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Research Article
Isolation and Molecular Identification of Two Chitinase Producing Bacteria from Marine Shrimp Shell Wastes

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Abstract

Background and Objective: Chitinase enzymes have a various application in the field of environmental, biotechnology and medical aspects. This study aimed to the production of the chitinolytic enzymes from different species of bacteria. **Materials and Methods:** Bacterial isolation from different habitats was carried out on agar medium containing chitin as carbon and nitrogen sources. The obtained bacteria (20) were characterized and screened again in chitin broth medium. **Results:** Out of 20 bacterial isolate, 2 new isolates, belonged to *Streptomyces laurentii* SN5 and *Cellulosimicrobium funkei* SN20, were the most active in chitin degradation compared to the other isolates. They have been characterized for the first time for their chitinase activity. They were identified using 16S rRNA gene analysis and in the liquid medium, the 2 isolates have enzyme activities of 0.533 and 0.537 U mL^{-1}, respectively. The maximum chitinase production was obtained when those bacterial strains were grown in Luria-Bertani (LB) broth amended with 1% colloidal chitin, for 1 day and at temperature of 30°C. The optimum pH value for chitinase production was pH 7 for both *S. laurentii* and *C. funkei*. The enzyme has been purified using Sephadex G-100 and DEAE-Cellulose chromatography column and found to have a similar molecular size of ~50 kDa. **Conclusion:** Those two bacterial species could be used in chitinase production and in the environmental recycling of disposable chitin wastes such as chitin from shrimp shell waste.

Key words: Chitin, chitinase, bacterial isolation, 16S rRNA, *Streptomyces*, *Cellulosimicrobium funkei*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Chitin is a biopolymer that resembles to cellulose and composed of unbranched linear chain of glucosamine with an acetyl group[1]. Chitin is insoluble substrates, found in fungal cell walls and exoskeletons of insect and crustaceans. Each year, 1011 t of the chitin is produced[2] and most of those quantities are wastes such as those of crustacean shell wastes[3]. One way to recycle the unused chitin in the environments is to degrade it into low molecular weight products through enzymes called chitinases[4]. Chitin degradation, the strictly regulated process, have a role in carbon and nitrogen cycles, reduced marine wastes and produced valuable products[5]. N-acetyl glucosamine and chitin oligosaccharides that are produced mainly from chitin degradation, have significant roles in many industrial, agricultural, pharmaceutical and medical uses[6]. In addition, these enzymes have been utilized in other fields rather than chitin degradation such as in the production of bioinsecticide, single cell protein and in the control of malaria transmission[7,8]. Chitinases are classified into three types: endo-chitinase, exo-chitinase and β-N-acetyl glucosaminidase[9]. Most chitin hydrolases belong to glycoside hydrolase which have 3-dimensional structures and substrate-binding patterns[10,11]. However, various organisms including bacteria, fungi, insects, plants, animals and human possess this enzyme for various functions which are:

- Reshaping of their matrices at different developmental stage
- Hydrolysis of the chitin to be used as a source of energy
- As defense against chitin coating microorganisms[12,13]

Nevertheless, the production of this enzyme implies a change in the activity of different species. Therefore, several studies are reporting the chitinolytic activity of several species producing them. Bacteria are one of the main mediators of the chitin in the environment and the most investigated source as they could be cultivated in a short period of time and the production could be in a large-scale manner[14]. Bacterial chitinases has carbohydrate-binding module, increased enzymes binding to chitin and a fibronectin type III, caused enzyme stability[15] which is clear in *Serratia marcescens*[16]. Several bacterial species have been investigated for the production of chitinolytic enzymes such as *Serratia marcescens*, *Bacillus* sp. and *Aeromonas* sp.[17,18]. *Aeromonas* sp. from aquatic environments is efficient in chitinase production[19,20]. Also, *Aeromonas* sp. PTCC 1691 which was isolated from the waste of shrimp shells produce high levels of extracellular chitinase in medium containing chitin as the main carbon source[21,22]. The crude chitinolytic enzymes from this bacterium was commercially used to prepare N-acetyl glucosamine from chitin wastes. Thermostable chitinase has wide applications and was produced[23] by *Streptomyces* sp. F-3. Chitinases from bacterial culture filtrates were precipitated by ammonium sulphate followed by gel filtration through column chromatography. Different chitinase enzymes have been purified from the genus *Streptomyces*. Four chitinases were purified from *Streptomyces olivaceoviridis*[24]. All reported *Streptomyces* chitinases have high molecular weight ranging from 30-68 kDa while a low molecular weight enzyme is being reported for the first time from the novel halophilic *Streptomyces chilikensis*[25] RC1830. Bacterial chitinases had high molecular weight ranged from 20-120 kDa[19]. Screening and studying the properties of chitinases have become important topics of many researches. This research aimed to isolate and characterize chitinolytic enzymes from local potent strains, isolated from the local area of Jeddah, Saudi Arabia.

