International Journal of Ecology and Environmental Sciences 49: 425-434, 2023
ISSN: 2320-5199 (Online); https://doi.org/10.55863/ijees.2023.2781

Production and Partial Characterization of Alkaline Chitinase from Bacterial Strain *Aeromonas dhankensis* PZ6 Isolated from Tithal Coastline of Valsad, Gujarat, India

PARTH ZAVERI[1], NOOPUR GOYAL[2]AND PAYAL PATEL[2]
[1]*Department of Microbiology, School of Sciences, Gujarat University, Ahmedabad, 380009*
[2]*M.G Science Institute, Navrangpura, Ahmedabad, 380009*
E-mail: pkkzaveri@gmail.com, payal1311@gmail.com, noopurgoyal19@gmail.com
***Corresponding author**

ABSTRACT
Chitin is the most widespread renewable polymer present in ocean and land. It is a crucial source of carbon and nitrogen for many marine organisms. The goal of this research was to identify and enhance the production of chitinase from bacterial isolates from the Tithal coastline in Valsad, Gujarat, India. A total 100 different soil samples were screened using colloidal chitin agar medium for chitinolytic activity. Based on maximum zone of chitin hydrolysis PZ6 isolate was selected for submerged fermentation (SmF). Optimization of chitinase production in reference to production time, carbon source, nitrogen source, inoculum size, pH, temperature, and metal ions were investigated. Highest chitinase production observed with 0.8 % (w/v) colloidal chitin;8 pH 35°C temperature under shaking conditions after 24 hours. The most favored nitrogen and carbon sources were yeast extract and glucose. The growth rate increased when medium was supplemented with Co^{+2} metal ions. The molecular weight of purified chitinase as determined by SDS-PAGE technique was 60 kD. The results suggest possible use of this bacterial chitinase as a potent biocontrol agent for plant pathogenic fungi and as a tool for sustainable conversion of natural abundant chitinous waste into various value-added products.

Key words: Chitin, Chitinolytic, Optimization, SDS-PAGE, Chitinous waste.

INTRODUCTION

Henri Braconnot a French chemist first discovered chitin in the early nineteenth century in France (Crini 2019).Chitin, in its pure state, is translucent, pliable, resilient, and quite tough (Abidin et al. 2020). It is the most abundant and insoluble amino-polysaccharide occurring in nature following cellulose, consisting of β1-4 linked n-acetyl d-glucosamine residues. Chitin is always found cross-linked to other structural components such as proteins and glucans. It is present in shells, exoskeletons, and stomach linings of arthropods (crustaceans and insects) and lobsters consist of chitin (Swiontek-Brzezinska et al. 2007). It is a major structural component of yeast and several fungi forming part of cell wall. Chitin exists in several zygomycetes species in its deacetylated form as chitosan (Martinou et al. 1995)

Chitinases (EC3.2.1.14) are enzymes that hydrolyze chitin, and they are synthesized in bacterial, viral and fungal cells as well as in insects, plants and animals. In nature majority of chitin degradation is mediated by microbes like bacteria and fungi having chitinase enzyme and contribute to carbon and nitrogen biogeochemical cycles (Setia 2015); but the degradation depends on temperature, pH and the succession stage of the degradation process (Beier and Bertilsson 2013). Hence, potential microbial chitinase is needed for biodegradation and conversion of huge chitin waste into economically viable products (Yadav et al. 2019)

Several chitinolytic bacteria were reported as chitinase producers like *Serratia marcescens bjl 200* (Monreal and Reese 1969), (Sun et al. 2019), *Bacillus* sp. (Lee et al. 2007, Itoh and Kimoto 2019), *Paenibacillus* sp. *Ik5* (Kusaoke et al. 2017), *Listeria monocytogenes* (Halbedel et al. 2019), *Saccharomyces cerevisiae* (Hurtado-Guerrero and van Aalten 2007). Additionally, actinomycetes such as *Streptomyces* sp. (Dahiya et al. 2006), *Streptomyces griseus hut6037* (Ralph Berger and Reynold 1958), *Streptomyces plicatus* (Robbins et al. 1988), reported as potent producer of chitinases. Chitin modified products holds great economic value due to their versatile biological activities and chemical applications in medical (Iber et al. 2022) and pharmaceutical areas (Hoell et al. 2010).

Chitooligosaccharides(cos) and their N-acetylated analogues are useful for applications in various biological activities such as antimicrobial activity, and immune-enhancing effects (Rinaudo 2006). Some chitooligosaccharides have been reported to possess antitumor activity (Rinaudo 2006) and (Patil et al. 2000).It can also be widely used in various areas including the agricultural, biological, and environmental fields (Veliz et al. 2017)such as applications in water treatment, cosmetics and toiletries, healthcare and medical, agrochemicals, food and drinks, as natural biocontrol agent and others. The search for extracellular chitinase producing microorganisms is in demand along with enhanced enzyme productivity by designing culture medium and optimizing fermentation conditions is need of time to further reduce the overall economic cost. The objective of the study was to identify optimize inoculum and fermentation parameters for higher productivity of chitinase by isolate PZ6.

MATERIAL AND METHODS

Colloidal chitin preparation

The modified Hsu and Lockwood method was used to prepare colloidal chitin by using chitin powder (Hi Media) (Hsu and Lockwood 1975). For colloidal chitin making 40 g of chitin powder was slowly mixed with 600 ml of strong hydrochloric acid and held at 30^0C for 60 min with vigorous stirring. Chitin was precipitated as a colloidal suspension in 2 litres of water. The suspension was recovered using suction filtration on coarse filter paper and then washed with 5 litres of distilled water. The procedure was repeated three times until the pH of the suspension reached 4. The loose colloidal chitin was utilized as a substrate following the process as mentioned above (Wang et al. 2013).

Isolation and screening of chitinase producing bacteria

A total number of 100 soil samples were collected aseptically from diverse regions of Gujarat. This soil samples were collected from the fish market lal Darwaja Ahmedabad, Dastan farm Ahmedabad, Gandhinagar, and coastlines areas of Khambhat, Somnath, Tithal, Valsad Gujarat. The minimal agar medium containing colloidal chitin was used for screening for chitinase-producing bacteria. Bacterial isolates were selected on the basis of a larger chitin hydrolysis zone after 96 hours of incubation. Based on maximum zone of chitin hydrolysis *Aeromonas dhankensis* PZ6 isolate was selected and further tested for maximum enzyme production in *colloidal chitin* broth (CCB).

