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Full Length Research Paper

Optimization of cultural conditions for production of chitinase by *Bacillus laterosporous* MML2270 isolated from rice rhizosphere soil

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A total of 39 chitinolytic bacteria were isolated from 77 rhizosphere soil samples collected from different crop fields in Tamil Nadu state, India. Among them, a strain designated as MML2270, which produced highest chitinolytic activity in primary and secondary screening in colloidal chitin agar was selected and later identified as *Bacillus laterosporous*. The production of chitinase by *B. laterosporous* was optimized using different growth media, substrate concentrations, pH, temperature and incubation period. The maximum chitinase production was observed in yeast nitrogen based medium (YNB) amended with 0.3% colloidal chitin at pH 8.0 and 35°C after four days of inoculation. Under this optimized growth condition, *B. laterosporous* MML2270 produced a total chitinase activity of 59.05 units/ml as against only 19.7 units/ml in the initial YNB medium stage, which is a three-fold increase.

Key words: Chitinolytic bacteria, *Bacillus laterosporous*, chitinase, culture conditions.

INTRODUCTION

Chitin, a β-1,4-linked polymer of N-acetyl-D-glucosamine, is one of the most abundant polysaccharides in nature, next to cellulose. It is a major cell wall constituent of higher fungi belonging to chitridiomycetes, ascomycetes, basidiomycetes and deuteromycets, insect exoskeletons and crustacean shells (Flach et al., 1992; Felse and Panda, 1999). Interestingly, the presence of chitin in the cyst wall of human pathogen, *Entamoeba histolytica* was demonstrated (Das et al., 2006). Therefore, chitinases, the hydrolytic enzymes that specifically degrade chitin, are gaining much attention worldwide (Pichyangkura et al., 2002; Gkargkas et al., 2004; Makino et al., 2006; Wang et al., 2006). Chitinases (EC 3.2.1.14) are produced by several bacteria (Mabuchi et al., 2000; Someya et al., 2001; Wen et al., 2002; Huang et al., 2005; Hobel et al., 2005; Ajit et al., 2006) actinomycetes (El-Tarabily et al., 2000; Akagi et al., 2006), fungi (Mathivanan et al., 1998; Viterbo et al., 2001) and also by higher plants (Mauch et al., 1984; Collinge et al., 1993; Graham and Sticklen, 1994; Ancillo et al., 1999; Takakura et al., 2000; Troedsson et al., 2005; Matsushima et al., 2006). These chitinases are used in various applications such as biological control of fungal pathogens (Chernin et al., 1997; Mathivanan et al., 1998; Someya et al., 2001; de la Vega et al., 2006; Chang et al., 2007), preparation of oligosaccharides and N-acetyl D-glucosamine (Pichyangkura et al. 2002; Wang et al., 2006; Makino et al., 2006) and protoplasts from filamentous fungi (Balasubramanian et al., 2003; Prabavathy et al., 2006; Yano et al., 2006). Microorganisms produce the chitinase primarily for assimilation of chitin as carbon and (or) nitrogen source (Flach et al., 1992; Kupiec and Chet, 1998).

Chitinases have been isolated from variety of bacteria including *Bacillus* spp. and some of them are reported to produce multiple forms of chitinases with different molecular masses (Vaidya et al., 2001; Wen et al., 2002;

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Someya et al., 2003; Woo and Park, 2003; Dahiya et al., 2005; Ajit et al., 2006). In this present study a number of chitin degrading bacteria were isolated from soil samples collected from agricultural fields of Tamil Nadu, India. Among them, a bacterial isolate designated as MML2270, which later identified as *Bacillus laterosporus* was found to produce high amount of chitinase. Although chitinase production was reported in different species of *Bacillus* such as *Bacillus amyl-oliquefaciens* (Sabry, 1992), *Bacillus cereus* (Chang et al., 2007), *Bacillus circulans* (Chen et al., 2004), *Bacillus licheniformis* (Waldeck et al., 2006), *Bacillus megaterium* (Sabry, 1992), Bacillus pabuli (Frandberg and Schnurer, 1994), *Bacillus stearothermophilus* (Sakai et al., 1994), *Bacillus subtilis* (Wang et al., 2006), *Bacillus thuringiensis* sub sp. *aizawai* (de la Vega et al., 2006), *B. thuringiensis* sub sp. *kurstaki* (Driss et al., 2005), our literature survey revealed that there was no report on the production of chitinase by *B. laterosporus*. Therefore, this isolate was selected and its growth conditions were standardized in order to optimize the chitinase production.

MATERIALS AND METHODS

Preparation of colloidal chitin

Colloidal chitin was prepared from the chitin flakes (Sigma Chemicals Company, USA) by the method of Mathivanan (1995). The chitin flakes were ground to powder and added slowly to 10 N HCl and kept overnight at 4°C with vigorous stirring. The suspension was added to cold 50% ethanol with rapid stirring and kept overnight at 25°C. The precipitate was collected by centrifugation at 10000 rpm for 20 min and washed with sterile distilled water until the colloidal chitin became neutral (pH 7.0). It was freeze dried to powder and stored at 4°C until further use.