MATERIALS AND METHODS

Preparation of colloidal chitin: Shrimp shell wastes were collected during summer 2017 from Fish market of Jeddah, washed several times with water, dried and powdered at Microbiological Laboratory, Faculty of Science, KAU. Colloidal chitin was prepared from chitin that was extracted from shrimp shell wastes based on a method of Saima *et al.*[26] with some alteration. Briefly, 40 g of the isolated chitin was added to 600 mL of concentrated hydrochloric acid (HCl) under vigorous stirring at room temperature. Then, the chitin was precipitated from the mixture by the addition of 1600 mL cold water. The chitin was then appearing as a colloidal suspension and collected by centrifugation at 4500 rpm for 20 min. The collected material has been washed several times with distilled water to restore neutral pH and was then used for analysis and media preparation.

Infra red spectra (IR) and x-ray diffraction (XRD) analysis: The isolated chitin sample was characterized using the Perkin Elmer Infrared Spectroscopy device version 10.03.09 from wavelength of 4000-400 cm^{-1} at King Fahad Medical Research Center. Degree of acetylation (DA) of the purified chitin was calculated using absorbance (A) ratio through the following equation[27]:

$$DA\ (\%) = \frac{A1655}{A3450} \times 100$$

The crystallinity of the isolated chitin has been characterizing using Ultima IV X-ray Diffractometer (Rigaku Corporation, Japan) at Center of Nanotechnology, King Abdulaziz University. The peaks of XRD were collected at scan rate of 4 degree min^{-1} with scan range from 5-40 degree. The crystallinity of the polymer was calculated using the following[28] formula:

$$CrI_{110} = \frac{I_{110} - I_{am}}{I_{110}} \times 100$$

Where:

I_{110} = Maximum intensity at $2\theta \cong 19$

I_{am} = Intensity of amorphous diffraction at $2\theta \cong 17$

Samples collection for isolation of chitinolytic bacteria: Samples were aseptically collected from different sources in Jeddah, Saudi Arabia. The sample sources were soil samples (local garden soil, marine soil and a rhizosphere soil), shrimp shell wastes and a naturally died insect. To screen for chitinase producing bacteria, a colloidal chitin agar (CCA) medium was used, which is consist of the following (g L^{-1}): KH_2PO_4 3.0, Na_2HPO_4 6.0 , NaCl 0.5, yeast extract 0.05, NH_4Cl 1.0 and agar 15.0 in addition to colloidal chitin 1% (w/v)[29]. Colonies that form a clear zone or that with the heaviest growth after incubation at 37°C for 3 days were considered as the chitinase producers. The bacteria were then purified to obtain a pure culture through the streak plate method. For preservation, the isolated species were maintained for several months using slant agar and in 50% glycerol for a longer period of time.

Growth in liquid medium: The colonies with the larger clearing zone or with the heaviest growth on chitin agar medium were selected for cultivation on colloidal chitin broth medium. At first, the pre-culture was prepared in a 100 mL flask containing 20 mL of a sterile nutrient broth media that were inoculated with a loop full of the pure bacterial strain under the sterilized conditions. The culture was incubated for 24 h at 37°C. The optical density of the bacterial growth was then measured at 540 nm using spectrophotometer and the absorbance was adjusted to the desired optical density. About 2 mL of the preculture (4×10^6 CFU mL^{-1}) was used to inoculate 20 mL of the colloidal chitin broth medium which consists of (g L^{-1}): 3.0 g KH_2PO_4, 6.0 g Na_2HPO_4, 1.0 g NH_4Cl, 0.05 g yeast extract, 0.5 g NaCl and 1% (w/v) colloidal chitin. The inoculated medium was incubated at 37°C for 1 day and bacterial cells were collected by centrifugation at 4500 rpm for 20 min at 4°C. The filtrate contained the crude chitinase enzyme was assayed at 540 nm using a colorimetric method[30]. One unit of the chitinase activity was described as the quantities of the enzyme by which 1 µmol of N-acetyl glucosamine are generated from the substrate each min under the reaction conditions. Absorbance was described as the mean of three independent experiments ±standard deviation of the mean.

Assay of chitinase activity: A colorimetric method was used for determination of the chitinase activity[30]. In short, 0.5 mL of the culture filtrate or crude enzyme extract was added to 0.5 mL of a 1.5% colloidal chitin in 0.1 M phosphate buffer (pH 7.5), the mixture was kept at 50°C for 1 h, boiled for 10 min in a boiling water bath, followed by centrifugation for 5 min at 3000 rpm. Subsequently, 0.5 mL of DNSA reagent (Dinitrosalicylic acid) and 0.5 mL of the previous mixture were mixed and kept in a boiling water bath for 10 min. The DNSA reagent react with the resulting reduced sugar giving yellow to orange color which was measured at 540 nm against control.

