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Evaluation of bioactivities of the bacterial strain *Bacillus velezensis* B26

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ABSTRACT

Bacillus species are widely recognized as effective biocontrol agents utilized for suppressing plant pathogens. These species isolated from diverse habitats exhibit antifungal activity against several plant pathogens. Although complete genome sequences of several *Bacillus* species have been reported, exploration of the bioactivities of the *Bacillus velezensis* strains remains limited. In this study, we aimed to assess the bioactivities exhibited by the bacterial strain *B. velezensis* B26. Through our screening process, we identified *B. velezensis* B26 as a producer of chondroitinase enzyme, additionally demonstrating chitinase, glucanase, amylase, and gelatinase activities. Significantly, we unveil the potent antifungal capabilities of the *B. velezensis* B26 against opportunistic fungal pathogen *Candida albicans* and emerging fungal pathogen *Saccharomyces cerevisiae.*

INTRODUCTION

The most common glycosaminoglycans (GAGs) include chondroitin sulphate (CS), dermatan sulphate, heparan sulphate, and keratin sulphate (KS). GAGs are large polysaccharides, consisting of repeating disaccharide units of amino sugar either N-acetyl D-galactosamine (D-GalNAc) or N-acetyl D-glucosamine (D-GlcNAc), and uronic acid, either glucuronic acid or iduronic acid. KS consists of galactose instead of uronic acid [1–3]. CS proteoglycans (CSPGs) consist of core proteins covalently bonded to CS-GAG. CSPGs abundant in the extracellular matrix, play roles in neurological diseases. Elevated CSPGs impede repair and support cancer progression. Chondroitinase ABC, a bacterial enzyme from *Proteus vulgaris*, offers a promising approach to degrade CSPGs and address associated conditions [1,2,4–8]. In this study, for the first time, we have identified a bacterial strain *Bacillus velezensis* B26 which produces chondroitinase enzyme. In addition to chondroitinase activity, *B. velezensis* B26 strain exhibited chitinase, glucanase, amylase, and gelatinase activities. Chitinases enzymes belonging to a diverse group, are capable of digesting large chitin polymers into their constituent acetyl glucosamine units. These enzymes exhibit variability in their catalytic mechanisms, structures, and substrate specificities [9]. Several bacterial isolates have been identified as chitinase producers, underscoring the significant potential of chitin degradation in diverse applications such as waste management, biocontrol, and even osteoarthritis treatment [10]. Glucanases are hydrolytic enzymes that degrade the polysaccharide chains of glucans. They are primarily classified based on the specific

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linkage they target, including β-1,3, β-1,4, or α-1,3 glucanases. Bacterial glucanases hold immense biotechnological importance, finding extensive applications in animal feed and the brewing industry [11,12].

Even though fungal diseases affect a significant population worldwide that is estimated to be around 1 billion annually, there is a general lack of awareness with respect to the threat posed by them in public [13]. An increase in co-morbid conditions such as cancer, AIDS, and diabetes also leads to higher susceptibility to fungal infections. Overall, *Candida spp*. is one of the most prevalent fungal pathogens causing both superficial and invasive diseases. With the limited number of drug classes available and the rising emergence of infections, there is an urgent necessity to start exploring novel targets against the pathogens [14]. In this research work, for the first time, we have identified that *B. velezensis* B26 strain exhibits several enzymatic activities, and importantly showed antifungal activity against *Candida albicans (C. albicans)* and *Saccharomyces cerevisiae (S. cerevisiae)*.

MATERIALS AND METHODS

Materials

Nutrient Broth, NB (M002-100G), Luria Bertani broth, LB (M1725-500G), Sabouraud Dextrose Broth (MH033-100G), MR-VP broth (M070S-100G), Simmon's Citrate Agar ((M099-100G), Urea Broth base (M111-100G), Meat Extract (RM002-500G), Skim Milk Agar (M763-500G), SIM medium (M181-500G), Agar powder (GRM026P-500G), CS A (TC040-5G), Bovine Serum Albumin Factor V (TC194-5G), Glucose (TC130-100G), Gelatin (TC041-100G), Starch (GRM198-500G), and N-acetyl D glucosamine (NAG) (TC081-10G), carboxy methyl cellulose (CMC) (MB138-100G), and Chitin (GRM1356-100G) all the compounds listed were purchased from HiMedia Laboratories, India.