Production of chitinase enzyme

The bacterial culture was inoculated in a 75ml of fermentation media. Medium consists of (g l^{-1}): NaCl, 0.5g; $KH_2PO_{4,}$3g; NH_4Cl, 1g; $Na_2HPO_{4,}$6g; yeast extract, 0.5g; agar powder, 15g and 1% (w/v) colloidal chitin.(Farag et al. 2016, Monreal and Reese 1969)The bacterial culture was grown for 96 hours at 35°C, 120 rpm on shaking incubator. The pH of the fermentation medium was adjusted to 8. After incubation period the fermentation media was centrifuged at 8000 rpm for 5 minutes and the supernatant of fermentation broth was collected.

Chitinase enzyme assay and protein estimation

The protein content was estimated by Lowry's method by using bovine serum albumin (BSA) as standard in 20–200 µg/ml concentration (Sharma et al. 2021). Enzyme activity of chitinase was estimated based on the release of reducing sugar from colloidal chitin. Crude sample, precipitated enzyme, and purified enzyme (0.5ml) was added with equal volume of 1% (w/v) colloidal chitin in 0.1 M phosphate buffer with pH 7. After incubation at 37°C for 1 hour the reaction mixture was added with 1ml of DNS reagent and boiled for 5 min. One unit (u) of chitinase activity considered as the production of one ìmol of N-Acetyl-D-glucosamine (GlcNac) per minute using a standard curve of N-Acetyl-D-glucosamine (GlcNac) (Zhang et al. 2016).

Optimization of fermentation condition for chitinase production

One optimised parameter was followed while other parameters underwent iterative optimization, one factor at a time. For the investigation of culture conditions and chitinase production the following variables were tested viz., inoculum size (1, 2, 3, 4 and 5%), incubation temperatures (25,30,35,40 and 45°C), pH (4, 6, 8, 10, 12), source of carbon (glucose, sucrose, lactose, mannitol, fructose), source of nitrogen (yeast extract, peptone, urea, ammonium sulphate, gelatine), For the metal ion optimization, the medium was supplemented with chloride salts of the selected metal except for copper for which sulphate was used, and their supplementation was as 0.1mg/L of the medium (Co^{2+}, Ca^{2+}, Cu^{2+}, Mg^{2+}, Mn^{2+}).

Enzyme purification

The concentration of ammonium sulphate used in the study was 25, 35, 50, 65 and 75% (Farag et al. 2016). For this process the crude enzyme solution was divided in five equal fractions of 10ml each and to these fractions' ammonium sulphate was added according to the saturation concentration. That is to the fractions of crude enzyme solution 1.34, 1.94, 2.91, 3.98 and 4.76gm of ammonium sulphate was added to obtain the saturation concentration of 25, 35, 50, 65 and 75%, respectively. The mixture was incubated at 4°C for 5 hours to allow the protein precipitation. The precipitated protein was collected by centrifugation of the content at 8000 rpm for 15min. 50 mM phosphate buffer was used to dissolve the centrifuged protein pellet. After collection of precipitated protein pellets from all concentrations they were pooled together to obtain the final precipitated protein sample of chitinase from each source. This precipitated chitinase enzyme sample was used for protein quantification by Lowry's assay and enzyme activity determination with DNS reagent. This precipitated protein sample was dialyzed against the same buffer that is 50mm phosphate buffer for overnight at 4°C. The dialyzed protein solution was used as purified fraction in purification study (Beygmoradi et al. 2018, Libantová et al. 2009).

Enzyme purification via Sephadex G-100 column

The protein solution obtained after dialysis was applied on the gel filtration column (2.5 x 45 cm) of Sephadex G-100 pre-equilibrated with 50mm phosphate buffer of pH 7. Then 5ml of concentrated enzyme solution was loaded on top of the column bed and elution was carried out with 50mm phosphate buffer. The fractions of 5ml were collected at a flow rate of 30ml/hour. Protein concentrations were determined by Lowry's method (Kim et al. 2003).

SDS-PAGE analysis of Chitinase enzyme

SDS-PAGE was performed to determine the molecular weight of enzyme. The precipitated and purified chitinase enzyme sample from each source was loaded on the SDS-PAGE gel composed of 12% separating gel and 5% stacking gel. The gel solutions were prepared according to the given composition and poured in the gel casting tray and allowed to solidify (Patel et al. 2010). The enzyme samples were mixed with sample buffer (gel loading dye) and incubated at 95°C for 5 min. After incubation, the samples were loaded into wells on the stacking gel. The samples were allowed to run alongside protein marker till the end of the separating gel. Then the gel was stained with 0.1% CBB R250 dye followed by de-staining to visualize the protein bands (Narayana and Vijayalakshmi 2009)

Statistical analysis

Design expert software 8.0.7.1 was used for statistical analyses at 5% level ($P = 0.05$). Data of chitinase production were fed into the design expert software and analyzed by it. The software has the function by which we can predict the production of chitinase within studied rang of all medium components. All the experiments were repeated three times (Akeed et al. 2020, Vaidya et al. 2003).

RESULTS AND DISCUSSION

Obtaining chitinase producing strain

A total 100 different soil samples were screened. Also, different soil samples screened from the Valsad coastline area using colloidal chitin agar medium for chitinolytic activity. Out of these, PZ6 isolate designated. Chitinase has been previously isolated from *Aeromonas* sp. (Halder et al. 2013, Korany et al. 2019, Stumpf et al. 2019). In the present investigation bacterial strain *Aeromonas dhankensis* PZ6 was identified as a potent chitinase producer on the basis of zone (14mm zone size) of chitin hydrolysis on an agar plate supplemented with 1% colloidal chitin and incubated at 35°C for 96 hours. Figure 1 shows PZ6 soil sample collection sites (Valsad, Gujarat (Latitude: 20°35'47.26''N, Longitude 72° 53' 58. 39''E).

Figure 1. PZ6 soil sample collection site Valasad Gujarat

Growth and chitinase production profile

Bacterial growth and chitinase synthesis increased from 24 to 96 hours of incubation (Fig. 2). After 96 hours of incubation the maximum enzyme activity was found, simultaneously bacterial growth entered into stationary phase and enzyme activity declined during 120 and 144 hours of incubation. Maximum enzyme activity 4.7 U/ml was obtained at 96 hours (Hungund et al. 2022).