Identification of the isolated bacterial strains

Morphology and microscopic examinations: The morphological characteristics of the grown colonies were determined followed by Gram staining to define the bacterial type.

Molecular identification using 16S rRNA gene analysis: Genomic DNA was extracted from 1-day culture broth using Gene JET Genomic DNA purification kit (Thermo Fisher Scientific Inc., USA). The extracted DNA was checked for its integrity and concentration using 0.5% agarose gel electrophoresis and nanodrop spectrophotometer (Thermo Fisher Scientific Inc., USA). The 16S rDNA gene was amplified using a universal primer, forward fD1 5′-AGAGTTTGAT CCTGGCTCAG-3′ and revers primer rP2 5′-ACGGCTACCT TGTTACGACTT-3′ (Macrogen Inc., Korea) as described before[31]. Sequencing of the amplified product was performed by Macrogen Inc., (Korea) and the resulted sequences were analyzed to identify the bacteria based on the highest percent of nucleotide sequence similarities. The phylogenetic tree was constructed using Phylip program.

Optimization of culturing conditions for enzyme production

Effect of media type on chitinase production: Four different media were used to study their effect on chitinase production[13]. The tested media were colloidal chitin broth (CCB), nutrient broth containing 1% colloidal chitin (NB),

Luria-Bertani (LB) broth containing 1% colloidal chitin and chitin mineral broth medium (CM) consist of the following (%): colloidal chitin, 2, $MgSO_4.7H_2O$, 0.05, NaH_2PO_4, 0.5. Each culture consists of 20 mL of the media inoculated with 2 mL of the prepared pre-culture (4×10^6 CFU mL^{-1}). The culture was then incubated for 24 h at 37°C in a rotary shaker, followed by centrifugation at 4500 rpm for 20 min and the supernatant was used to determine the chitinase activity as previously stated.

Effect of incubation time, temperature and medium pH on chitinase production: To investigate the effect of incubation time on chitinase production, the bacterial cultures were incubated at the previously optimized medium and its activity was determined every day (up to 4 days). In order to determine the optimum temperature that results in a maximum enzyme production, the inoculated cultures of previously optimized condition were incubated at different temperatures (25, 30, 37, 40 and 45°C). Moreover, the effect of pH was studied by culturing the bacteria at the previously optimized condition at varying pH values (pH 4, 6, 7, 8 and 10) adjusted using 1 M NaOH or HCl. At the end of the growth period, chitinase assay was performed using the stated protocol.

Purification of chitinase enzymes and SDS-PAGE analysis: The chitinase enzyme has been purified from the selected chitinase producing bacteria by precipitation of the total protein from the culture filtrate using 60-70% ammonium sulfate. Then, the sample was dialyzed against 200 volume of distilled water at 4°C under shaking for 3 days. The sample was then concentrated by lyophilization and the proteins were separated using Sephadex G-100 and DEAE-Cellulose chromatography columns. The active fractions were pooled and concentrated by lyophilization. The molecular weight of the purified enzymes was determined using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using 5% (w/v) stacking gel and 12 % (w/v) resolving gel[32]. The gel was stained with Coomassie brilliant blue and the molecular weight was determined by comparing it with standard protein marker.

Statistical analysis: The IBM SPSS statistics program version 19 was used to calculate the mean±standard deviation value and to determine whether there were any differences among samples. Analysis of variance (ANOVA) and t-test ($p<0.05$) were applied to the obtained results.

RESULTS

Chitin was collected from shrimp shell wastes and was used for colloidal chitin preparation. Colloidal chitin was characterized using X ray and IR spectra. The XRD analysis of the isolated chitin showed two strong peaks at degree of 9.1 and 19.1. However, four faints peaks were observed at 17.5, 20.6, 23.4 and 26.3° (Fig. 1a) and the crystallinity percent were calculated to be 63.7%. The IR spectra showed peaks at 1654, 1621 and 1543 cm^{-1} and the degree of acetylation was calculated to be 189.1% (Fig. 1b).

After preparing of chitin (Fig. 2a), it was used for bacterial isolation. Total of 20 morphologically different bacterial strain was able to grow on colloidal chitin agar (CCA) medium. Based on chitin degradation ability, the bacterial colonies that had clear zone or heaviest growth on CCA medium were selected for chitinase production. The selected bacteria were further grown on colloidal chitin broth medium and assayed