Starch agar plate (Starch agar medium-2.5 g in 100 ml distilled water); Nutrient gelatin medium (Peptone-0.5 g, Beef extract-0.3 g, Gelatin-12 g, andAgar-1.5 g in 100 ml distilled water); Skim milk agar (Tryptone-5 g, Yeast extract-2.5 g, Glucose-1 g, SM powder-28 g, and Agar-15 g in 1,000 ml purified/distilled water).

Media composition

Colloidal chitin minimal salt (CCMS) (Colloidal chitin-10 g, yeast extract-1 g, Ammonium sulphate-2.5 g, Dipotassium phosphate-0.7 g, Monopotassium phosphate-0.3 g, Magnesium sulphate pentahydrate-0.5 g, sodium chloride-1 g, ferrous sulphate heptahydrate-0.01 g, zinc sulphate-0.001 g, manganese (III) phosphate-0.001 g, Calcium chloride-0.4 g, Urea; 0.3 g, Agar; 20 g and finally pH is adjusted to 7 [15].

Screening for identification of chondroitinase-producing microorganism

Soil specimens from a depth of 3–5 cm of the surface, were collected from 2 locations, one chicken poultry farm and one chicken carcass, from Manipal, Udupi (Location: 13.325922, 74.804554) using sterile tubes. Sample collected from chicken poultry farm labeled as CPF-I. Three samples were collected from chicken carcasses named as CHCS – I, CHCS – II, and CHCS – III. Characteristics of all the soil samples were observed.

Primary screening

Soil samples (1 gram) were screened for organisms capable of utilizing CS based on the rapid plate method [16]. The soil in poultry farm (CPF-I) is powdery, and thus it was directly sprinkled on nutrient agar containing 0.04% w/v CS (filter sterilized with 0.22 µ filter). The three soil samples collected from chicken carcass (CHCS – I, II, III) were suspended in 100 ml sterile water and shaken well for 30 minutes. Further samples were serially diluted and added 1 ml to 18 ml nutrient agar containing 0.04% w/v filter sterilized CS (CNA). All these plates were incubated at 37°C for 24 hours. Each isolate was streaked separately on the surface of CNA containing 0.04% w/v of CS and 1% w/v bovine serum albumin fraction V. The plates were incubated for 3 days at 37°C. Glacial acetic acid (2N) was added to each of the plates and incubated for 10 minutes. The formation of a clear zone around the colony indicates the breakdown of CS, indicating chondroitinase-producing organism. The isolates giving the largest clear zones were selected for the production of chondroitinase. Gram staining was carried out for all the positive isolates.

Identification of the isolate

The identification of isolates was performed at the sequencing facility of the National Centre for Microbial Resource (NCMR), National Centre for Cell Science, Pune. Genomic DNA was extracted using the standard phenol/chloroform extraction method [17], followed by PCR amplification of the 16S rRNA gene using universal primers 16F27 [5'-CCA GAG TTT GAT CMT GGC TCA G-3'] and 16R1492 [5'-TAC GGY TAC CTT GTT ACG ACT T-3']. The resulting amplification product was purified through PEG-NaCl precipitation and then directly sequenced using ABI® 3730XL automated DNA sequencer (Applied Biosystems, Inc., Foster City, CA) in accordance with the manufacturer's instructions. Importantly, sequencing was performed bi-directionally to ensure that each position was read at least twice. The assembly of the sequence was performed using a Lasergene package followed by a comparison to sequences from the type material using NCBI BLAST for tentative identification. The amplified gene was sequenced and the identification was performed by using EzBioCloud Database, with the confidence in identification restricted by both the availability and the extent of homology shown by the ~1,200 bp sequence of the sample in comparison to its closest neighbor in the database. The sequencing and identification were performed by NCMR-NCCS Pune, India [18].