Isolation of chitin degrading bacteria

A total of 77 different soil samples were collected from agricultural fields in six different southern districts of Tamil Nadu State, India. Chitin degraders were isolated by serial dilutions of soil samples and plated on 0.5% colloidal chitin agar (CCA) medium. After 48 h of incubation at room temperature, the isolates capable of degrading chitin with distinct zone of clearance on CCA were selected and sub cultured in NB slants and maintained.

Primary screening of chitin degrading bacteria

Primary screening was performed by spot inoculating all the chitin degrading bacterial isolates on CCA using toothpick heads of 2 mm diameter and incubated at room temperature. The zone of clearance due to chitin hydrolysis was recorded up to 5 days. The bacterial isolates producing clear zones over 0.5 cm alone were selected and subjected to secondary screening.

Secondary screening of chitin degrading bacteria

Secondary screening was performed with the culture filtrates of the 11 selected bacterial isolates using well diffusion method. All the 11 isolates were grown in Nutrient broth (NB) containing 1% colloidal chitin. One ml of each test bacterial inoculum with 0.5 OD was inoculated to 100 ml of medium and incubated at 100 rpm in a rotary shaker at room temperature. After two days of incubation, the cultures were harvested, centrifuged at 10000 rpm for 15 min at 4°C and the supernatant was collected. Colloidal chitin (0.5%) agar plates were prepared and wells were made using 9 mm sterile cork borer. Culture filtrate of each isolate was placed at 100 µl in each well and incubated at 37°C. After 12 h, the development of clear zone around the well was observed.

Identification of chitinolytic bacterium MML2270

The identification of bacterial isolate MML2270 was carried out by the methods suggested in the Bergey's Manual of Systematic Bacteriology (Williams et al., 1989).

Optimization of culture conditions

Medium

Three different media namely NB, Luria Bertaini broth (LB) and yeast nitrogen base broth (YNB) amended with 1% colloidal chitin were used to determine the growth and chitinase production. 1 mL inoculum of *B. laterosporus* MML2270 with 0.5 OD was inoculated with 100 ml of each medium and incubated at 100 rpm in a rotary shaker at room temperature. After two days of incubation, the cultures were harvested, centrifuged at 10000 rpm for 15 min and the supernatant was used for chitinase assay.

Substrate concentration, pH and temperature

B. laterosporus MML2270 was grown at different concentrations (0.1 - 0.5%) of colloidal chitin amended YNB broth to determine the optimum concentration of substrate for chitinase production. In addition, the bacterium was grown at different pH (4 - 10) and temperature (20 - 40°C) in YNB amended with 0.3% of colloidal chitin to determine the optimum pH and temperature for chitinase production. After two days of growth, the cultures were harvested, centrifuged and the supernatant was used for chitinase assay.

Incubation period

B. laterosporous was grown in YNB broth with optimized growth conditions (0.3% colloidal chitin, pH 8.0 and temperature 35°C) up to 10 days. At every two days interval, the production of chitinase in the culture filtrate was assayed.

Chitinase assay

The reaction mixture contained 1 ml of 0.1% colloidal chitin in sodium acetate buffer (0.05 M, pH 5.2) and 1 ml culture filtrate was incubated at 37ºC for 2 h in a water bath with constant shaking. Suitable substrate and enzyme blanks were included. Chitinase activity was assayed by the colorimetric method of (Reissig et al., 1955). The reaction was terminated by adding 0.1 ml of 0.08 M potassium tetraborate, pH 9.2 to 0.5 ml of reaction mixture and then boiled in a water bath for 3 min. Then 3 ml of diluted p-dimethylaminobenzaldehyde (p-DMAB, Sigma Chemicals Company, USA) reagent was added and again incubated at 37ºC for 15 min. The released product in the reaction mixture was read at 585

Figure 1. Clear zone produced by the isolate MML2270 due to chitin hydrolysis in 0.5% colloidal chitin agar.