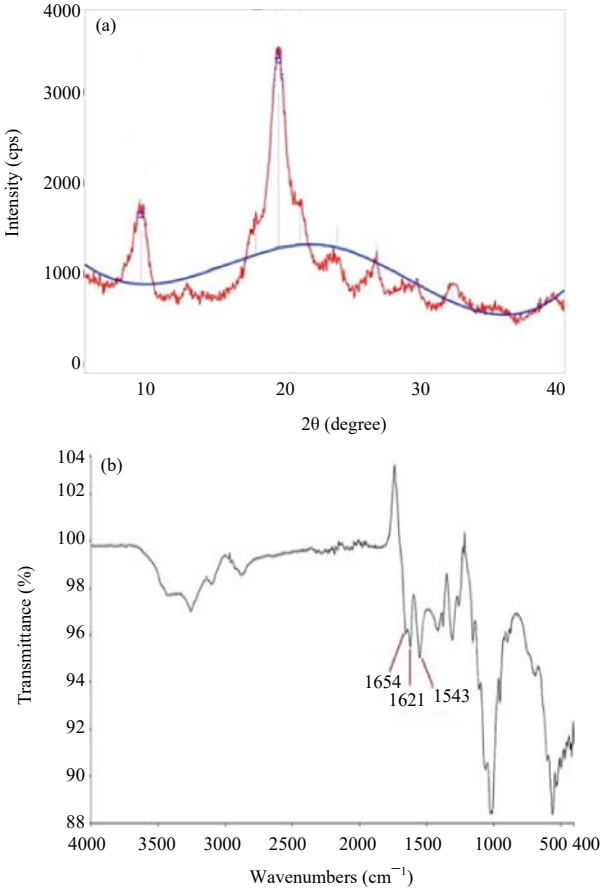

Fig. 1(a-b): (a) X-ray diffraction of the purified chitin and (b) IR spectra of the purified chitin from shrimp shell wastes

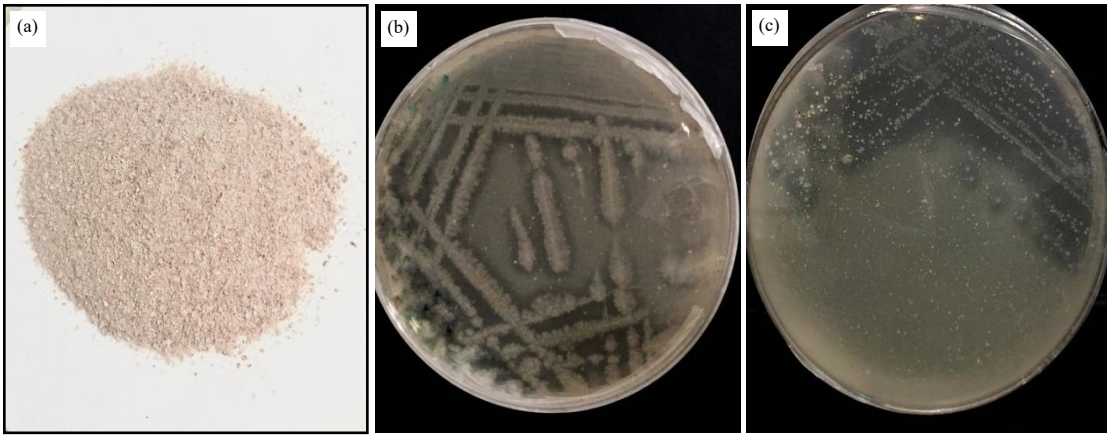

Fig. 2(a-c): (a) Purified chitin, (b) Growth of the isolate SN5 and (c) SN20 on chitin agar medium

Table 1: Chitinase production on solid and broth media by the selected bacterial isolates

Strain numbers	Sources	Clear zone on solid media (mm)	Growth (Absorbance 540 nm)	Enzyme activity (U mL^{-1}) on broth media
SN4	Rhizospheric soil	2	0.108±0.005	0.294*
SN5	Shrimp shell waste	3	0.136±0.003	0.533
SN6	Rhizospheric soil	3	0.121±0.009	0.444*
SN18	Local garden soil	2	0.003±0.003	0.201*
SN12	Shrimp shell waste	1	0.122±0.030	0.310*
SN20 (control)	Shrimp shell waste	3	0.159±0.007	0.537

*Significant difference at $p \leq 0.05$ compared to control

for their chitinase activity. A day later of incubation, the bacterial isolates that showed the maximum activity in colloidal chitin broth were isolates SN5 and SN20 (Fig. 2b and c). Using t-test at $p \leq 0.05$, no significant differences were found between the 2 isolated bacteria in chitinase production in liquid medium (Table 1), thus they were selected for further studies.

Identification of the selected bacterial isolates: After Gram staining, the isolates SN5 appeared under the light microscope as Gram-positive filamentous bacterium. Their colonies have a size of 3-5 mm diameter on nutrient agar with brown color and brownish back ground pigment. However, on the agar plate, isolates SN20 have appeared as yellow, round and a smooth colony with a size of 2-3 mm. Under the light microscope, it was a Gram-positive bacterium with a cocci-rod shape.