Enzymatic activities

Chondroitinase enzyme activity assay

The enzyme activity was assessed by its ability to lyse chondroitin-4-sulphate to produce unsaturated disaccharides. Overnight grown primary culture (*Bacillus velezensis* B26 strain) was inoculated in the secondary medium, grown

overnight at 37ºC for 48 hours in nutrient broth with 0.05% CS, and the colony count was adjusted to 1 × 10^8 cfu/ml using a 0.5 McFarland solution for the collection of bacterial supernatants. Briefly, varying concentrations of bacterial cell-free supernatant with chondroitinase enzyme and substrate were incubated in 20 mM Tris-HCl buffer, pH 7.5, at 37°C and the relative amount of unsaturated disaccharides was recorded by using spectrophotometer (BioTek Synergy H1 Multimode Reader, Agilent) (UV 232 nm) for 20 minutes at 37ºC. In this assay, EmM, the millimolar absorption coefficient of unsaturated disaccharides 5.1 L/(mol·cm) was used in the calculations. One international unit is defined as the amount of protein needed to form 1 µmol of 4,5-unsaturated uronic acid/minute [19]. Enzyme activity (Units/ml) = (ΔA232nm/minute test)/[(EmM) (0.1)]

Amylase activity

Bacterial cells were spotted on the starch agar plate. The plate was incubated at 37°C for 48 hours following which the plate was flooded with Gram's iodine using a dropper to check for a zone of clearance around the colony [20,21].

Caseinase activity

The organism was spotted on a skim milk agar plate and incubated at 37°C for 24 hours in order to observe a zone of clearance around the colony [22].

Gelatinase activity

The organism from the 24-hour-old culture broth was stab inoculated into the nutrient gelatin medium and incubated at 37°C. After every 24 hours, the test tube was kept in the refrigerator (4-8°C) for 20 minutes and then checked for liquefaction of the medium [20,21].

Chitinase assay and glucanase assay

Preparation of colloidal chitin, chitinase, and glucanase assay was performed as reported [15,23].

Preparation of colloidal chitin

Chitin (5 g) was mixed thoroughly in 50 ml of concentrated HCL by stirring for 30 to 50 minutes. Chitin colloidal suspension was prepared by mixing with 500 ml of ice-cold water with continuous stirring. The colloidal chitin (CC) obtained was filtered using muslin cloth and washed repeatedly until the suspension pH was 6.5. The collected CC was air-dried and stored at 4ºC [15].

Chitinase assay and glucanase assay

Overnight grown primary culture (*Bacillus velezensis* B26 strain) was inoculated in the secondary medium (CCMS broth) for chitinase production and CCMS broth with CMC (1%) for glucanase production. The cells were maintained at 37ºC and 150 rpm. Samples were collected after 24-hour and enzyme activity was performed by the DNS method. For chitinase enzyme activity, soluble fractions obtained from the bacterial cells were incubated with 1%CC as a substrate and the released glucose molecule was monitored by measuring absorbance at 450 nm. Similarly, for glucanase enzyme activity, soluble fraction obtained from the bacterial cells was incubated with 1% CMC as a substrate and the released glucose molecule was monitored by measuring absorbance at 450 nm. One unit of enzyme was defined as the amount of enzyme required to release 1 µM glucose per minute at 37˚C.

Growth curve

A 24-hour culture broth was inoculated in a fresh LB broth to prepare 0.5 McFarland equivalent broth culture. 1 ml of this culture was added to 150 ml of fresh sterile LB broth and incubated at 37°C at 150 rpm. Samples were taken at regular intervals in order to measure their optical density values at 540 nm. A fresh LB broth was used as blank. The samples were taken at 1, 2, 4, 8, 12, 24, 48 and 60 hours, respectively. The assay was done twice and an average of the optical density values were recorded. A graph was plotted with sampling time on the X-axis and optical density on the Y-axis, with the values corresponding to their respective sampling times.