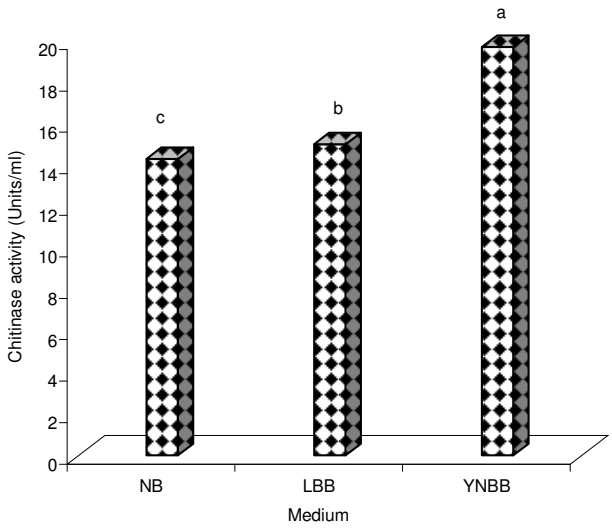

Figure 2. Production of chitinase by *B. laterosporous* MML2270 in different media [Nutrient broth (NB) Luria Bertaini broth (LBB) and yeast nitrogen base broth (YNBB)] amended with 0.1% colloidal chitin. Values are mean of three replicates. Values with different letters are significantly different at 5% level.

nm in a spectrophotometer (Hitachi, Japan). Chitinase activity was determined using N-acetylglucosamine (Sigma Chemicals Company, USA) as the standard.

One unit of chitinase activity was defined as the amount of enzyme, which produces 1 µ mole of N-acetylglucosamine in 1 ml of reaction mixture under standard assay condition (Mathivanan et al., 1998).

Statistical analysis

The experimental data were analyzed statistically by one-way ANOVA using Agres Statistical Software, Version 3.01 (1994).

RESULTS

Isolation of chitinolytic bacteria

A total of 39 different chitinolytic bacteria were isolated from 77 soil samples collected from six districts of Tamil Nadu state, south India. Of which, 16 were isolated from the soils with the pH between 6.0 and 6.5 and remaining bacteria were obtained from pH 7.0 to 7.8 (Table 1).

Among the 39 chitinolytic bacteria, only 11 isolates produced zone of clearance over 0.5 cm. Interestingly, a bacterial isolate obtained from the rice rhizosphere soil, designated as MML2270 remarkably hydrolyzed the colloidal chitin and produced a prominent and maximum clear zone in CCA plate (Figure 1).

Among the 11 chitinolytic bacteria tested in the secondary screening, the culture filtrate of the rice rhizosphere isolate MML2270 produced maximum clear zone of 1.1 cm in CCA. The clear zones due to hydrolysis of colloidal chitin by the culture filtrates of the remaining 10 bacteria were ranged between 0.6 and 0.8 cm (Table 2).

Results of Table 3 revealed that the isolate MML2270 is a Gram-positive, endospore forming, rod shaped bacterium with catalase and oxidase positive reactions. Based on the physiochemical characteristics, the isolate MML2270 was identified as *B. laterosporous*.

Optimization of culture conditions for chitinase production

Among three media tested, YNB supported high chitinase production of 19.7 units/ml as compared to 15.0 and 14.3 units/ml, respectively in LBB and NB (Figure 2).

Result on the effect of different concentrations of colloidal chitin on chitinase production is presented in Figure 3. Among five different concentrations tested, colloidal chitin at 0.3% considerably enhanced the chitinase activity (26.12 units/ml) followed by 0.2% (24.12 units/ml). Beyond 0.3%, the substrate concentrations decreased the enzyme activity.

Among different pH tested, pH 8.0 in YNB medium favoured the chitinase production at the maximum of 32.0 units/ml as against 0 – 27.1 units/ml in rest of the pH. Interestingly, there was no chitinase production at pH 4.0 (Figure 4). Qualitative assay determined by hydrolysis of colloidal chitin has also revealed that the chitinase production was high in culture filtrate of *B. laterosporus* at pH 8.0 (Figure 5) than the culture filtrates of other pH.

Among different temperature tested, *B. laterosporus* produced maximum chitinase activity of 42.93 units/ml at 35°C. The chitinase activity in rest of the temperature ranged between 2.11 and 29.33 units/ml. It has been observed that in both the lower and higher temperatures (20 and 40°C), the chitinase activity was sharply de-

Table 1. Isolation of chitinolytic bacteria from rhizosphere soils in different districts of Tamil Nadu, India

Sampling location	Number of soil samples	Name of the crop fields	pH of the soils	Number of chitinolytic bacteria isolated
Thanjavur	13	Rice, banana and sugarcane	6.2 - 6.5	7
Chengalpattu	12	Rice and groundnut	7.2 - 7.6	5
Karur	13	Cotton, turmeric and rice	7.0 - 7.3	5
Coimbatore	13	Rice, cotton and sugarcane	7.2 - 7.6	9
Madurai	14	Rice	6.0 - 6.3	9
Puthukottai	12	Rice and groundnut	7.6 - 7.8	4
Total	77			39

Table 2. Secondary screening of chitinolytic bacteria on colloidal chitin agar.