The amplicons of 16S rDNA genes for both isolates were sequenced and based on the sequence similarities, isolate SN5 was identified as *Streptomyces laurentii* strain ATCC 31255 (accession number: AP017424.1) with sequence similarity percent of 99%, whereas isolate SN20 was identified as *Cellulosimicrobium funkei* strain W6122 (accession number: NR_042937.1) with sequence similarity percent of 99%. *Streptomyces laurentii* has a high level of similarity with other *Streptomyces* sp. with the highest percent to *S. laurentii* (Fig. 3). The same thing is with *C. funkei* as appear in its phylogenetic tree (Fig. 4), in that it has a high degree of similarity with other *Cellulosimicrobium* sp. and the highest degree of similarity to *C. funkei*.

Optimization of culturing conditions: It was observed that LB broth medium with 1% colloidal chitin has supported the production of chitinase enzyme by the two tested isolates. The enzyme activity in both medium was 0.673 and 0.899 U mL^{-1} for isolate SN5 and SN20, respectively (Fig. 5). The other media contained lower enzyme activity, thus, LB broth medium with 1% colloidal chitin was used to grow bacteria for different incubation time. Maximum activity was recorded after incubating the tested bacterial isolates for 1 day (Fig. 6). Later on, the activity of the enzyme was gradually decreased. Moreover, 5 different temperatures has been selected to investigate the enzyme production by the two selected bacteria. Among all the tested temperature, 30°C have supported the activity of the enzyme to be 0.705 U mL^{-1} for isolate SN5 and 1.315 U mL^{-1} for isolate SN20. However, above and below this temperature, the activity of the enzyme was decreased in both strains (Fig. 7). The optimum pH value that

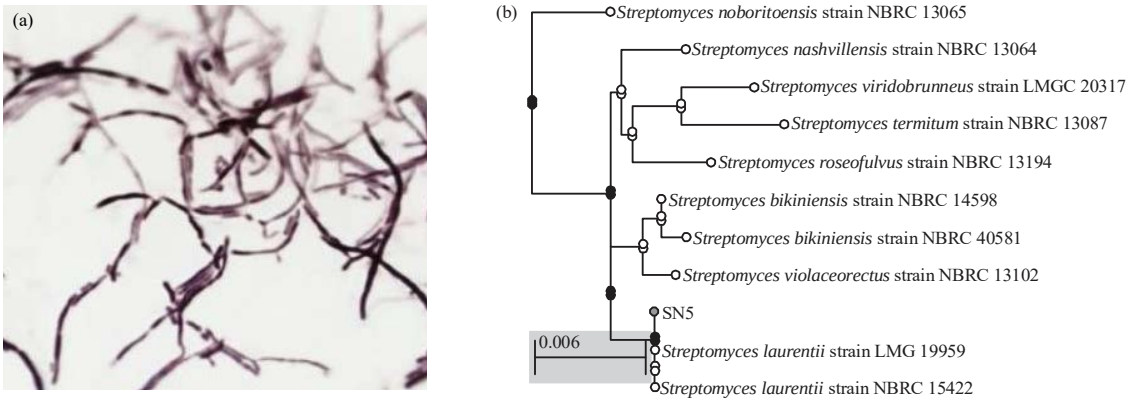

Fig. 3(a-b): (a) Gram stained bacterium SN5 udder light microscope (x1000) and (b) Phylogenetic tree constructed based on 16S rDNA gene of SN5 with other *Streptomyces* species

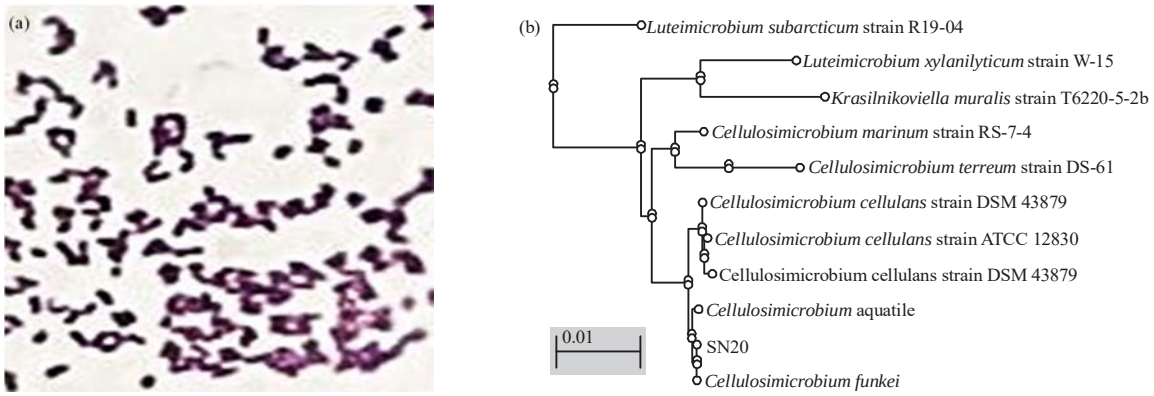

Fig. 4(a-b): (a) Gram stained bacterium SN5 udder light microscope (x1000) and (b) Phylogenetic tree constructed based on 16S rDNA gene of SN20 with other *Cellulosimicrobium* species

