. The inhibition zones (mm) around discs were measured by the transparent ruler.

Total proteins

The amount of proteins was determined spectrophotometrically according to (Chatterjee and Mukherjee, 2014), with Bovine serum albumin (BSA) as the standard.

IAA activity

IAA activity was measured by the method described by (Lohani et al., 2004). The intensity of the pink colour was measured at 530 nm, and values were compared with those of the standard curve of IAA. The activity of all the hydrolytic enzymes is expressed as a standard International Unit (IU). The culture filtrates of the cultures of the cyanobacterial strains were also scanned in the UV-VIS range using a Specord model spectrophotometer, and the peaks were recorded for the identification of novel compounds.

Measurement of hydrolytic enzymes

Chitosanase activity

The cell-free culture filtrates were analyzed for chitosanase activity (EC 3.2.1.4) by the spectrophotometric method using glycol chitosan as the assay substrate. One unit of chitosanase activity was defined as µsmoles of N-Acetyl glucosamine released per min under assay conditions (Chang et al., 2007).

Protease activity

Protease activity was assayed using casein as a substrate and measured by the liberation of tyrosine from the incubation mixture (Thangam and Rajkumar, 2002). The intensity of the blue colour was measured at 660 nm using a spectrophotometer against the standard curve of Tyrosine and expressed as an International unit (IU), which was calculated as µmoles of tyrosine liberated min–1.

Filter paperase/FPase (exo-β-1, 4-glucanase; EC 3.2.1.91) and CMCase activity (EC 3.2.1.4)

The enzyme activities of both enzymes were assayed spectrophotometrically using filter paper and carboxymethyl cellulose as substrates, respectively (Ezeilo et al., 2022). Reducing sugars liberated were estimated at 575 nm against the standard curve of Glucose. One unit of enzyme represents I µmoles of glucose liberated per ml of culture filtrate per min.

Xylanase activity

The xylanase activity was measured spectrophotometrically at 575 nm using xylan as substrate (Lim et al., 2013)

against the standard curve of xylose. One unit of xylanase activity represents I µmole of xylose liberated per ml of culture filtrate per min.

Cellobiase (β-D-glucosidase; EC 3.2.1.21) and Cellobiohydrolases activity

The cellobiase and cellobiohydrolases activity was determined spectrophotometrically at 430 nm by the method outlined by (Wood and Bhat, 1988) against the standard curve of p-nitrophenol.

Statistical analysis

Analyses were performed in at least three independent experiments. Analysis of variance (ANOVA) was applied to verify whether means from independent experiments within each given variant were significant at level $p < 0.05$. Data shown in the figures are means and standard errors (s.e.), using asterisks to present the significant differences.

Results

Antifungal activity of two cyanobacteria strains

Preliminary analyses of the culture filtrates of two cyanobacterial strains against the selected phytopathogenic fungi (as measured by disc diffusion assay) revealed that both strains produced a zone of inhibition of varying diameter, ranging from 20-35 mm at *Desmonostoc alborizicum* and ranging from 32-61 mm at *Neowestiellopsis persica* A1387.

Neowestiellopsis persica was observed to be highly potent in terms of its fungicidal activity, as it produced a much larger zone of inhibition than *Desmonostoc alborizicum*.

The largest and smallest diameter of the zone of inhibition, 61.66 ± 0.6 and 32.07 ± 0.8 mm, were recorded by *Neowestiellopsis persica* against *Macrophomina phaseolina* and *Phytophthora capsici*, respectively. In contrast, the largest and smallest diameter of the zone of inhibition, 35.71 ± 0.7 and 20.01 ± 0.3 mm, were recorded by *Desmonostoc alborizicum* against *Fusarium solani* and *Macrophomina phaseolina*, respectively.

IAA (indole acetic acid) hormone and soluble protein content

According to Table 2, there was a significant difference in the amount of IAA hormone and the content of soluble protein in the extracts of cyanobacteria *Neowestiellopsis persica* A1387 and *Desmonostoc alborizicum* ($p<0.05$). IAA production and soluble protein were also observed in both cyanobacterial strains, which was higher in *Desmonostoc alborizicum* in comparison to *Neowestiellopsis persica* A1387.