Bacterial isolate	Zone of clearance (cm)
MML2265	0.6
MML2266	0.6
MML2267	0.7
MML2268	0.7
MML2269	0.6
MML2270	1.1
MML2271	0.6
MML2272	0.7
MML2273	0.8
MML2274	0.6
MML2275	0.6

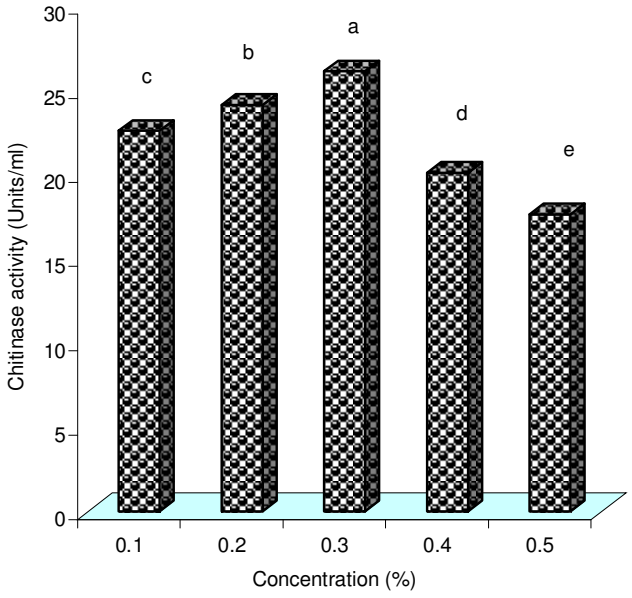

Figure 3. Production of chitinase by *B. laterosporous* MML2270 in different concentrations of colloidal chitin amended yeast nitrogen base broth (YNBB). Values are mean of three replicates. Values with different letters are significantly different at 5% level.

Table 3. Physiochemical characteristics of the isolate MML2270.

Test	Result
Gram staining	+ve
Motility	+ve
Citrate	-ve
Urease	-ve
Glucose	+ve
TSI	Acid butt/alkaline slant/H2S-ve/Gas-ve
Arabinose	-ve
Fructose	-ve
Lactose	+ve
Sucrose	-ve
Maltose	-ve
Starch	-ve
Chitin	+ve
Gelatin	+ve
Indole	-ve
Nitrate	+ve
Endospore	+ve
Tween 20	-ve
Tyrosine	+ve

creased (Figure 6).

It was observed that *B. laterosporus* MML2270 produced maximum chitinase of 59.05 units/ml on 4th day, when the bacterium grown in all the other standardized parameters such as YNB medium, 0.3% colloidal chitin as substrate, pH 8.0 and temperature 35°C. The chitinase activity was declined in subsequent ages and only 19.67 units/ml activity was measured on 10th day (Figure 7).

DISCUSSION

Previous reports have shown that species of *Bacillus* are known to produce chitinolytic enzymes (Wen et al., 2002; Chen et al., 2004; Driss et al., 2005; Waldeck et al., 2006; Chang et al., 2007). However, this is the first report on the production of chitinase by *B. laterosporus*. In this

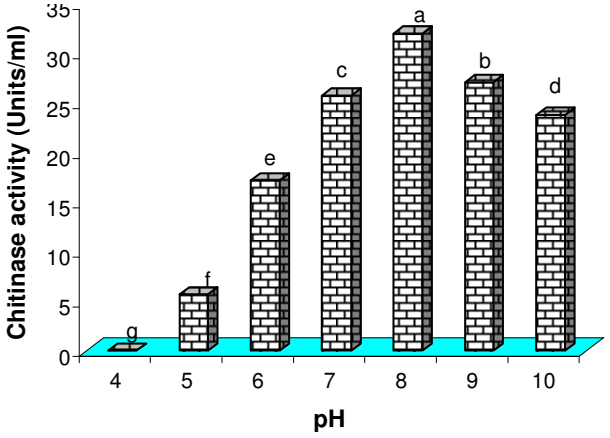

Figure 4. Production of chitinase by *B. laterosporous* MML2270 in yeast nitrogen base broth (YNBB) amended with 0.3% colloidal chitin in different pH. Values are mean of three replicates. Values with different letters are significantly different at 5% level.

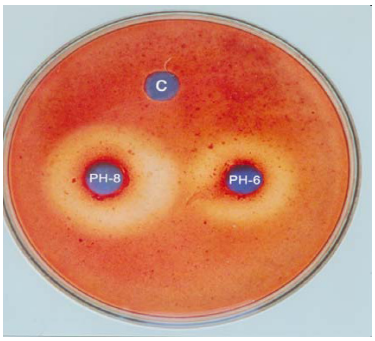

Figure 5. Clear zone produced by the culture filtrates of *B. laterosporous* MML2270 obtained after growth in 0.3% colloidal chitin amended YNBB with initial pH of 8.0 and 6.0. C: Sterile medium.