Bacterial cell-free supernatant preparation

Broth cultures were centrifuged at 6,000 rpm for 10 minutes following which the supernatant was collected and cell debris was discarded. The supernatant was then filter sterilized (0.22 µm) to remove any further remaining bacteria. The filtered supernatant was stored at −20ºC until used further.

Antifungal activity

The cell-free supernatant was tested for its antifungal activity against fungi using the cup plate method [24]. The overnight cultures of *C. albicans* and *S. cerevisiae* were adjusted to 0.5 Mc Farland and were spread on SDA plates. A borer of 6 mm diameter was used to create 4 wells in the plates. 50 µl of bacterial cell-free supernatant were collected and added to the wells after 1 hour, 6 hours, and 24 hours of incubation, respectively. Additionally, 50 µl of Amphotericin B (100 µg/ml) was added to the fourth well as a control.

0.5 McFarland equivalent suspension of *C. albicans* and *S. cerevisiae* in SDB were swabbed individually over SDA plates. Subsequently, following which four wells were made using a sterile cork borer in each plate. 50 µl of cell-free supernatant collected after 1, 6, and 24 hours of incubation were added into the wells of each respective plate. As a control, 50 ul of Amphotericin B (100 µg/ml) was added to one well on every plate. The plates were refrigerated for 30 minutes for the diffusion of suspensions into the media and then incubated at 28ºC for 24 hours.

RESULTS

Isolation and identification of chondroitinase-producing *B. velezensis B26* strain from soil samples

The four soil samples, namely CPF-I, CHCS-I, CHCS-II, and CHCS-III were collected from Manipal, Udupi, Karnataka, India. Out of these samples, only 29 morphologically distinct bacterial isolates were obtained using enrichment media. These 29 isolates were checked for chondroitinase production using the rapid plate method [16]. Among these, 8 isolates exhibited clear zones around their growth (Fig. 1a), identifying them as chondroitinase-producing bacterial isolates. The prominent chondroitinase-

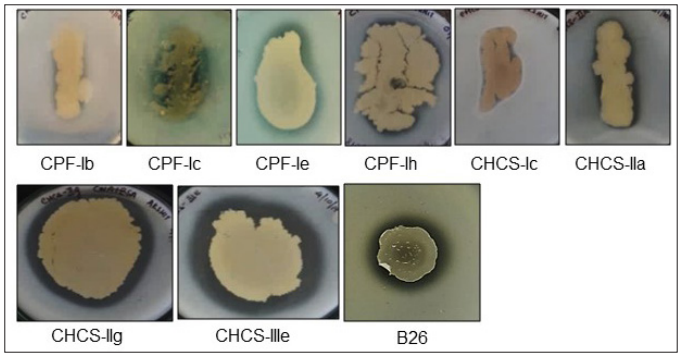

Figure 1. Identification of chondroitinase-producing bacterial isolates from soil samples.
a. Among the four samples-CPF-I, CHCS-I, CHCS-II, and CHCS-III, 8 isolates were found to be producing clear zones around their growth areas. This clear zone serves as an indicator of the chondrotinase production. The isolates were cultured on nutrient agar supplemented with 0.04% w/v of CS and 1% w/v bovine serum albumin, followed by incubation at 37ºC for 3–4 days.

Table 1. Chondroitinase enzyme activity of *B. velezensis* B26 strain.