Results of hydrolytic enzymes

According to Table 3, there was a significant difference ($p<0.05$) in the two cyanobacterial extracts of *Neowest-*

Table 1. Fungicidal activity of the purified extract of *Neowestiellopsis persica* A1387 and *Desmonostoc alborizicum* in terms of zone of inhibition. Values indicate the diameter of inhibition zone in mm. The results are Means ± SE.

Tabela 1. Fungicidna aktivnost prečiščenih ekstraktov cianobakterija *Neowestiellopsis persica* A1387, *Desmonostoc alborizicum* in cone inhibicij. Vrednosti predstavljajo premer cone inhibicije v mm. Rezultati so povprečne vrednosti ±SN.

	zones of growth inhibition (mm)	
Fungal strain	***Neowestiellopsis persica***	***Desmonostoc alborizicum***
Alternaria alternata	28.57±7.0^{b}	43.07±7.0^{b}
Fusarium solani	35.71±7.0^{d}	33.57±8.0^{a}
Fusarium oxysporum	33.16±8.0^{c}	45.57±8.0^{c}
Macrophomina phaseolina	20.01±3.0^{a}	61.66±6.0^{d}
Verticillium dahliae	29.11±7.0^{b}	41.91±7.0^{b}
Phytophthora capsici	31.57±8.0^{c}	32.07±8.0^{a}

iellopsis persica A1387 and *Desmonostoc alborizicum*. Protease, FPase, and Xylanase enzyme activity were significantly higher in the cyanobacterial extract of *Desmonostoc alborizicum* ($p<0.05$).

While CMCase (1.55 fold), cellobiohydrolases (1.85 fold), cellobiase (1.63 fold), and chitosanase activity (4.21) was significantly higher in *Neowestiellopsis persica* A1387 cyanobacterial extract ($p<0.05$) in comparison to *Desmonostoc alborizicum*.

Totally, protease and chitosanase activity were significantly higher in *Desmonostoc alborizicum* and *Neowestiellopsis persica* A1387, respectively.

Table 2. Mean results of IAA production (µmol/g) and protein accumulation (mg/g) in *Neowestiellopsis persica* A1387 and *Desmonostoc alborizicum*. The results are Means ± SE. Different letters indicate a significant difference at the 0.05 level.

Tabela 2. Povprečne vrednosti za sintezo IAA (µmol/g) in akumulacijo proteinov (mg/g) pri cianobakterijah *Neowestiellopsis persica* A1387 in *Desmonostoc alborizicum*. Rezultati so povprečne vrednosti ±SN. Različne črke predstavljajo statistično značilno razliko pri $p < 0,05$.

	Neowestiellopsis persica	*Desmonostoc alborizicum*
IAA (indole acetic acid) hormone (µmol/g)	40.78 ± 1.03^{b}	60.63 ± 0.98^{a}
soluble protein content (mg/g)	0.22 ± 0.00^{b}	0.25 ± 0.00^{a}

Table 3. Screening the activity of selected hydrolytic enzymes of *Neowestiellopsis persica* A1387, *Desmonostoc alborizicum*. The results are Means ± SE. Different letters indicate a significant difference at the 0.05 level.

Tabela 3. Pregled aktivnosti izbranih hidrolitičnih encimov pri cianobakterijah *Neowestiellopsis persica* A1387 in *Desmonostoc alborizicum*. Rezultati so povprečne vrednosti ±SN. Različne črke predstavljajo statistično značilno razliko pri $p < 0,05$.