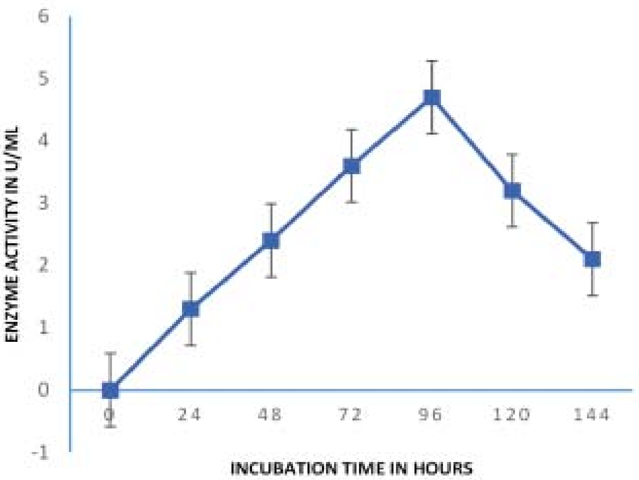

Figure 2. Growth and chitinase production profile of bacterial isolate PZ6

Optimization of chitinase production by one variable at a time (OVAT)

Effect of inoculum size

High enzyme production results from small inoculum sizes because they increase surface area to volume ratio and improve dissolved oxygen distribution. On the other hand if the inoculum size is too small insufficient numbers of bacteria are produced, which results in low enzyme production. To increase the production of enzymes, it is important to maximise the size of the inoculums(Kumar 2017). In our study 5% of the inoculum size gave maximum enzyme production (1.44U/ml)(DF=5, F-value=35.389, $P < 0.0001$) was observed. (Fig. 3).

Effect of incubation temperature

Since the organism is a mesophile very less growth hence low amount of enzyme production was observed at higher temperature of 45°C. Maximum enzyme production (1.45U/ml) (DF=4, F-value=1760.740, $P < 0.0001$) was observed at 35°C (Liu et al. 2019, Saima et al. 2013) (Fig. 4).

Effect of substrate concentration

Considerable increase in enzyme yield was achieved with substrate concentration. Various concentrations of substrate were investigated for enzyme yield and maximum enzyme production (1.52U/ml) (DF=5, F-value=40.221, $P<0.0001$) was achieved with 0.8% colloidal chitin (Fig.5) (Kumar 2017).

Effect of medium pH

At lower and higher pH values the isolate showed minimal growth, resulting in less enzyme production. In the pH range of 6 to 8 the organism grew well and pH 8 was found to be best under our experimental conditions (1.55U/ml) (DF=5, F-value=311.465, $P<0.0001$) was observed.(Fig. 6) (Saima et al. 2013, Liu et al. 2019).

Effect of carbon sources

It was found that some chitinase was produced when various carbon sources were used (Hungund et al. 2022). Added carbon sources have influential impact on chitinase production.Maximum production was achieved with glucose, giving the highest enzyme yield (2.12U/ml) (DF=7, F-value=1860.780, $P<0.0001$) while mannitol had least effect (Fig.7).This indicate furthure utilization of other raw and low cost carbon sources can be employed for cost effective fermentation.Addition of sugars was found to be beneficial in comparision with no sugar(Shivalee, Lingappa, and Mahesh 2018).

Effect of nitrogen source

The enzyme production is substantially impacted by nitrogen supplies just like carbon sources. Chitin can act as both source carbon and nitrogen in medium. The impact of additional nitrogen source was studied

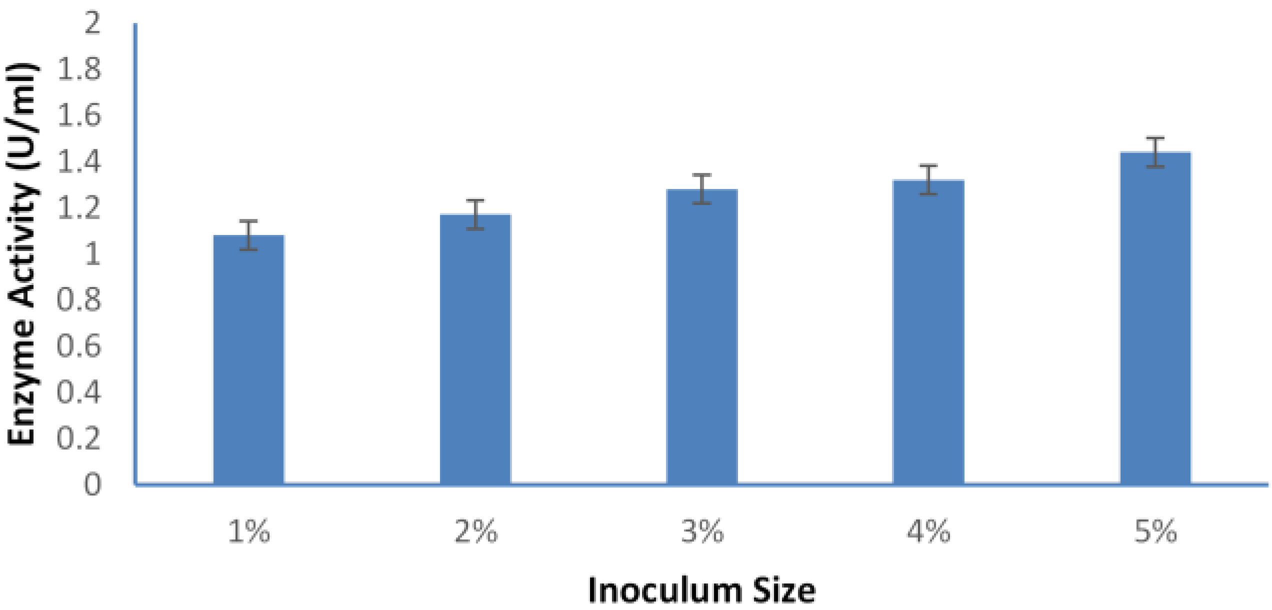

Figure 3. Influence of inoculum size on chitinase production by bacterial isolate PZ6

