Bacillus licheniformis strain UKCH17 from northwestern Indian Himalayas: Characterization of chitinolytic enzyme and determination of its antifungal potential

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Bacterial biocontrol agents are viable alternatives to chemical pesticides with target specificity and ecological safety. Here, we have identified a pesticidal microbial agent, an extracellular chitinase producing Bacillus strain (designated as UKCH17) from Uttarakhand soils, a biologically rich Northwestern Indian Himalayas ecosystem. The 16S rRNA gene sequence of UKCH17 showed 99% similarity with sequences of *B. licheniformis* available in the public domain. The culture supernatants have a maximum chitinase activity of 99 U/mL after 4 days of culturing. The isolate showed strong antifungal activity manifested in the form of progressive mycelia degradation of *Rhizoctonia solani*, *Fusarium solani* and *Sclerotium rolfsi* in dual culture plates. Mycelial deformities like constrictions and protoplast leakage were observed in microscopic studies. However, it showed little growth reduction in *Helicoverpa armigera* when fed on surface contaminated diet. The electrophoretic analysis showed a monomeric 70 kDa active enzyme having an optimum pH and temperature of 5 and 50ºC, respectively. The enzyme also obeyed Michaelis-Menten kinetics, the Km and kcat values being 0.387 mg colloidal chitin/mL and 6335 s⁻¹, respectively. Partial characterization of chitinase gene also confirms the family 18 status of glycosyl hydrolase from UKCH17. Above all, seed bacterization improved germination percentage and other plant growth characteristics of *Pisum sativum* in sick pots of *R. solani* and *S. rolfsi* suggesting its utilization as potent antifungal biocontrol agent.

**Keywords:** Biocontrol agent, Chitinase, *Helicoverpa armigera*, Indian Himalayas, Mycelial degradation, Seed bacterization

Chitin is an insoluble linear homopolymer of N-Acetyl-D-glucosamine residues, linked by β 1-4 linkage and is the second most abundant polysaccharide in nature, after cellulose1,2. It is an integral constituent of the integument and gut lining of arthropods and the cell walls of fungi, which are the major limiting factor of agricultural production. The chitin in these organs provides structural rigidity and act as physical barrier against invading microorganisms. So, chitin metabolism is an important biological function3 and any extraneous agents targeting chitin may have adverse effects on growth and development of these chitin containing organisms4 either directly or indirectly.

Chitinases (EC 3.2.1.14) are the degrading enzymes produced by the chitin containing organisms which are used for the carbohydrate catabolism. The studies on a variety of prokaryotic and eukaryotic organisms including higher plants reported the presence of these hydrolytic enzymes as well. The company of chitinolytic enzymes, despite of lack of chitin as a structural component in these organisms confirms some additional functions which range from nutrition to protection1. Among different chitinolytic organisms, microbes especially, *Bacillus* spp. are well known chitinase producers with high levels of chitinolytic activity2. The potential application of these enzymes comes from their established direct toxicity to a variety of plant pathogenic fungi4,2 and synergism with Cry toxins in control of phytophagous insect pests6. Therefore, chitinases are of great pest management interest and all chitinolytic bacteria have the potential to be used as biological control agents1. These enzymes are also used in the generation of fungal protoplasts to degrade the fungal cell wall and in human health care such as making ophthalmic preparations7.

The omnipresence of the substrate, its degrading enzymes and practical utility of both in agriculture and industrial sectors leads to the worldwide
exploration studies on chitinolytic organisms. Amongst, different chitinolytic organisms, *Bacillus* genus occupy a prime position in characterization, cloning and practical application of their chitinases. Besides, some species and strains of the genus are known for its biocontrol potential and plant growth promotion activities. Studies reported the existence of potent strains with substantial biocontrol activity against plant pathogenic fungi (PPF) and insect pests. It is noteworthy that location specific pest management options were benefitted from native biocontrol options. Keeping in view the vast diversity of *Bacillus* genus, Northwestern Himalayas was also investigated for the existence of potent chitinolytic *Bacillus* species. The present study investigated biocontrol potential and enzyme characteristics of a native *B. licheniformis* strain UKCH17 isolated from Uttrakhand Himalayas, India.