SL.no.	A 0 (UV 232 nm absorbance at 0 minute)	A 20 (UV 232 nm absorbance at 20 minute)	Chondroitinase enzyme activity (U/ml)
1	0.269	0.419	0.17
2	0.139	0.601	0.25
3	0.139	0.605	0.25
4	0.147	0.782	0.27

producing capacity was noticed in the CHCS-IIIe isolate among the 8 isolates, further it was selected for investigation. The isolated CHSCS-IIIe strain was carefully purified by multiple streaking, and individually isolated colonies were designated as B26. This strain, B26, was further subjected to confirm its chondroitinase-producing potential, as evidenced by the presence of a clear zone around the cell growth area (Fig. 1a). B26 cells were grown in nutrient broth supplemented with 0.04% w/v CS at 37ºC for 48 hours. Subsequently, cells were harvested and collected supernatant was subjected to enzyme assay to estimate the amount chondroitinase present in the extracellular soluble fraction. The average activity chondroitinase enzyme activity was found to be 0.235 ± 0.04 U/ml (Table 1). To identify the nature of the organism, gram staining, and biochemical tests were performed (Supplementary Fig. S1, S2; Supplementary Table. S1). To reveal its taxonomic identity, the 16s rRNA gene sequence of the isolated strain was analyzed and identified. The result revealed that the identified strain exhibited 100% similarity with *B. velezensis* CR-502(T) (Accession no. AY603658) (Table 2). Thus, the strain B26 was identified as *B. velezensis*.

Bacillus velezensis B26 exhibits enzymatic activity. To assess the enzymatic activity of *B. velezensis* B26 cells, we performed an amylase assay using a starch agar medium. The organism was introduced at the center of a starch agar plate and allowed to incubate at 37°C for 48 hours. Subsequently, the plate was flooded with iodine solution. The appearance of a distinct clear zone around the colony served as evidence of the occurrence of starch hydrolysis and the consequent production of the amylase enzyme (Fig. 2a). Similarly, the evaluation of

Table 2. The identification of bacterial sample B26 through analysis of its 16S rRNA gene sequencing.

PRN	Strain No.	Closest neighbour*		% Similarity
		Taxonomic designation	Accession No.	
B_APR_21_003	B26	*Bacillus velezensis* CR-502(T)	AY603658	100

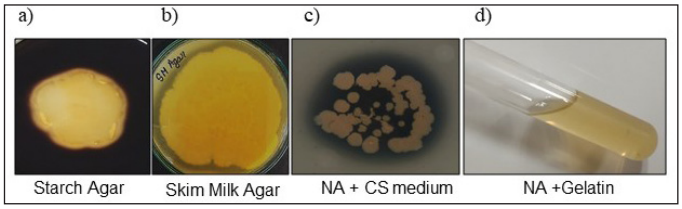

Figure 2. *Bacillus velezensis* B26 cells exhibit enzymatic activities. *Bacillus velezensis* B26 cells were introduced at the center region of different agar plates: a. Starch agar plate, b. Skim milk agar plate, and c. Nutrient Agar plate supplemented with 1% w/v bovine serum albumin and 0.04% w/v CS. Followed by incubation at 37°C for 48 hours, clear zones around the colony growth signifying starch hydrolysis by (a) amylase enzyme, (b) proteolytic enzyme, and (c) chondroitinase activity, (d) For the evaluation of gelatinase activity, *B. velezensis* B26 cells were stab cultured in a test tube with nutrient agar containing gelatin and incubated at 37°C. After 48 hours of incubation, the nutrient gelatin medium had undergone liquefaction, indicating gelatinase enzyme production.