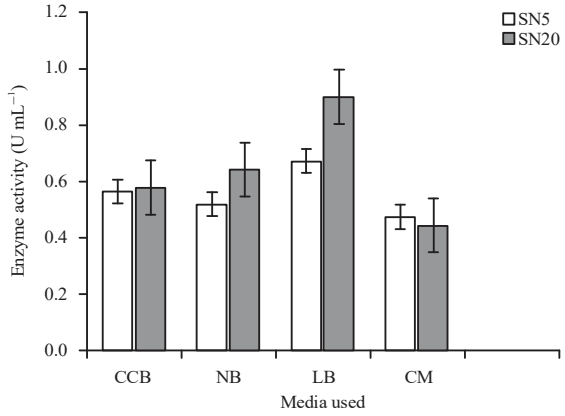

Fig. 5: Effect of different media on chitinase production by isolates SN5 and SN0

CCB: Colloidal chitin broth medium, NB: Nutrient broth containing 1% colloidal chitin, LB: Luria-Bertani medium, CM: Chitin mineral broth medium

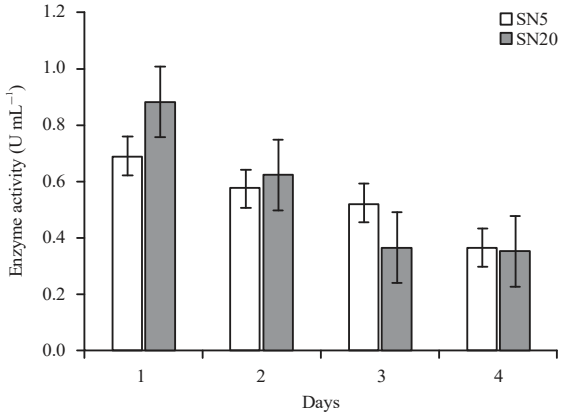

Fig. 6: Effect of incubation period on chitinase production in broth medium by the selected isolate SN5 and SN20

supports the maximum production of the enzyme have been investigated (Fig. 8). The two isolates SN5 and SN20 showed maximum chitinase activities of 0.746 and 1.315 U mL^{-1}, respectively at pH 7. The enzyme activity has been highly decreased as the pH value increased but slightly decreased at acidic pH values.

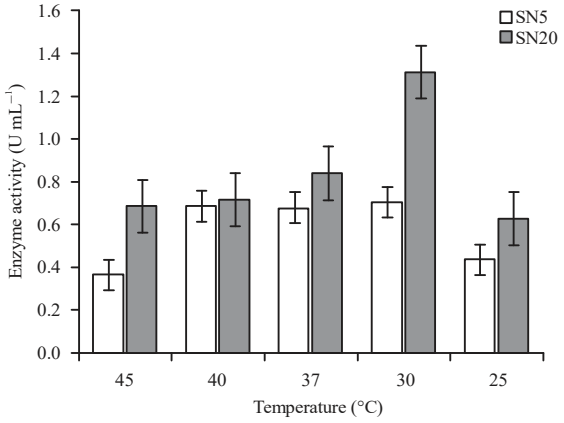

Fig. 7: Effect of incubation temperatures on chitinase production in broth medium by the selected isolate SN5 and SN20

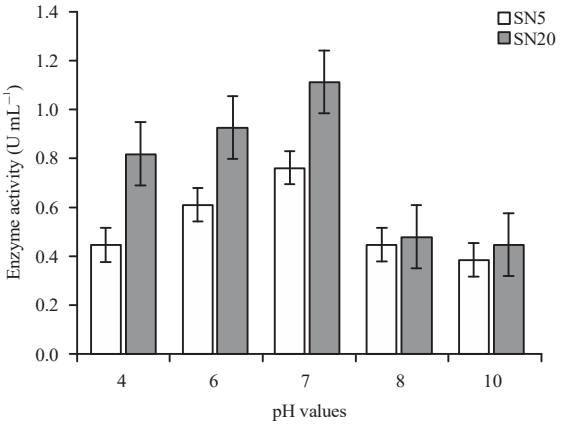

Fig. 8: Effect of the pH values on chitinase production in broth medium by the isolate SN5 and SN20

Purification and molecular weight determination of chitinase: The isolates SN5 and SN20 were grown in LB broth medium at pH7 for 24 h at 30°C, then the cells were collected and the filtrate was used as crude enzyme which was obtained by NH_4SO_4 precipitation. Sephadex G-100 and DEAE- cellulose columns chromatography were used for enzyme purification and the elution profiles of the 2 columns for the two bacterial extracts were the same. Elution profiles of the isolate SN5 were shown in Fig. 9a, b, respectively. The most active fractions with the highest chitinase activity have been selected after each type of column and concentrated by lyophilization. The purified enzyme in the pooled fractions from DEAE-cellulose column has been analyzed using SDS-PAGE analysis. As illustrated in Fig. 10, a single band was detected for both purified enzyme samples with a molecular mass of ~ 50 kDa.