	***Neowestiellopsis persica* A1387**	***Desmonostoc alborizicum***
Protease activity (mg/g)	5.60 ± 0.13^{a}	4.27 ± 0.01^{b}
Filter papers activity (µmol/hour mg)	2.59 ± 0.10^{a}	2.49 ± 0.20^{b}
CMCase activity (µmol/hour mg)	0.47 ± 0.10^{b}	0.76 ± 0.10^{a}
Cellobiohydrolases activity (µmol/hour mg)	4.69 ± 0.14^{b}	8.70 ± 0.24^{a}
Cellobiase activity (mg/g)	2.99 ± 0.00^{b}	4.88 ± 0.16^{a}
Xylanase activity (mg/g)	3.70 ± 0.12^{a}	2.85 ± 0.50^{b}
Chitosanase activity (mg/g)	1.79 ± 0.23^{b}	7.54 ± 0.65^{a}

Discussion

Cyanobacteria are known to be an important determinant of allelopathic activity, besides leading to severe monetary losses as a result of soil-borne pathogens. Extensive screening programs for bioactive compounds in cyanobacteria have revealed the presence of unique peptides with cyclic structures (cyanopeptolins, depsipeptides), besides linear peptides isolated from a number of strains belonging to genera *Microcystis*, *Nostoc* and *Anabaena* (Welker and Von Döhren, 2006). The analyses of the culture filtrates are currently in progress, as UV scans revealed the presence of novel compounds. Although toxins were the main subject of research on toxic cyanobacterial blooms, the isolation of bioactive compounds other than biotoxins has now been recently stressed (Anderson et al., 2021).

The production of phytohormones, earlier considered as a trait of the plant kingdom, is also widespread among soil and plant-associated prokaryotes (Sergeeva et al., 2002), especially those involved in plant-microbe symbiotic or associative interactions or plant pathogenesis. Microorganisms inhabiting the rhizosphere of various plants are likely to synthesize and release auxin as a secondary metabolite because of rich supplies of substrates exuded from the roots compared with non-rhizosphere soils (Sarwar and Kremer, 1995) (Sureshbabu et al., 2016).

The result of this study showed that the *Desmonostoc*

alborizicum strain exhibited 1.43 fold higher IAA production than *Neowestiellopsis persica* A1387. In contrast, *Neowestiellopsis persica* A1387 has more effect on interactions with the fungal pathogens.

It has been suggested up to 80% of bacteria isolated from the rhizosphere can produce IAA. The capacity for IAA biosynthesis was found in the representatives of free-living and symbiotic cyanobacteria of the genera *Nostoc*, *Chlorogloeopsis, Calothrix, Plectonema, Gloeothece, Anabaena, Cylindrospermum* and *Anabaenopsis* (Tsavkelova et al., 2006).

Cyanobacteria produce a variety of enzymes (chitosanase, protease, xylanase, and cellobiase) with antifungal activity and twenty-four families of protease inhibitors involved in several human, animal, and plant metabolic pathways (Righini et al., 2022). The culture filtrates are rich in many substances that can display interesting antifungal activity. In particular, several families of metabolites with high antimicrobial activity were isolated from various strains of cyanobacteria (Demay et al., 2019). Culture filtrates of several cyanobacteria species with fungicidal activity produced one or more hydrolytic enzymes, such as proteases, chitosanases, exo-β-1,4-glucanases, and carboxy-methyl cellulase (Righini et al., 2022). For *Anabaena variabilis* ATCC 29413, the gene putatively responsible for chitosanase and antifungal activities was attributed to the glycoside hydrolase 3-like family (Gupta et al., 2010).

All these enzymes are known to be involved in the digestion of fungal or oomycetes cells. For example, chitosan and chitosan-glucan complexes were found in the mycelia of *Aspergillus niger* and *Fusarium moniliforme* (Razak et al., 2018). The use of organic solvents in the extraction process affects the antifungal activity of cyanobacterial extracts (Abedin and Taha, 2008). For example, in the extract of *Microcystis* aeruginosa obtained with diethyl ether were identified the butylated hydroxytoluene and methyl ester of hexadecanoic acid, which has antifungal activity against *Aspergillus* spp., *Fusarium* spp. and *Penicillium* sp. (Deyab et al., 2019). Another important antifungal activity against *A. flavus* was observed in the methanol extract of *Anabaena* spp., *Nostoc* sp. and *Scytonema* sp. The identification of the macrolide scytophycin and the presence of the glycolipopeptide hassallidin extracted from the *Anabaena* strains elucidated the antifungal activity (Shishido et al., 2015).

