SHORT COMMUNICATION

Molecular characterisation of *Bacillus* chitinase for bioconversion of chitin waste

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In this work chitin was extracted chemically from shrimp shells. Seventeen *Bacillus* isolates were screened for chitinolytic activity. The chitinolytic strains of *Bt* were screened at different temperatures and pHs for their hydrolytic potentials. By using a pair of specific primers, endochitinase gene was amplified from SBS Bt-5 strain through PCR, and then cloned into pTZ57 TA cloning vector and transferred in *Escherichia coli* DH5\(^a\) strain. The sequenced gene (GenBank Accession No: HE995800) consists of 2031 nucleotides capable of encoding 676 residues. The protein consisted of three functional domains with a calculated molecular mass of 74.53 kDa and a pI value of 5.83. The amino acid sequence of chi gene showed 99% similarity to the genes of *Bt* MR11 endochitinase, *Bt* serovar kurstaki chitinase (*kch*), *Bt* strain MR21 endochitinase and *Bacillus cereus* B4264.

**Keywords:** N-acetyl-D-glucosamine; chitin; shrimp shells; endochitinase; chitinolytic bacteria

1. Introduction

Chitin is a biopolymer naturally present in the exoskeleton of Crustacea, Insecta, Arachnida, kingdom Fungi, the phyla Porifera and Cnidaria (Gardner & Blackwell 1975; Kaya et al. 2014). Although all these organisms have chitin in their bodies, crab and shrimp shells are the main commercial sources of chitin (Gardner & Blackwell 1975). Chitin degrading activity has been repeated for several species of the genus *Bacillus* including (*brevis, megaterium, licheniformis* and *thuringiensis*) (Watanabe et al. 1990). Chitinases are produced by *Bacillus thuringiensis*, but in smaller quantities, and the enzyme yields can be increased by genetic manipulation...
(Casique-Arroyo et al. 2007). In GenBank database (www.ncbi.nlm.nih.gov), at least 21 chitinase gene sequences from B. thuringiensis have been deposited; however, characterisation of only six of the encoded proteins has been completed (Zhong et al. 2003). In this study, chitin was purified from the exoskeleton of shrimps and chitin degrading Bacillus sp. is screened and the one with high chitinolytic activity was selected. The specific gene was identified, amplified, cloned and characterised. Hence this study is a significant step forward in utilisation of B. thuringiensis as a source of chitin degradation enzyme.

2. Results and discussion

2.1. Purification and qualitative analysis of chitin

In this work, 2.13 g of dried purified chitin powder was obtained from 5 g of dried shells and the percentage recovery was 42%. The FT-IR analysis confirmed the presence of α chitin (Supplementary Figure S1). Deproteination with 2% NaOH, and demineralisation with 5% HCl at 50°C, in terms of final ash and chitin content and yield, have been documented as best (Pinelli-Saavedra et al. 1998). Chitinases from B. thuringiensis are poorly studied (Ramirez et al. 2002), although interest has slowly increased due to their potential role as bio-control agents for insects and plant-pathogenic fungi, as well as a means to exploit shrimp wastes (Ramirez et al. 2003).

2.2. Chitinolytic activity at different pHs

Ratio between hydrolysis zone and growth of the chitinolytic B. thuringienesis showed various trends at different pHs after 24, 48, 72 and 96 h of incubation. CMBL Bt-4 (1.33 ± 0.015) and CMBL Bt-5 (1.39 ± 0.027) showed maximum zones at pH 8.5 and 10.0, respectively, after 96 h. SBS Bt-3 and SBS Bt-5 showed optimal chitin digestion (1.1 ± 0.002) and (1.1 ± 0.004) at neutral pH 7.0. SBS Bt-1 produced maximum zone of clearance (1.07 ± 0.002) at 5.5 pH. (Supplementary Table S1)

2.3. Chitinolytic activity at different temperatures

Chitinolytic bacterial strains were cultured at four different temperatures and incubated for 96 h. At 30°C all the strains produced less but very clear and defined chitin hydrolysis zones. SBS Bt-4 showed highest hydrolysis zone at 25°C, SBS Bt-1 at 30°C and SBS Bt-5 at 37°C. At 45°C incubation after 96 h growth diminished and a clear zone was produced showing only lines of growth in some strains such as SBS Bt-1, SBS Bt-5 and SBS Bt-6, perhaps due to release of endospores from vegetative cells (Supplementary Table S1).

2.4. DNA isolation and amplification of chitinase gene

Genomic DNA isolation of bacterial strains was confirmed by running the samples on 1% agarose gel, and eight DNA samples of positive strains were subjected to PCR amplification using the designed primers of the chitinase gene. SBS Bt5 yielded chitinase gene fragment of approximately 2300 bp in length by PCR amplification.

2.5. Restriction T/A cloning and sequencing of chiB gene

The amplified chi gene was cloned in pTZ57R/T cloning vector and transformed into Escherichia coli DH5α cells. Positively screened clones were confirmed by double restriction fragmentation using Eco RI and Hind III restriction enzymes (Supplementary Figure S2).
Plasmids were prepared from the confirmed positively screened clones and sequenced using M13 forward and reverse primers. The sequence of the chitinase gene was deposited in GenBank under Accession number: HE995800.

2.6. Protein structure of endochitinase

The predicted protein has 676 amino acids, consisting of three domains and had maximum similarity with ChiB from the predicted pI value of 5.83 and molecular weight of 74.53 kDa. Total number of negatively charged residues (Asp + Glu) and positively charged residues (Arg + Lys) are 71 and 64, respectively. Formula of endochitinase deduced from protparam is C$_{3334}$H$_{5064}$N$_{882}$O$_{1037}$S$_{14}$. The estimated half-life of protein is >10 h (E. coli, in vivo). The instability index (II) computed is 21.33, so the protein is classified as stable (http://web.expasy.org/cgibin/interpro_sacc/interpro_sacc).

3. Conclusion

The chitinolytic strains of Bt. were screened at different temperatures and pHs for their hydrolytic potentials. It was found that endochitinase gene consists of 2031 nucleotides capable of encoding 676 residues. The protein consisted of three functional domains with a calculated molecular mass of 74.53 kDa and a pI value of 5.83. The amino acid sequence of chi gene showed 99% similarity to the genes of Bt MR11 endochitinase, Bt serovar kurstaki chitinase (kchi), Bt strain MR21 endochitinase and Bacillus cereus B4264. Hence the strains observed in this work has a good potential of chitin degradation.

Supplementary material

Experimental details relating to this paper are available online, alongside Supplementary Table S1 and Supplementary Figures S1 and S2 http://dx.doi.org/10.1080/14786419.2015.1040789.

Disclosure statement

No potential conflict of interest was reported by the authors.

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