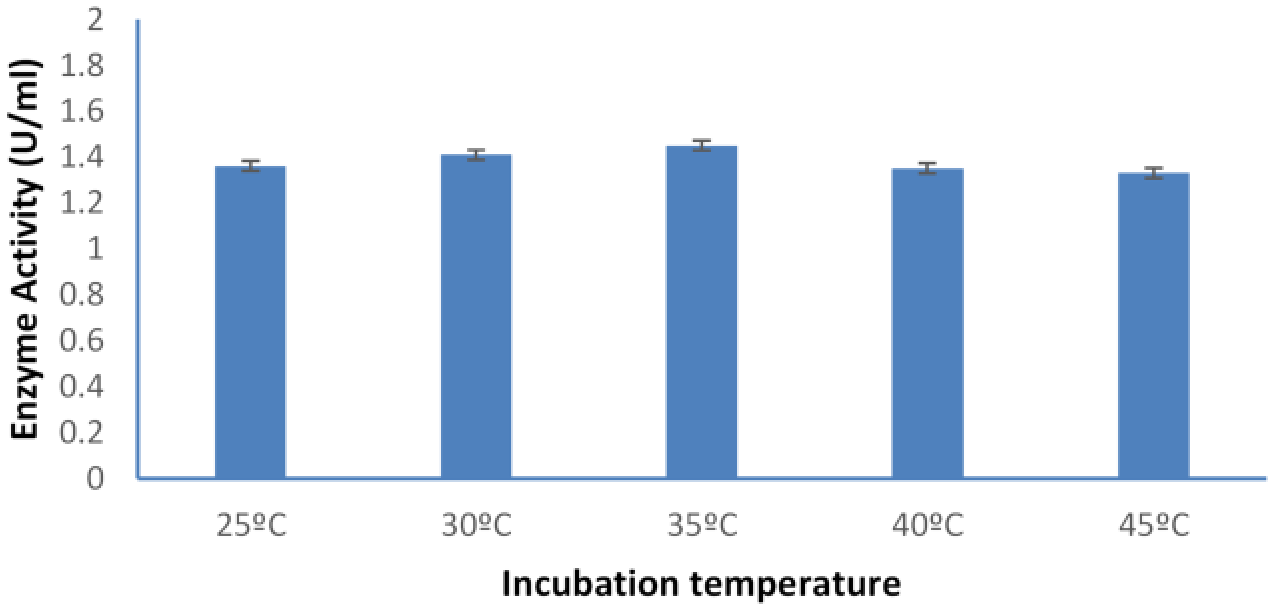

Figure 4. Influence of incubation temperature on chitinase production by bacterial isolate PZ6

in addition to chitin (Cheba et al. 2018). Among all majority nitrogen sources under study peptone increased the enzyme production and produced highest enzyme yield (2.68 U/ml) (DF=4, F-value=1123.940, P<0.0001) (Fig. 8).

Effect of metal ions

Various concentrations of metal ions were investigated for enzyme yield. Concentration of cobalt (0.1mg/ml) gave maximum enzyme production (3.4U/ml) (DF=4, F-value=180.211, P<0.0001) was observed. (Fig.9) (Cheba et al. 2016).

Purification of chitinase by ammonium sulfate precipitation

The chitinase enzyme was partially purified by precipitation with different concentration of ammonium sulphate. Maximum specific enzyme activity 0.724 U/mg/ml was obtained in the fraction having 65% of ammonium sulphate, whereas in crude extract it was found to be 0.653 U/mg/ml. The enzyme activity of purified enzyme increased (Table 1) and hence, specific enzyme activity increased by 41% (Hungund et al. 2022).

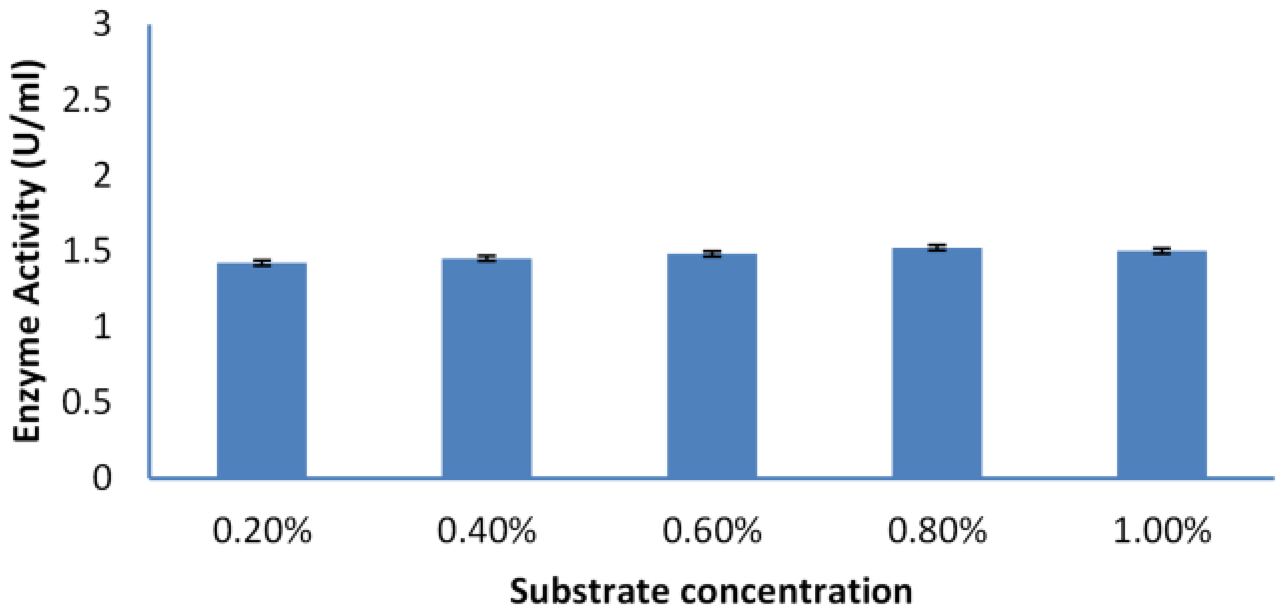

Figure 5. Influence of substrate concentration on chitinase production by bacterial isolate PZ6