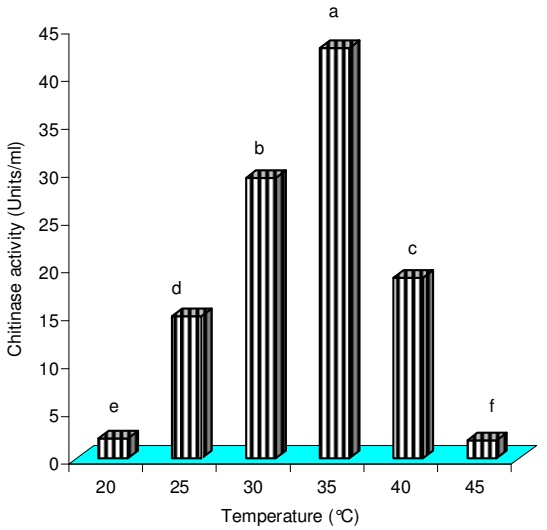

Figure 6. Effect of different temperature on chitinase production by *B. laterosporous* in yeast nitrogen base broth (YNBB) amended with 0.3% colloidal chitin at pH 8.0. Values are mean of three replicates. Values with different letters are significantly different at 5% level.

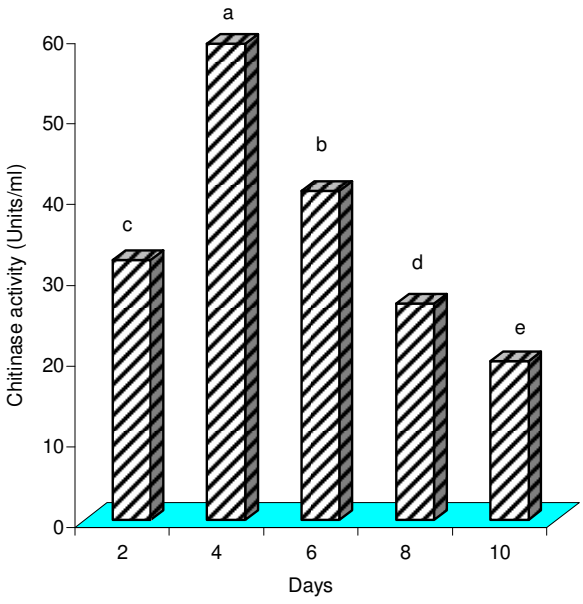

Figure 7. Production of chitinase by *B. laterosporous* with standardized cultural conditions at different ages. Values are mean of three replicates. Values with different letters are significantly different at 5% level.

present study, 39 different chitinolytic bacteria were isolated from rhizosphere soils as already demonstrated the presence of more number of chitin degrading bacteria in agricultural fields (El-Tarabily et al., 2000; Mabuchi et al., 2000; Nawani and Kapadnis, 2003). Although a large number of chitinase-producing bacteria were isolated from soils with different pH (Nawani and Kapadnis, 2003), in the present study, we have isolated all the 39 chitinolytic bacteria from the soils with a narrow pH of 6.0 to 7.8.

Among the 39 isolates, 11 bacteria produced clear zones of more than 0.5 cm by hydrolyzing the colloidal chitin, which accounts 28% of the total chitinolytic bacterial isolates. The presence of chitinolytic bacteria in the crop rhizosphere soils is highly beneficial as they could suppress the plant pathogenic fungi near the root zone

and provide sustainable plant protection against root diseases. Further, we evaluated all the 11 short-listed chitinolytic bacteria in secondary screening using their culture filtrates and selected an isolate designated as MML2270 as it hydrolyzed the colloidal chitin at the maximum in the qualitative assay performed with colloidal chitin agar. This has clearly indicated the possible secretion of high amount of extracellular chitinase by the isolate MML2270. Therefore, further studies were designed to optimize the production of chitinase. In addition, identification of the isolate MML2270 was also carried out, as it is essential to establish the identity of the organism. Various physiological and biochemical tests were carried out as outlined in the Bergey's Manual of Systematic Bacteriology (Williams et al., 1989). Results of the above tests have revealed that the isolate MML2270 is *B. laterosporous*.

Culture medium is a key factor for the growth as well as metabolites production by microorganisms. Among three media tested, colloidal chitin amended YNB supported high chitinase production than LBB and NB. Concentration of colloidal chitin is another vital factor as it is reported to induce the chitinase production in several microorganisms (Frandberg and Schnurer, 1994; Mathivanan et al., 1997, 1998; Soiuza et al., 2005). At 0.3% concentrations, colloidal chitin significantly enhanced the chitinase activity. The same concentration of colloidal chitin has already been reported as optimum for chitinase production in *Fusarium chlamydosporum* (Mathivanan et al., 1998). However, addition of colloidal chitin at 0.5% and above induced the maximum chitinase production in *Bacillus* sp. NCTV2 (Wen et al., 2002), *Alternaria alternata* (Sharaf, 2005) and *Trichoderma harzianum* (Sandhya et al., 2005).