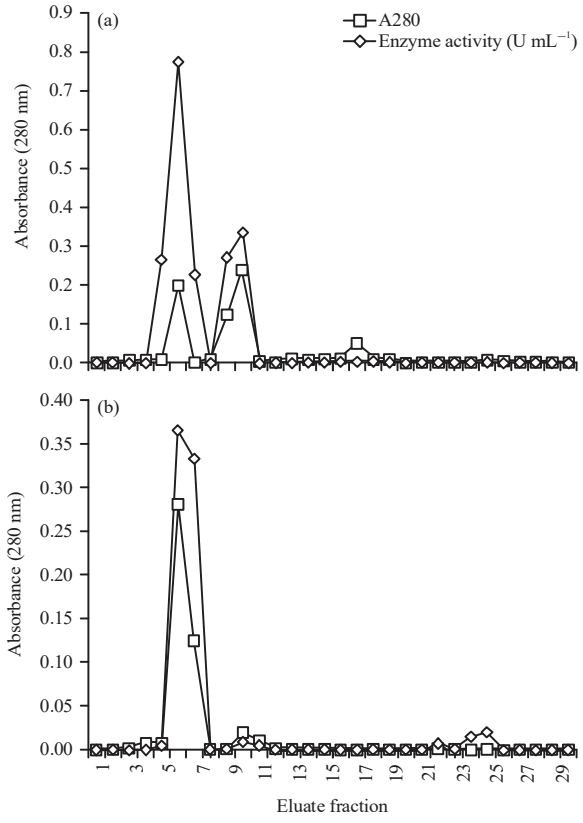

Fig. 9(a-b): Elution profile of chitinase of the (a) Isolate SN5 after Sephadex G-100 chromatography and (b) DEAE-Cellulose chromatography

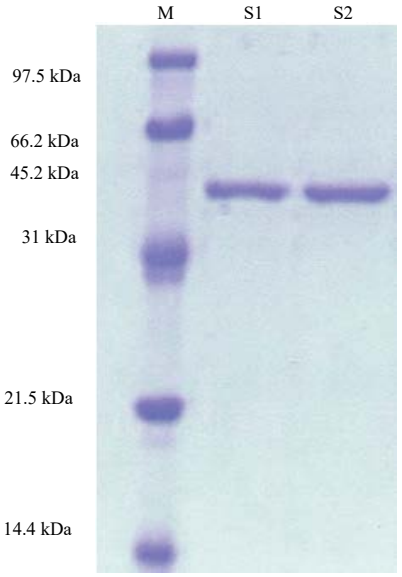

Fig. 10: SDS-PAGE analysis of the purified enzymes after DEAE-cellulose chromatography
M: Protein molecular weight marker, S1: Purified enzyme of isolates SN5, S2: Purified enzyme of isolates SN20

DISCUSSION

Several morphologically different bacteria were screened for their ability to produce chitinase using chitin as the only carbon and nitrogen sources. The bacteria are considered as one of the most significant mediators in degradation and hydrolysis of the chitin in the natural systems as the aquatic one[33]. Chitin could be hydrolyzed into products of different sizes through chitinolytic enzymes which have numerous applications as fungicide, pesticide and in various medical applications[34,35]. However, it has been reported that the expression of the chitinolytic enzyme by bacteria is regulated by an inducer and repressor system, by which chitin is act as inducer and most often glucose is as a repressor[36]. Thus, the colloidal chitin was used as a substrate in the present study to induce the production of chitinases from different bacteria. Chitinase production was detected by the presence of clear zone around the bacterial colonies. Isolates SN5 and SN20 were found to have the higher and similar chitinase activity when grown in colloidal chitin broth medium, thus they were selected for further investigation. The 16S rRNA gene was amplified using the PCR and sequenced in order to identify these bacterial species. Isolate SN5 was identified as *Streptomyces laurentii* strain ATCC 31255, whereas isolate SN20 was similar to *Cellulosimicrobium funkei* strain W6122*. Streptomyces* is a genus of Gram-positive bacteria[37] of Streptomycetaceae family that contained over 500 species[38]. *Streptomyces* mostly inhabitant in soil and have a significant role in soil ecology since they have a filamentous hyphae that enable them to recycle the natural biopolymer such as chitin and cellulose[39]. However, *Cellulosimicrobium* species are also a Gram-positive bacteria that belong to Promicromonosporaceae family, which is broadly found in water and soil[40]. However, several bacterial genera have been isolated and identified for the production of chitinolytic enzymes such as *Sphingomonas* sp., *Acinetobacter* and *Bacillus* sp.[41-43]*.* In addition, the production of chitinases has been detected in a number of *Streptomyces* sp. such as *S. antibioticus*, *S. plicatus* and *S. aureofaciens*[44]. Nevertheless, the production of chitinolytic enzymes was characterized for the first time for *S. laurentii* and *C. funkei* in this research. The activity of chitinases have been reported in *S. violascens*[45] NRRL B27004 and maximum chitinase activity of 0.1523 (U mL^{-1}) was obtained after 72 h of incubation in the colloidal chitin medium. Also, a maximum chitinase production of 0.058 U mL^{-1} was reported for *S. griseorubens* at 40°C after 6 days of incubation[46]. In addition to that, *S. rubiginosus* was identified a as chitinase producer with maximum activity[47] of 2.79 U mL^{-1}. On other hands, limited number of researches was conducted to characterize the chitinases from *Cellulosimicrobium* sp.[48]. Also, *C. cellulans* had shown to have a chitinase activity of 6.9 U mL^{-1} when grown at 25°C for 3 days[49].