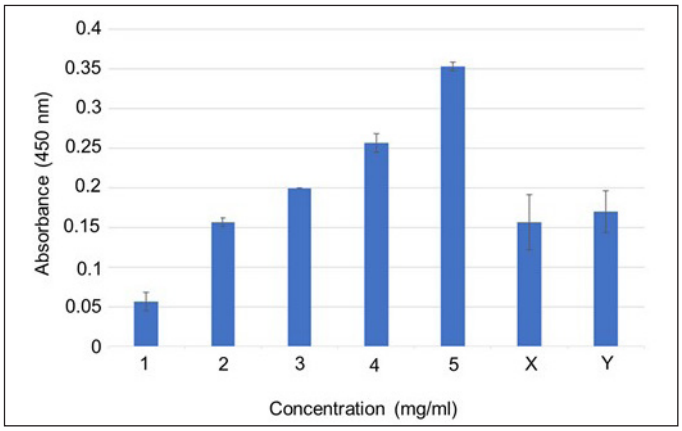

Figure 3. Estimation of chitinase and glucanase enzyme activities. For chitinase enzyme activity, soluble fractions obtained from the bacterial cells were incubated with colloidal chitin as a substrate and the released glucose molecule was monitored by measuring absorbance at 450 nm. Similarly, for glucanase enzyme activity, soluble fraction obtained from the bacterial cells was incubated with carboxymethyl cellulose as a substrate and the released glucose molecule was monitored by measuring absorbance at 450 nm. Varying glucose concentrations are employed to plot the standard curve. X-axis represents glucose concentration and Y-axis indicates absorbance at 450 nm. Chitinase and glucanase activity is extrapolated from the standard plot. X indicates supernatant obtained from the bacterial cells incubated with colloidal chitin substrate, and Y represents supernatant obtained from the bacterial cells incubated with carboxymethylcellulose.

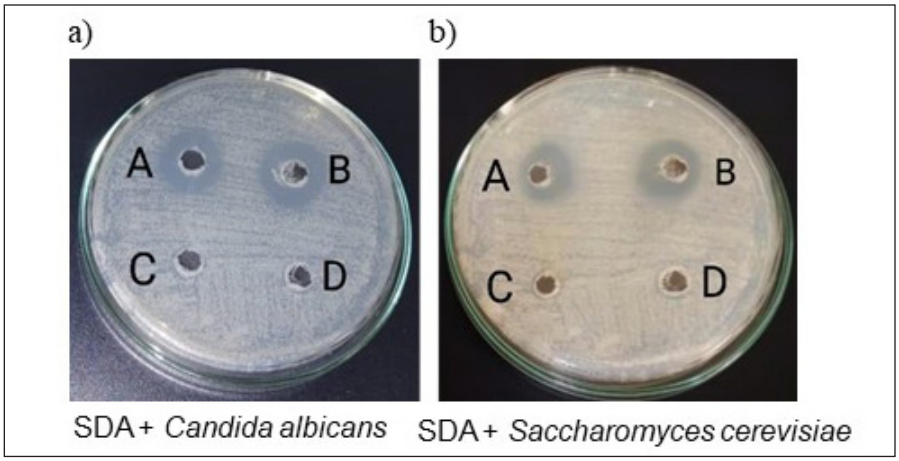

Figure 4. Antifungal potential of *B. velezensis* B26 extract. (a) 0.5 McFarland equivalent suspension of *C. albicans* and (b) *S. cerevisiae* in SDB were swabbed individually over SDA plates. 50 ul of Amphotericin B (100 µg/ml) was added to one well on every plate (Control). Well A. Amphotericin B (100 **µ**g/ml)—50 **µ**l, Well B. 6-hour supernatant sample—50 **µ**l, Well C. 1-hour supernatant sample—50 **µ**l, Well D—24-hour supernatant sample—50 **µ**l. Following the incubation period, a significant zone of clearance was visibly discerned around both the 6-hour incubated supernatant well and the control well, indicating potential antifungal activity.

Table 3. *Bacillus velezensis* B26 enzyme activity.