The pH of the culture medium is playing important role in chitinase production. Majority of the bacteria reported to produce maximum level of chitinase at neutral or slightly acidic pH and whereas fungi mostly secret it in acidic conditions (Ulhoa and Peberdy, 1991; Kovacs et al., 2004; Zhang et al., 2004; Sharaf, 2005). In contrast, *B. laterosporus* MML2270 produced highest chitinase at pH 8.0 and interestingly it failed to produce chitinase at pH 4.0. Similar optimum pH of 8.0 for chitinase production was reported in *B. pabuli* K1 (Frandberg and Schnurer, 1994). *B. laterosporus* produced high chitinase activity at 35°C, in which good bacterial growth has also been recorded (data not presented). Further, maximum production of chitinase was recorded on 4th day with all the standardized parameters such as YNB medium, 0.3% colloidal chitin as substrate, pH 8 and temperature 35°C. By standardizing the above cultural conditions, the production of chitinase has been increased to three fold from 19.7 units/ml at initial YNB medium stage to 59.05 units/ml at the final stage. Analysis of the results has revealed that the potential of any microbial culture could be increased for the production of different metabolites by adapting suitable culture techniques.

The role of chitinase in the biological control of various fungal pathogens has already been established (Gunaratna and Balasubramanian, 1994; Mathivanan et al., 1998; Chen et al., 2004; Huang et al., 2005; Chang et al., 2007). Since the chitinase producing *B. laterosporus* MML2270 was originally isolated from the rice rhizosphere, this bacterium could be an ideal candidate for biological control of rice pathogens. In this line further studies are in progress to purify and characterize the chitinase in order to study its role in the control of rice pathogens.

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REFERENCES

Agres Statistical Software Version 3.01. (1994). USA: Pascal International Software Solutions.

Ajit NS, Verma R, Shanmugam V (2006). Extracellular chitinases of fluorescent pseudomonads antifungal to *Fusarium oxysporum* f. sp. *dianthi* causing carnation wilt. Curr. Microbiol. 52: 310-316.

Akagi K, Watanabe J, Hara M, Kezuka Y, Chikaishi E, Yamaguchi T, Akutsu, H, Nonaka T, Watanabe T, Ikegami T (2006). Identification of the substrate interaction region of the chitin-binding domain of *Streptomyces griseus* chitinase C. J. Biochem. (Tokyo). 139: 483-93.

Ancillo G, Witte B, Schmelzer E, Kombrink E (1999). A distinct member of the basic (class I) chitinase gene family in potato is specifically expressed in epidermal cells. Plant Mol. Biol. 39: 1137-1151.

Balasubramanian N, Annie Juliet A, Srikalavani P, Lalithakumari D (2003). Release and regeneration of protoplasts from *Trichothecium roseum*. Can. J. Microbiol. 49: 263-268.

Chang WT, Chen YC, Jao CL (2007). Antifungal activity and enhancement of plant growth by *Bacillus cereus* grown on shellfish chitin wastes. Bioresour. Technol. 98: 1224-1230.

Chen CY, Wang YH, Huang CJ (2004). Enhancement of the antifungal activity of *Bacillus subtilis* F29-3 by the chitinase encoded by *Bacillus circulans* chiA gene. Can. J. Microbiol. 50: 451-454.

Chernin LS, De La Fuente L, Sobolev V, Haran S, Vorgias CE (1997). Oppenheim AB, Chet I. Molecular cloning, structural analysis, and expression in *Escherichia coli* of a chitinase gene from *Enterobacter agglomerans*. Appl. Environ. Microbiol. 63: 834-839.

Collinge DB, Kragh KM, Mikkelsen JD, Nielsen KK, Rasmussen U, Vad K (1993). Plant chitinases. Plant J. 3: 31-40.

Dahiya N, Tewari R, Tiwari RP, Hoondal GS (2005). Chitinase from *Enterobacter* sp. NRG4: Its purification, characterization and reaction pattern. Electronic J. Biotechnol. 8: 134-145.

Das S, Van Dellen K, Bulik D, Magnelli P, Cui J, Head J, Robbins PW, Samuelson J (2006). The cyst wall of Entamoeba invadens contains chitosan (deacetylated chitin). Mol. Biochem. Parasitol. 148: 86-92.

De la Vega LM, Barboza-Corona JE, Aguilar-Uscanga MG, Ramirez_lepe M. (2006). Purification and characterization of an exochitinase from *Bacillus thuringiensis* subsp. *aizawai* and its action against phytopathogenic fungi. Can J. Microbiol. 52: 651-657.

Driss F, Kallassy_Awad, M, Zouari N, Jaoua S (2005). Molecular characterization of a novel chitinase from *Bacillus thuringiensis* subsp. *kurstaki*. J. Appl. Microbiol. 99: 945-53.