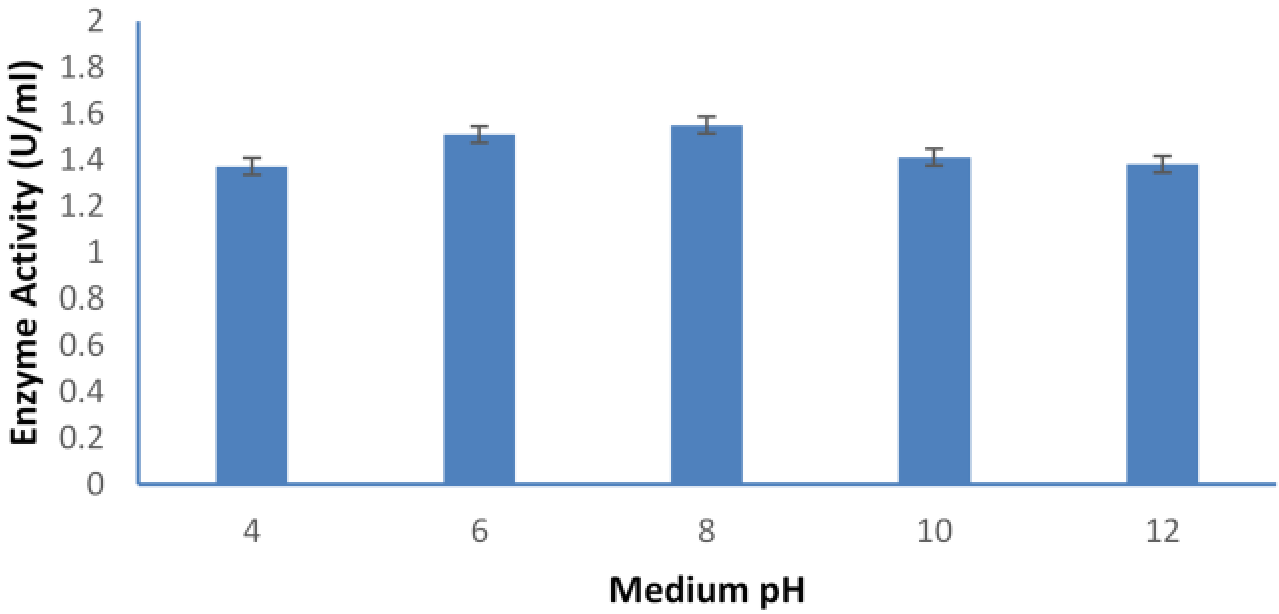

Figure 6. Influence of pH on chitinase production by bacterial isolate PZ6

Table 1. Purification of chitinase for bacterial isolate PZ6

Sample name	Total enzyme activity (U/ml)	Concentration of protein (mg/ml)	Specific activity (U/mg/ml)
Crude enzyme	3.47U	5.308	0.653
Precipitated enzyme (With ammonium sulphate)	3.52U	4.857	0.724
Purified enzyme (Dialyzed enzyme)	3.58U	3.891	0.920

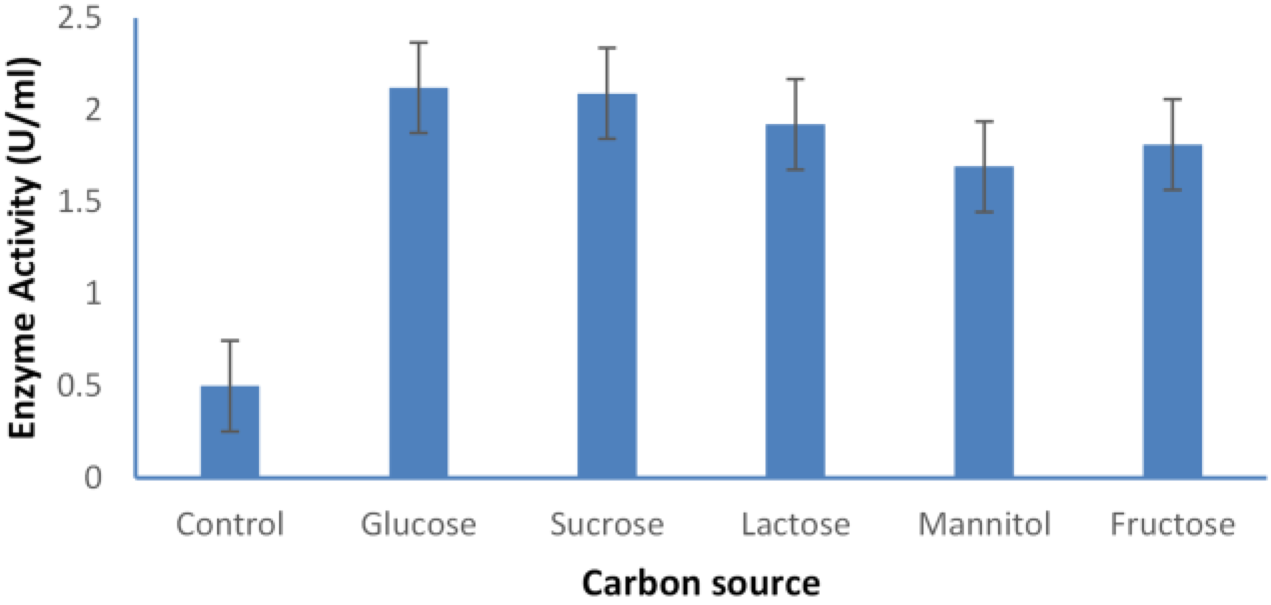

Figure 7. Influence of carbon source on chitinase production by bacterial isolate PZ6

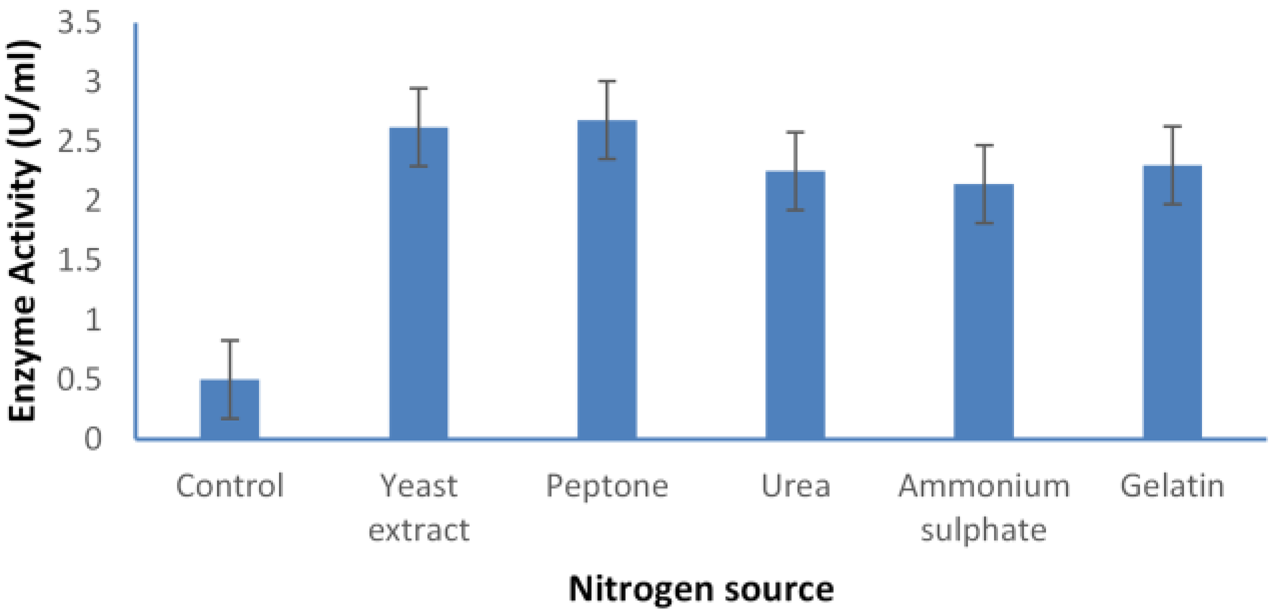

Figure 8. Influence of nitrogen sources on chitinase production by bacterial isolate PZ6

SDS-PAGE for Molecular Weight Determination

The SDS-PAGE analysis represents that the size of chitinase enzyme from our bacterial isolate lies 60kDa. The position of protein band in purified sample showed slightly above the 54kDa ladder band and very lower to the 71kDa band, hence the size of the enzyme is approximately 60kDa for chitinase obtained from PZ6 isolate (Fig.10).