S. No.	Enzyme assay	Substrate	Enzyme activity Experiment-1 (U/ml)	Enzyme activity Experiment-2 (U/ml)	Enzyme activity Experiment-3 (U/ml)	Enzyme activity-Average
1.	Chitinase	Colloidal chitin	1.1	0.8	1.3	1.06 ± 0.25 U/ml
2.	Glucanase	Carboxymethylcellulose	1.5	1.2	1.2	1.3 ± 0.17 U/ml

proteolytic activity was conducted through the skim milk agar method. A clear zone around the colony growth suggests that *B. velezensis* B26 exhibited proteolytic activity (Fig. 2b). In the context of chondroitinase activity, the *B. velezensis* B26 strain was assessed by introducing it into a medium composed of nutrient agar supplemented with 1% w/v bovine serum albumin and 0.04% w/v CS. The appearance of a clear zone around the colony validated the production of chondroitinase (Fig. 2c). Additionally, an evaluation of gelatinase activity was carried out by observing gelatin medium liquefaction. The selected bacterial cells were stab cultured into the nutrient agar with gelatin and liquefaction of the medium was observed, indicating the gelatinase enzyme production (Fig. 2d). Additionally, for the evaluation of chitinase and glucanase activities, the DNS method was employed [15,23]. In this method, B26 cells were harvested and the collected supernatant was subjected to enzyme assay to estimate the amount of enzymes present in the extracellular soluble fraction. Optical density was measured to calculate the content of sugar present in the sample after its incubation of soluble fraction with the colloidal chitin and carboxymethyl cellulose substrates for chitinase and glucanase assays, respectively. By correlating the optical density of the samples with the standard curve established using standardized glucose concentrations (Fig. 3). Enzyme quantities within the samples were estimated to be 1.06 ± 0.25 U/ml for chitinase and 1.3 ± 0.17 U/ml for glucanase (Table 3). Enzyme activity (µmol/minute ml) or (U/ml) = (Concentration of product of the reaction) (µmol /ml) ×Total Reaction Volume (ml) / [(Reaction time (minute)) × (Enzyme volume(ml))].

Table 4. Antifungal activity of *B. velezensis* B26 against *C. albicans*.

	Diameters of zone of inhibition (mm)			
Samples	**Amphotericin B**	**Supernatant (1 hour)**	**Supernatant (6 hour)**	**Supernatant (24 hour)**
Set-1	20	–	15.66	–
Set-2	21.33	–	16.66	–
Average	20.6 ± 0.66	–	16.16 ± 0.50	–

Bacillus velezensis B26 extract exhibits antifungal activity

To assess the antifungal efficacy of the *B. velezensis* cells, a sample was collected during the lag phase, log phase, and stationary phase (Supplementary Table. S2). The samples were subjected to an antifungal assay against *C. albicans* and *S. cerevisiae* (which serve as commonly employed model fungal organisms for experimentation). While no discernible inhibition zones were noted for the samples from the lag and stationary phases, a clear inhibition zone emerged against both fungal organisms in the log phase sample (Fig. 4a, b, and Table 4 and 5).

Table 5. Antifungal activity of *B. velezensis* B26 against *S. cerevisiae.*

Samples	Diameters of zone of inhibition (mm)			
	Amphotericin B	Supernatant (1 hour)	Supernatant (6 hour)	Supernatant (24 hour)
Set-1	19.33	–	20.66	–
Set-2	15.66	–	16.67	–
Average	14.49 ± 2.5	–	18.66 ± 2.8	–

This observation suggests the production of the antifungal component occurs during the intense multiplication stage of cellular growth.

DISCUSSION

In this study, we show that *B. velezensis* B26 strain isolated from soil samples exhibited enzymatic and antifungal activity against *C. albicans,* a predominant human fungal pathogen, as well as *S. cerevisiae*, an emerging opportunistic pathogen.

Previous studies have isolated chondroitinase-producing bacteria from different aquatic and terrestrial environments using different screening methods [25,26]. However, our study for the first time reported isolation of a chondroitinase-producing *B. velezensis* B26 strain from soil samples. This finding is significant because chondroitinase enzyme exhibits therapeutic potential against several pathological processes [6]. Furthermore, our study revealed that *B. velezensis* B26 strain exhibits several ranges of enzymatic activities including amylase, gelatinase, chitinase, and glucanase. Amylase and gelatinase are commonly used in the food industry [27]. Chitinase and glucanase are employed in bioconversion of agricultural waste into important by-products [28,29]. Thus, *B. velezensis* B26 strain underscores its potential applications in both the food and pharmaceutical industries.