El-Tarabily KA, Soliman MH, Nassar AH, Al-Hassani HA, Sivasithambaram K, McKenna F, Hardy GE St J (2000). Biological control of Sclerotinia minor using a chitinolytic bacterium and actinomycetes. Plant Pathol. 49: 573-583.

Felse PA, Panda T (1999). Regulation and cloning of microbial chitinase genes. Appl. Microbiol. Biotechnol. 51: 141-151.
Flach J, Pilet PE, Jolles P (1992). What's new in chitinases research? *Experientia* (Basel) 48: 701-716.
Frandberg E, Schnurer J (1994). Chitinolytic properties of *Bacillus pabuli* K1. J. Appl. Bacteriol. 76: 361-367.
Gkargkas K, Mamma D, Nedev G, Topakas E, Christakopoulos P, Kekos D, Macris BJ (2004). Studies on N-acetyl-ß-D-glucosaminidase produced by *Fusarium oxysporum* F3 grown in solid-state fermentation. Process Biochem. 39: 1599-1605.
Graham LS, Sticklen MB (1994). Plant chitinases Can. J. Bot. 72: 1057-1083.
Gunaratna KR, Balasubramanian R (1994). Partial purification and properties of extracellular chitinase produced by *Acremonium obclavatum*, an antagonist to groundnut rust, *Puccinia arachidis*. World J. Microbiol. Biotechnol. 10: 342-345.
Hobel CF, Hreggvidsson GO, Marteinsson VT, Bahrani-Mougeot F, Einarsson JM, Kristjansson JK (2005). Cloning, expression, and characterization of a highly thermostable family 18 chitinase from *Rhodothermus marinus*. Extremophiles 9: 53-64.
Huang CJ, Wang TK, Chung SC, Chen CY (2005) Identification of an antifungal chitinase from a potential biocontrol agent, *Bacillus cereus* 28-9. J. Biochem. Mol. Biol. Sci. 38: 82-88.
Kovacs K, Szakacs G, Pusztahelyi T, Pandey A (2004). Production of chitinolytic enzymes with *Trichoderma longibrachiatum* IMI 92027 in solid substrate fermentation. Appl. Biochem. Biotechnol. 118:189-204.
Kupiec CR, Chet I (1998). The molecular biology of chitin digestion Curr. Opin. Biotechnol. 9: 270-277.
Mabuchi N, Hashizume I, Araki Y (2000). Characterization of chitinases exerted by *Bacillus cereus* CH. Can. J. Microbiol. 46: 370-375.
Makino A, Kurosaki K, Ohmae M, Kobayashi S (2006). Chitinase-catalyzed synthesis of alternatingly N-deacetylated chitin: a chitin-chitosan hybrid polysaccharide. Biomacromolecules, 7(3):950-957.
Makino A, Ohmae M, Kobayashi S (2006). Synthesis of Fluorinated Chitin Derivatives via Enzymatic Polymerization. Macromol Biosci. 6: 862-872.
Mathivanan N (1995). Studies on extracellular chitinase and secondary metabolites produced by *Fusarium chlamydosporum*, an antagonist to *Puccinia arachidis*, the rust pathogen of groundnut. Ph. D. thesis, University of Madras, Chennai, India.
Mathivanan N, Kabilan V, Murugesan K (1997). Production of chitinase by Fusarium chlamydosporum, a mycoparasite to groundnut rust, Puccinia arachidis. Indian J. Exp. Biol. 35: 890-893.
Mathivanan N, Kabilan V, Murugesan K (1998). Purification, characterization and anti-fungal activity from *Fusarium chlamydosporum*, a mycoparasite to groundnut rust, *Puccinia arachidis*. Can. J. Microbiol. 44: 646-651.
Matsushima R, Ozawa R, Uefune M, Gotoh T, Takabayashi J (2006). Intraspecies Variation in the Kanzawa Spider Mite differentially Affects Induced Defensive Response in Lima Bean Plants. J. Chem. Ecol. [Epub ahead of print]
Mauch F, Hadwiger LA, Boller T (1984). Ethylene: symptom, not signal for the induction of chitinase and β-1,3-glucanase in pea pods by pathogens and elicitors. Plant Physiol. 76: 607-611.
Nawani NN, Kapadnis BP (2003). Chitin degrading potential of bacteria from extreme and moderate environment. Indian J. Exp. Biol. 41: 248-254.
Pichyangkura R, Kudan S, Kultiyawong K, Sukwattanasinitt M, Aiba S (2002). Quantitative production of 2-acetamido-2-deoxy-D-glucose from crystalline chitin by bacterial chitinase. Carbohydr. Res. 337: 557-559.
Prabavathy VR, Mathivanan N, Sagadevan E, Murugesan K, Lalithakumari D (2006). Intra-strain protoplast fusion enhances carboxymethyl cellulase activity in *Trichoderma reesei*. Enzyme Microb. Technol. 38: 719-723.