### Materials and Methods

**Sampling, isolation and maintenance of culture**

The soil sample used for isolation of the bacteria, *Bacillus* strain UKCH17, was collected from native agricultural fields of Balta, an intensive vegetable growing village located in Almora district (29°36’ N and 79°40’ E), Uttarakhand of the N.W. Indian Himalayas, at an altitude of 1860 m above mean sea level. An aliquot (100 µL) of heat shocked soil and sterile distilled water mixture (1:100 weight/volume) of the sample was spread on to chitinase detection (CHD) agar media and incubated at 30ºC for 3 days. The colony exhibiting prominent degradation of colloidal chitin around the bacterial growth was further purified and maintained on nutrient agar slants and 25% glycerol stocks at −80ºC. All the subcultures and further studies were initiated from overnight grown nutrient broth cultures of the bacterium. The isolate was submitted to National Agriculturally Important Microbial Culture Collection (NAIMCC) of ICAR-National Bureau of Agriculturally Important Microorganisms (NBAIM) with accession number NAIMCC-B-02001.

**Molecular identification of the bacterial strain**

The chitinolytic bacterial isolate, *B. licheniformis* strain UKCH17 was identified using 16S ribosomal RNA gene sequence. Genomic DNA was prepared from overnight grown culture using CTAB buffer and further purified using phenol/chloroform extraction procedure. The 16S rRNA gene was amplified using *Bacillus* specific primers 16S rRNA(F) (5’-CAGGCCCTAACACATGCAAGTC-3’) and 16S rRNA(R) (5’-GGCCGCTGTGTACAAAGGC-3’) as reported earlier. The PCR reaction mixture consisted 100 ng of total DNA, 1 µM each forward and reverse primer, 1.5 mM MgCl₂, 300 µM dNTPs, and 2.5 U of *Taq* DNA polymerase in a final volume of 50 µL. The amplification was performed in a thermal cycler (Biorad) programmed to an initial denaturation at 94ºC for 5 min, followed by 35 cycles of 94ºC for 1 min, 45ºC for 1 min and 72ºC for 2 min, and a final polymerization step of 72ºC for 10 min. The amplification was confirmed by electrophoresis of 10 µL of PCR product in 1% agarose gel followed by visualizing the band under UV after ethidium bromide staining. The 16S rRNA amplicon was sequenced by an automated DNA sequencer (ABI 377) using the Big Dye terminator kit (Applied Biosystems) as per manufacturer’s instructions. The obtained sequence was compared to the 16S rRNA sequences in the GenBank databases by BLASTN. The phylogenetic tree was constructed with the neighbor-joining (NJ) method of MEGA 5.0 software to obtain evolutionary relationships. The confidence level of each branch was tested by bootstrapping 100 replicates generated with a random seed.

**Colloidal chitin preparation**

Commercial chitin flakes (Himedia) was used for colloidal chitin preparation according to Berger and Reynolds.

**Kinetics of chitinase production**

The *Bacillus* strain UKCH17 was grown in CHD medium in a rotary incubator (150 rpm) at 30ºC for 8 days. An aliquot was harvested, on daily basis and was analyzed for bacterial growth by measuring OD₆₆₀. The enzyme activity was also measured from the supernatant which was obtained by centrifuging the culture at 10000 rpm for 15 min.

**Isolation of chitinases**

The enzyme was purified from 4 days old culture of the strain, grown in CHD medium at 30ºC and 150 rpm. The cell free supernatant was obtained by centrifugation at 10000 rpm for 20 min and was mixed with four volumes of ice cold acetone. The mixture was left overnight at −20ºC and precipitated proteins were collected by centrifugation at 10000 rpm for 10 min at 4ºC. The protein pellet was washed twice with 80% ethanol and dissolved in appropriate volume of 50 mM phosphate buffer (pH 7.0) and dialyzed extensively against the same buffer at 4ºC. The protein content of the dialyzed enzyme sample
was estimated by standard Bradford method and stored at −80°C for further study.