CONCLUSIONS

As a part of vigorous searching for microbial tool to recycle naturally plentiful chitin, microbial chitinase consider to be a key solution. In our study we have characterized chitinase obtained from bacterial isolate PZ6. The optimization study demonstrated most efficient production of chitinase in a medium supplemented with glucose (1g/ml), peptone (1g/ml)

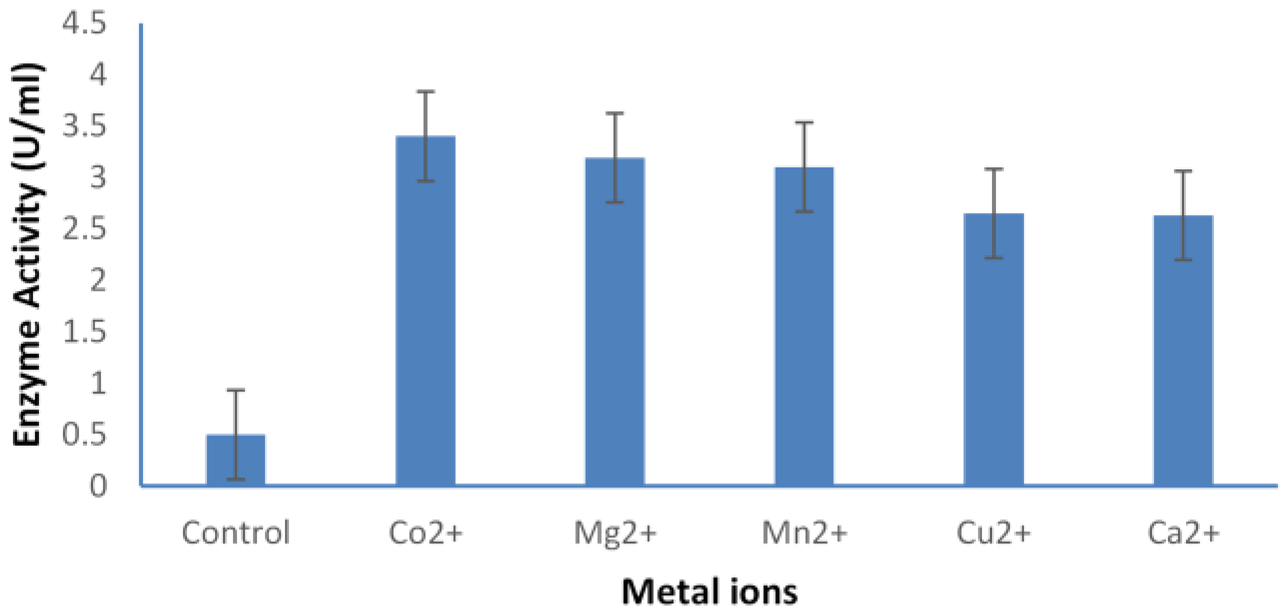

Figure 9. Influence of metal ions on chitinase production by bacterial isolate PZ6.

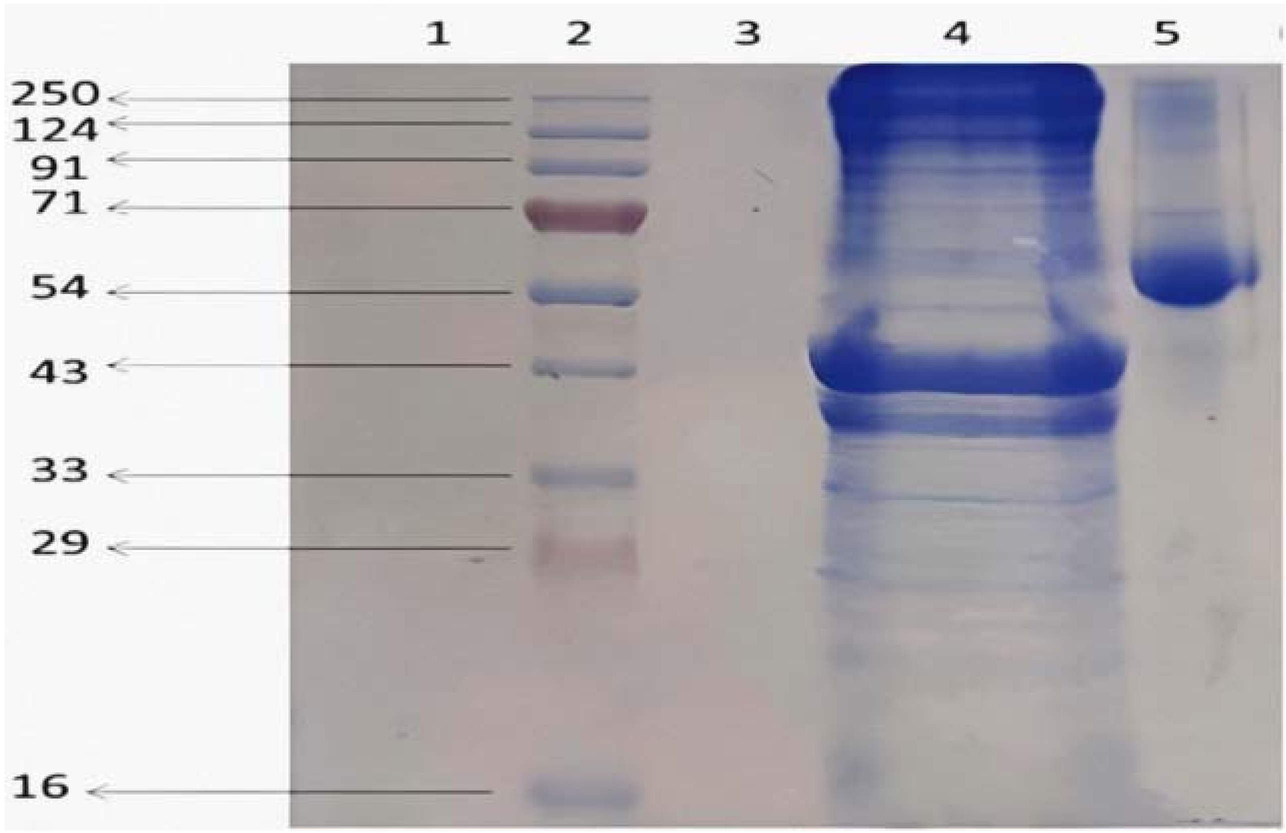

Figure 10. SDS-PAGE analysis for partial purification of chitinase enzyme, where lane 2- protein ladder with protein size in kDa; lane 4 and lane 5- crude and purified enzyme from bacterial chitinase.

and cobalt chloride (0.1mg/ml). Additionally, the alkaliphilic strain of bacteria was capable of producing highest level of enzyme at 0.8% (w/v) of colloidal chitin substrate. With a broad range of pH and other factors demonstrated that this enzyme could be a suitable tool as biological catalyst with applications in biotechnological and pharma industries. This bacterial enzyme if optimized further could be a potential tool for bio control with maintaining sustainable ecology.