Phenols and polysaccharides contained in extracts from *Nostoc* spp. are involved in the antifungal activity against *R. solani* (Ismail and Ismail, 2011). In fact, a phenolic compound was isolated and purified from the chloroform extract of *Nostoc muscorum* with strong activity against *Aspergillus niger, A. flavus, Penicillium* sp., and *Fusarium microsporium* (Righini et al., 2022). Aqueous extracts have not been so widely investigated as the extracts obtained with organic solvents, even though they are safer for both humans and the environment. A recent study showed that soluble polysaccharides extracted from *Anabaena minutissima* aqueous extract reduced both colony growth and colony-forming units of *B. cinerea* (Righini et al., 2022). In the same extract, proteins, phycobiliproteins, chlorophylls, carotenoids, and antioxidant activities were also determined and correlated with the antifungal effect against the pathogenic fungus *Podosphaera xanthii* on cucumber detached cotyledons in vitro assay (Pérez-García et al., 2009).

On analyzing the overall performance of the strains in terms of biocontrol traits, strain *Neowestiellopsis persica* A1387 was observed to be the top-ranked strain in terms of diameter of zone of inhibition, CMCase activity, cellobiase, cellobiohydrolase and chitosanase activity, while was top-ranked in terms of cellobiohydrolase activity (8.70±0.2), besides exhibiting high levels of activity of other hydrolytic enzymes and inhibited potentially more the growth of fungi tested. While strain *Desmonostoc alborizicum* has more activity in protease activity, FPase (exo-β-1, 4-glucanase, xylanase activity, moreover the amount of total proteins and IAA activity was higher than *Neowestiellopsis persica* A1387.

Majidi et al., 2011, found maximum cellulase activity for *S. variabilis, K. rosea* and *S. maltophilia* was obtained after 72 h of fermentation with 0.091, 0.089 and 0.084 U mL^{-1} for CMCase and 0.079, 0.074 and 0.072 U mL^{-1} for FPase respectively (Samira et al., 2011). These results are in agreement with those of (Narasimha et al., 2006, and Niranjane et al., 2007), who found that carboxymethyl cellulose was the best carbon source, followed by cellulose for cellulase production. Higher production of cellulase when CMC serves as the substrate may be a result of the induction of the enzyme since cellulose is known to be a universal inducer of cellulase synthesis. The growth profile of the bacterial isolates during fermentation shows that the cellulase was being produced during the growth phase of the *S. variabilis, K. rosea* and *S. maltophilia* (Barzkar and Sohail, 2020).

The excretion of hydrolytic enzymes is known to be a common trait of plant pathogens/symbionts, which promotes a closer association with plant roots/target organisms and improves the stability of such associations. Chitosanases are known to selectively degrade chitosan/chitin by hydrolysis of the β-1, 4-glycosidic bonds that link N-acetyl glucosamine residues of chitin and form the basis for antifungal activity. The chitosanase/chitinase produced by *S. plymuthica* C48 inhibited spore germination and germ-tube elongation in *Botrytis cinerea*. The ability to produce extracellular chitosanases/chitinase is considered crucial for *Serratia marcescens* to act as an antagonist against *Sclerotium rolfsii* and for *Paenibacillus* sp. Strain 300 and *Streptomyces* sp. strain 385 to suppress *Fusarium oxysporum* f.sp. *cucumerinum* (Chaudhary et al., 2013). Although the role of cyanotoxins/peptides/phenolic molecules cannot be ruled out in terms of biocidal activity, it becomes evident from this study that hydrolytic enzymes are definitely contributing to the inhibition of phytopathogenic fungi.

Conclusions

The present investigation, for the first time, illustrates the activity of hydrolytic enzymes in two native cyanobacteria strains and their possible role in biocidal activity against phytopathogenic fungi. The potential of these strains in developing biocontrol agents is immense – as these strains possess abilities for the production of IAA and hydrolytic enzymes. Such multifaceted strains would possess a competitive edge over other rhizosphere microflora against phytopathogenic fungi and need to be explored for developing biocontrol agents.

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