Reissig JL, Strominger JL, Leoloir IF (1955). A modified colorimetric method for the estimation of N- acetylamino sugars. J. Biol. Chem. 217: 959-966.
Sabry SA (1992). Microbial degradation of shrimp-shell waste. J. Basic Microbiol. 32(2):107-111.
Sakai K, Narihara M, Kasama Y, Wakayama M, Moriguchi M (1994). Purification and characterization of thermostable beta-N-acetylhexosaminidase of *Bacillus stearothermophilus* CH-4 isolated from chitin-containing compost. Appl. Environ. Microbiol. 60(8): 2911-2915.
Sandhya C, Binod P, Nampoothiri KM, Szakacs G, Pandey A (2005). Microbial synthesis of chitinase in solid cultures and its potential as a biocontrol agent against phytopathogenic fungus Colletotrichum gloeosporioides. Appl. Biochem. Biotechnol. 127: 1-15.
Sharaf EF (2005). A potent chitinolytic activity of Alternaria alternata isolated from Egyptian black sand. : Pol J Microbiol.54: 145-51.
Someya N, Nakajima M, Hirayae K, Hibi T, Akutsu K (2001). Synergistic antifungal activity of chitinolytic enzymes and prodigiosin produced by the biocontrol bacterium *Serratia marcescens* strain B2 against the gray mold pathogen, *Botrytis cinerea*. J. Gen. Plant Pathol. 67:312-317.
Someya N, Numata S, Nakajima M, Hasebe A, Hibi T, Akutsu K (2003). Biological control of rice blast by the epiphytic bacterium *Erwinia ananas* transformed with a chitinolytic enzyme gene from an antagonistic bacterium, *Serratia marcescens* strain B2. J. Gen Plant Pathol . 69: 276-282.
Soiuza RF, Soares RM, Nascimento RP, Coelho RR, Gomes RC (2005). Effect of different carbon sources on endochitinase production by Colletotrichum gloeosporioides. Curr. Microbiol. 51:16-21.
Takakura Y, Ito T, Saito H, Inoue, T, Komari T, Kuwata S (2000). Flower-predominant expression of a gene encoding a novel class I chitinase in rice (*Oryza sativa* L.). Plant Mol. Biol. 42: 883-897.
Troedsson U, Olsson PA, Jarl-Sunesson CI (2005). Application of antisense transformation of a barley chitinase in studies of arbuscule formation by a mycorrhizal fungus. Hereditas. 142: 65-72.
Ulhoa CJ, Peberdy JF (1991). Regulation of chitinase synthesis in *Trichoderma harzianum*. J. Gen. Microbiol. 137: 2163-2169.
Vaidya RJ, Shah IM, Vyas PR, Chhatpar HS (2001). Production of chitinase and its optimization from a novel isolate *Alcaligenes xylosoxydans*: potential antifungal biocontrol. World J. Microbiol. Biotechnol. 17: 62-69.
Viterbo A, Haran S, Friesem D, Ramot O, Chet I (2001). Antifungal activity of a novel endochitinase gene (chit36) from Trichoderma harzianum Rifai TM. FEMS Microbiol. Lett. 200: 169-174.
Waldeck J, Daum G, Bisping B, Meinhardt F (2006). Isolation and molecular characterization of chitinase deficient Bacillus licheniformis strains capable of deproteinization of shrimp shell waste to obtain highly viscous chitin. Appl. Environ. Microbiol. 72(12): 7879-7885.
Wang SL, Lin TY, Yen YH, Liao HF, Chen YJ(2006). Bioconversion of shellfish chitin wastes for the production of Bacillus subtilis W-118 chitinase. Carbohydr Res. 341: 2507-2515.
Wen CM, Tseng CS, Cheng CY, Li YK (2002). Purification, characterization and cloning of a chitinases from *Bacillus* sp. NCTU2. Biotechnol. Appl. Biochem. 35: 213-219.
Williams ST, Sharpe ME, Holt JG (1989). Bergey's Manual of Systematic Bacteriology. Vol. 4. London:Williams and Wilkins.
Woo CJ, Park HD (2003). An extracellular *Bacillus* sp. chitinase for the production of chitotriose as a major chitinolytic product. Biotechnol Lett. 25: 409-412.
Yano S, Wakayama M, Tachiki T (2006). Cloning and expression of an alpha-1,3-glucanase gene from *Bacillus circulans* KA-304: the enzyme participates in protoplast formation of *Schizophyllum commune*. Biosci. Biotechnol. Biochem. 70: 1754-63.
Zhang J, Cai J, Wu K, Jin S, Pan R, Fan M. (2004). Production and properties of chitinase from *Beauveria bassiana* Bb174 in solid state fermentation. Ying Yong Sheng Tai Xue Bao. 15: 863-866.