Several parameters may affect the production of the enzyme, thus they should be investigated. In this study, the isolated strains give their maximum enzyme production when grown in LB broth medium amended with 1% colloidal chitin after 1 day of incubation. Similarly, *Bacillus subtilis* have shown to produce a high enzyme activity when grown in LB broth amended with colloidal chitin[50]. Also, *Serratia marcescens* XJ-01 had shown to have the optimum incubate time for 32 h while *Paenibacillus* sp. was found to have their maximum production after 1 day[51,52]. Longer incubation period support the higher chitinase production in other bacterial species such as *Cohnella* sp. A01, *S. rubiginosus* and *Bacillus laterosporous*[53]. The decrease in the activity of the bacterial strains in this research after 1 day may result from the depletion in nutrients or presences of toxicities that may interfere with the activity of the enzyme. On the another hand, the temperature of 30°C and pH 7 was found to support the maximum activity in both strains in this study. Also, *Enterobacter* sp. and *Zymomonas* sp. have the optimum temperature of 30°C and the optimum pH was 6 and 7, respectively[54,55]. Furthermore, these results are in agreement with the optimum temperature and pH for *Bacillus* sp. and *B. cereus* that have their maximum production at 30°C and[56] pH of 7. Moreover, *Streptomyces* sp. was shown to have the optimum culturing condition that supports the enzyme[57] production at 32°C and pH 5. A slightly higher temperature and pH value were reported as the optimum condition for *Streptomyces* sp.[58] In short, each species of bacteria have a different optimizing conditions that must be adjusted in order to reach the maximum enzyme production.

Chitinase enzyme of the isolated strains was purified first by precipitating total protein using ammonium sulfate, followed by separation using Sephadex G-100 and DEAE-cellulose chromatography. A single band with a similar molecular weight of ~50 kDa was obtained for each sample. A broad range of molecular sizes was detected for microbial chitinases as stated in the literature. *S. violaceusniger* have a chitinase with a molecular size of approximately 56.5 kDa[59], a ~38 kDa for *S. anulatus*[60], *B. cereus* IO8 have a size of 30 kDa[61] and a size of 72 kDa regarding the chitinase from *B. licheniformis*[62]. Generally, molecular sizes of bacterial chitinases are ranges from 20-120 kDa where for *Streptomyces* from 30-68 kDa[46]. Chitinase producing bacteria may be utilized as a biological control agent against chitin containing pathogen (as fungi) instead of harmful insecticide for protecting plants.

CONCLUSION

In this research, the chitinase enzymes have been also characterized from *S. laurentii* strain ATCC 31255 and *C. funkei* strain W6122. The size of the purified enzyme was found to be about 50 kDa. Those bacterial strains could be considered as a good source of the chitinolytic enzymes to be used in different applications, since they give a significant amount of the enzyme in a short period of time. They could be also mutated with the biotechnological protocols to even over-produce this enzyme. Additionally, those chitinase producing bacteria may be utilized as a biological control agent against chitin containing pathogen (as fungi) instead of harmful insecticide for protecting plants. The chitinase enzymes may also used in the recycling of the chitin and in a production of chito oligomers and N-acetylglucosamine, that have a significant role in many medical applications.

SIGNIFICANT STATEMENT

Crustacean shell wastes increased every year and degradation of these wastes by bacterial chitinases is very important. This study sheds light for the first time on the detection of chitinase from two new bacterial species which can be used in different biotechnological applications.

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