Authors' contribution: All authors contributed equally

Conflict of interest: Author declared no conflict of interest

REFERENCES

Abidin, N., Kormin, F., Anuar, N. and Abu Bakar, M. 2020. The potential of insects as alternative sources of chitin: An overview on the chemical method of extraction from various sources. International Journal of Molecular Sciences, 21(14), 1-25. https://doi/10.3390/ijms21144978.

Akeed, Y., Faiza, A. and Walid, N. 2020. Partial purification and characterization of chitinase produced by *Bacillus Licheniformis B307*. Heliyon, 6(5), e03858. https://doi/10.1016/j.heliyon.

Beier, S. and Stefan, B. 2013. Bacterial chitin degradation-mechanisms and ecophysiological strategies. Frontiers in Microbiology, 4(6), 1-12. https://doi/10.3389/fmicb.

Beygmoradi, A., Ahmad, H., Roohullah, H., Daniel, H. and Jesús, F. 2018. Marine chitinolytic enzymes, a biotechnological treasure hidden in the ocean. Applied Microbiology and Biotechnology, 102(23), 9937-9948. https://doi/10.1007/s00253-018-9385-7.

Cheba, B., Taha Ibrahim, Z., Ahmad, R. and Mohamad, H. 2018. Effect of nitrogen sources and fermentation conditions on *Bacillus* sp. *R2* chitinase production. Procedia Manufacturing, 22, 280-287. Elsevier B.V. https://doi/10.1016/j.promfg.2018.03.043

Cheba, B., Taha Ibrahim, Z., Mohamad, H. and Ahmad, R. 2016. Effect of metal ions, chemical agents, and organic solvent on *Bacillus* sp. *R2* chitinase activity. Procedia Technology, 22, 465-470. https://doi/10.1016/j.protcy.2016.01.090.

Crini, G. 2019. Historical review on chitin and chitosan biopolymers. Environmental Chemistry Letters, 17, 1623-1643. https://doi.org/10.1007/s10311-019-00901-0.

Dahiya, N., Rupinder, T. and Gurinder Singh, H. 2006. Biotechnological aspects of chitinolytic enzymes: A review. Applied Microbiology and Biotechnology, 71(6), 773-782. https://doi/10.1007/s00253-005-0183-7.

Farag, M., Hanan, M., Hassan, I. and Moustafa, S. 2016. Purification, characterization and antimicrobial activity of chitinase from marine-derived *Aspergillus Terreus*. Egyptian Journal of Aquatic Research, 42(2), 185-192. https://doi/10.1016/j.ejar.2016.04.004.

Halbedel, S., Rita P., Sangeeta, B., Sylvia, K., Eva, T., Gopala, N., Georg, A., Christina H., Friederike, S., Ariane P., Dirk S. and Antje, F. 2019. A *Listeria Monocytogenes ST2* clone lacking chitinase ChiB from an outbreak of non-invasive gastroenteritis. Emerging Microbes and Infections, 8(1), 17-28. https://doi/10.1080/22221751.2018.1558960.

Halder, K., Chiranjit, M., Arijit, J., Arpan, D., Tanmay, P., Das, M.P.K., Ranjan, P.B. and Chandra, M.K. 2013. Proficient biodegradation of shrimp shell waste by *Aeromonas hydrophila SBK1* for the concomitant production of antifungal chitinase and antioxidant chitosaccharides. International Biodeterioration and Biodegradation, 79, 88-97. https://doi/ 10.1016/j.ibiod.2013.01.011.

Hoell, I.A., Vaaje-Kolstad, G. and Eijsink, V.G.H. 2010. Structure and function of enzymes acting on chitin and chitosan. Biotechnology and Genetic Engineering Reviews, 27(1), 331-366. https://doi/ 10.1080/02648725.2010.10648156.

Hsu, S.C. and Lockwood, J.L. 1975. Powdered chitin agar as a selective medium for enumeration of *Actinomycetes* in water and soil. Applied Microbiology, 29, 422-426. https://doi/10.1128/am.29.3.422-426.

Hungund, S., Gururaj, T., Sharanappa, A. and Nagaraj, D.M. 2022. Production and partial purification of thermostable chitinase from *Bacillus cereus BSH-4* and its antifungal activity. Journal of Biochemical Technology, 13(2), 46-53. https://doi/10.51847/ralf1h5bce.

Hurtado, G.R. and van Aalten, D.M.F. 2007. Structure of *Saccharomyces cerevisiae* chitinase 1 and screening-based discovery of potent inhibitors. Chemistry and Biology, 14(5), 589-599. https://doi/10.1016/j.chembiol.2007.03.015.

Iber, B., Nor Azman, K., Donald, T. and Jenifer, W. 2022. A review of various sources of chitin and chitosan in nature. Journal of Renewable Materials, 10(4), 1097-1123. https://doi.org/10.32604/jrm.2022.018142.

Itoh, T. and Hisashi, K. 2019. Bacterial chitinase system as a model of chitin biodegradation. Pp. 131-151. In: Advances in Experimental Medicine and Biology, Springer.

Kim, K., Yong-Joon, Y. and Jong-Gi, K. 2003. Purification and characterization of chitinase from *Streptomyces* sp. *M-20*. Journal of Biochemistry and Molecular Biology, 36(2), 185-189. https://doi.org/10.5483/BMBRep.2003.36.2.185.

Korany, S.M., Mansour, A.N., El-Hendawy, H.H., Kobisi, A.N.A. and Aly, H.H. 2019. Entomopathogenic efficacy of the chitinolytic bacteria: *Aeromonas hydrophila* isolated from Siwa Oasis, Egypt. Egyptian Journal of Biological Pest Control, 29(1), 1-10. https://doi /10.1186/s41938-019-0116-x.

Kumar, A., Naveen Kumar, G., Steffy, A., Monika, R. and Gupta, N. 2017. Process optimization of extracellular chitinase production from *Bacillus* sp. isolated from fish waste dumping site application of nanofluids in plate heat exchanger view project. European Journal of

Pharmaceutical and Medical Research, 4(9), 474-480.

Kusaoke, H., Shoko S., Tamo, F. and Hisashi, K. 2017. Biochemical and biotechnological trends in chitin, chitosan, and related enzymes produced by *Paenibacillus IK-5 strain.* International Journal of Biological Macromolecules, 104, 1633-1640. https://doi/10.1016/j.ijbiomac.