Several other studies show that *Bacillus* species exhibit wide suppression against numerous fungal pathogens, majorly against phytopathogens. Importantly, recent studies suggest the effectiveness of *B. velezensis* against several fungal pathogens [30–35]. In addition to the production of antifungal metabolites, *B. velezensis* secretes fungal cell wall-digesting enzymes and bacterial volatiles (metabolites). The synthesis of these diverse compounds by *B. velezensis* significantly impacts the growth of fungal pathogens [35,36]. Notably, the synthesis of cyclic dipeptides (cyclo-(D-phenylalanyl-D-prolyl) and cyclic tetrapeptide (cyclo-(prolyl-valyl-alanyl-isoleucyl) by *B. velezensis* CE 100 strain shown to repress the spore germination and mycelial growth of *Colletotrichum gloeosporioides* [35,37]. Similarly, the *B. velezensis* CE 100 strain exhibits antifungal activity against phytopathogens that cause root rot disease [38]. Several studies report that chitinase and β-1,3-glucanase inhibit fungal growth, and both hydrolytic enzymes exhibit synergistic increase in antifungal activity.

In this study, we have demonstrated that *B. velezensis* B26 strain exhibits chondroitinase, chitinase, and glucanase enzyme activities. Chitinases, characterized by their ability to cleave glycosidic bonds within chitin molecules, are induced in plants as a defense mechanism against fungal infections and other stress factors [39,40]. Chitinases obtained from plants and bacterial sources exhibit immense potential as biocontrol agents of plant pathogenic fungi and insects [41–48]. For instance, *Bacillus cereus* YQ 308, a strain isolated from soil samples secreted chitinase enzyme on induction with shrimp and crab shell powder. The purified chitinase enzyme exhibited antifungal effects against fungi *Fusarium oxysporum* and *Pythium ultimum*. The utilization of chitinases and other enzymes for biological control against pathogenic fungi could serve as a viable alternative to chemical agents [49]. Plants secret both β-1,3-glucanase and various isoforms of chitinase to defend against various fungal pathogens. These enzymes work synergistically, hydrolyzing the fungal cell wall and inhibiting fungal growth [39,50]. Given this context, in addition to other antifungal components and metabolites, the synthesis of chitinase, and glucanase by the identified *B. velezensis* B26 strain might potentially aid in its antifungal activity. The antifungal properities of *B. velezensis* B26 strain highlight its potential as a biocontrol agent. Thus, *B. velezensis* B26 might be a promising candidate for developing new antifungal treatments. Further studies could be employed to isolate and characterize the specific compounds responsible for the antifungal activity.

CONCLUSION

This is the first study to identify and report the chondroitinase synthesis from *B. velezensis* B26. By exploring these novel sources, we hope to contribute to the broader understanding of chondroitinase enzymes and their potential biotechnological applications. We further show that besides chondroitinase activity, the *B. velezensis* B26 strain exhibits chitinase, glucanase, amylase, and gelatinase enzyme activity. Significantly, we reveal the potent antifungal capabilities of the *B. velezensis* strain B26 against *Candida albicans* and *Saccharomyces cerevisiae*. In conclusion, our findings contribute to the expanding usage of *B. velezensis B26* stain as a biocontrol agent and industrial enzyme producer.

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AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

All data generated and analyzed are included in this research article.

PUBLISHER'S NOTE

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USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

ACCESSION CODES

Bacillus velezensis B26 was received for public access deposit at NCMR allocated the accession number MCC 5370.

Whole genome sequence of *Bacillus velezensis* B26 strain submitted to GenBank (accession number: JAYKOV000000000).

SUPPLEMENTARY MATERIAL

The supplementary material can be accessed at the journal's website: [https://japsonline.com/admin/php/uploadss/4449_pdf.pdf].

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