Lee, Y., Ju Soon, Y., Soo Yeol, C., Young Choon, L., Young Su, C., Soon Cheol, A., Cheol Min, K. and Yong Lark, C. 2007. Cloning, purification, and characterization of chitinase from *Bacillus* sp. *DAU101*. Bioresource Technology, 98(14), 2734-2741.

Libantová, J., Terttu K., Jana, M., Ildikó, M. and Jan, S. 2009. Detection of chitinolytic enzymes with different substrate specificity in tissues of intact sundew (*Drosera rotundifolia* L.): chitinases in sundew tissues. Molecular Biology Reports, 36(5), 851-56. https://doi/10.1007/s11033-008-9254-z.

Liu, J., Ziyan, N., Jie, Z., Ping, S., Yin, T., Yue, G. and Qinying, W. 2019. Expression and characterization of two chitinases with synergistic effect and antifungal activity from *Xenorhabdus nematophila*. World Journal of Microbiology and Biotechnology, 35(7), 1-10. https://doi/10.1007/s11274-019-2670-5.

Martinou, A., Kafetzopoulos, D. and Bouriotis, V. 1995. Chitin deacetylation by enzymatic means: Monitoring of deacetylation processes. Carbohydrate Research, 273(2), 235-242. https://doi/10.1016/0008-6215(95)00111-6.

Monreal, J. and Reese, E.T. 1969. The chitinase of *Serratia marcescens*. Canadian Journal of Microbiology, 15(7), 689-696. https://doi/10.1139/m69-122.

Narayana, J.P. and Muvva, V. 2009. Chitinase production by *Streptomyces* sp.*ANU 6277*. Brazilian Journal of Microbiology, 40(4), 725-733. https://doi/10.1590/S1517-83822009000400002.

Patel, A., Vijay Kumar, S., Ravi Prakash, Y. and Arthur, M. 2010. Purification and characterization of a new chitinase from latex of *Ipomoea carnea*. Process Biochemistry, 45(5), 675-681. https://doi/10.1016/j.procbio.2009.12.016.

Patil, S., Vandana, G. and Deshpande, M.V. 2000. Chitinolytic enzymes: An exploration. Enzyme and Microbial Technology, 26(7), 473–483. https://doi/10.1016/S0141-0229(00)00134-4.

Ralph Berger, L. and Donald, M. 1958. The chitinase system of a strain of *Streptomyces griseus*. BBA - Biochimica et Biophysica Acta, 29(3), 522-534. https://doi/10.1016/0006-3002(58)90008-8.

Rinaudo, M. 2006. Chitin and chitosan: Properties and applications. Progress in Polymer Science, 31(7), 603-632. https://doi/10.1016/j.progpolymsci.2006.06.001.

Robbins, P.W., Albright, C. and Benfield, B. 1988. Cloning and expression of a *Streptomyces plicatus* chitinase (Chitinase-63) in *Escherichia coli*. Journal of Biological Chemistry, 263(1), 443-447. https://doi/10.1016/s0021-9258(19)57412-9.

Setia, I.N. and Suharjono. 2015. Chitinolytic assay and identification of bacteria isolated from shrimp waste based on 16S RDNA sequences. Advances in Microbiology, 05(07), 541-48. https://doi/10.4236/aim.2015.57056.

Sharma, N., Shuchi K. and Rajesh Singh, T. 2021. Isolation and characterization of extracellular enzyme (Glutaminase and Urease) producing bacteria isolated from soil samples of different regions of Gwalior, Madhya Pradesh, India. Research Journal of Biotechnology, 16(7), 122-129. https://doi/10.25303/167rjbt12221.

Shivalee, A., Lingappa, K. and Divatar, M. 2018. Influence of bioprocess variables on the production of extracellular chitinase under submerged fermentation by *Streptomyces pratensis* strain *KLSL55*. Journal of Genetic Engineering and Biotechnology, 16(2), 421-426. https://doi/ 10.1016/j.jgeb.2017.12.006.

Stumpf, A.K., Marina, V., Dirks-Hofmeister, M.E., Moerschbacher, B.M. and Bodo, P. 2019. Identification of a novel chitinase from *Aeromonas hydrophila AH-1N* for the degradation of chitin within fungal mycelium. FEMS Microbiology Letters, 366(1), fny294. https://doi/10.1093/femsle/fny294.

Sun, X., Yingjie, L., Zhennan, T., Yuanchao, Q., Huaiqiang, Z. and Lushan, W. 2019. A novel thermostable chitinolytic machinery of *Streptomyces* sp. *F-3* consisting of chitinases with different action modes. Biotechnology for Biofuels, 12(1), 1-12. https://doi/10.1186/s13068-019-1472-1.

Swiontek-Brzezinska, M., Lalke-Porczyk, E. and Donderski, W. 2007. Chitinolytic activity of bacteria and fungi isolated from shrimp exoskeletons. Oceanological and Hydrobiological Studies, 36 (3), 101-111. https://doi/10.2478/v10009-007-0019-0.

Vaidya, R.J., Macmil, S.L.A., Vyas, P.R. and Chhatpar, H.S. 2003. The novel method for isolating chitinolytic bacteria and its application in screening for hyperchitinase producing mutant of *Alcaligenes xylosoxydans*. Letters in Applied Microbiology, 36(3), 129-134. https://doi/10.1046/j.1472-765X.2003.01274.x.

Veliz, A., Pilar, H. and Hirsch, A.M. 2017. Chitinase-producing bacteria and their role in biocontrol. AIMS Microbiology, 3(3), 689-705. https://doi/10.3934/microbiol.2017.3.689.

Wang, K., Pei Sheng, Y., Qing, L.D., Qin Xi, W., Zhong, W. and Jie, P. 2013. Diversity of culturable root-associated/endophytic bacteria and their chitinolytic and aflatoxin inhibition activity of peanut plant in China. World Journal of Microbiology and Biotechnology, 29(1), 1-10. https://doi/10.1007/s11274-012-1135-x.

Yadav, M., Priynshi, G., Kunwar, P., Manish, K. and Nidhi, P. 2019. Seafood waste: A source for preparation of commercially employable chitin/chitosan materials. Bioresources and Bioprocessing, 6(1), art8. https://doi/10.1186/s40643-019-0243-y.

Zhang, A., Cong, G., Jing, W., Kequan, C. and Pingkai, O. 2016. An efficient enzymatic production of N-acetyl-d-glucosamine from crude chitin powders. Green Chemistry, 18(7), 2147-2154. https://doi/10.1039/c5gc02242h.

Received:23rd November 2022
Accepted: 13th February 2023