**Estimation of chitinase activity**

The enzyme activity of chitinases was assayed in a 500 μL reaction mixture containing equal volumes of enzyme solution and 50 mM acetate buffer (pH 5) containing 1% colloidal chitin as substrate. The reaction was performed by incubating the mixture at 50°C for 30 min followed by terminating the reaction by boiling at 100°C for 10 min. The remaining colloidal chitin was precipitated by centrifugation at 10000 rpm for 5 min and the supernatant was estimated for the released reducing sugars by modified Schales reagent. The absorbance was measured at 420 nm and the reducing sugar was estimated from a standard curve of N-acetyl-glucosamine (NAG). One unit (U) was defined as the amount of enzyme that released 1 μM of NAG per minute.

**Protein electrophoresis and identification of chitinases**

The supernatant proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) according to the discontinuous system of Laemmli. Ten micrograms of protein was subjected to electrophoresis using 12% separating gel and 5% stacking gel in a Bio-Rad mini-protein II cell assembly. The protein samples were mixed with gel loading buffer and boiled in a water bath for 5 min before loading on to gels. Electrophoresis was performed at a constant voltage of 100 volts and protein bands were visualized by Coomassie brilliant blue R-250 dye binding method.

The chitinolytic protein was identified after the electrophoresis of crude culture supernatant proteins on native PAGE gels at 4°C. Electrophoresis was performed under the same conditions as above except that sample buffer was devoid of mercaptoethanol and 10% separating gel was used. After protein separation, the gel was washed thoroughly with 50 mM acetate buffer (pH 5) and laid on to a substrate gel made of 2% agarose and 0.1% colloidal chitin. The assembly was incubated in a thermostatic chamber at 37°C for overnight to allow enzymatic activity. At the end of incubation, the agarase gel was recovered and stained for 15 min in chitin binding fluorescent dye solution (0.01% Calcefluor white M2R in 50 mM Tris HCl pH 8) followed by distaining in distilled water for 2 h. The lytic zones were visualized on UV illuminator and compared with simultaneously runned duplicate protein banding pattern.

**Sequencing and partial characterization of chitinase gene**

Genomic DNA of UKCH17 was isolated from overnight grown cultures by CTAB method of cell disruption followed by PCI purification as described by Sambrook and Russel (2001). The purified DNA was used as template for PCR amplification of chitinase gene fragment using previously described family 18 chitinase specific primers, GA1F (5'-CGTCGACATCGACTGGGARTDBCC-3') and GA1R (5'-ACGCCGGTCCAGCCNCKNCCRTA-3'). The PCR reaction mix (50 μL) contained 100 ng of total DNA, 1 pM each primer, 1.5 mM MgCl₂, 250 μM dNTPs, 15 μg bovine serum albumin and 0.6 U of Taq DNA polymerase. The reactions were performed in a thermal cycler (Applied Biosystems) with an initial 5 min denaturation step at 94°C followed by 30 cycles of amplification consisting of one min denaturation at 94°C, 45 s of annealing at 45°C, 2 min of extension at 72°C, with an extra extension step of 10 min at 72°C. The amplification was confirmed by investigating 10 μL of PCR product by electrophoresis in a 1% agarose gel.

After confirming the amplification, the PCR product was purified using gel elution columns (Sigma) and sequenced from Scigenome labs, Kochi, India. The obtained sequence was compared to the published sequences of family 18 chitinase genes in the GenBank databases by BLASTN. Nucleotide sequences of *B. licheniformis* and other *Bacillus* sps. (Accession numbers given along with results) were retrieved from NCBI and aligned with the Clustal Omega (1.2.1) multiple sequence alignment. Molecular evolutionary analysis was performed using the software MEGA4 (Molecular Evolutionary Genetic Analysis version 4) to study the patterns of nucleotide substitution within *B. licheniformis*. The chitinase gene sequence of UKCH17 was submitted to NCBI Gene Bank nucleotide sequence databases (Accession no. KX235873).

**Enzyme kinetic analysis**

The kinetic parameters of chitinase were assessed using colloidal chitin as the substrate. The enzyme activities at different concentrations of substrate (0.1 to 30 mg/mL) were estimated using constant volume of enzyme at 50°C for 10 min. The reaction was terminated by pelleting the colloidal chitin at 10000 rpm for 5 min and the released reducing sugars in the supernatant was measured using modified Schale’s reagent as described. All the assays were performed in independent triplicates. The $K_m$ and $V_{max}$
values were determined by Lineweaver-Burk plots using the Hyper32 software package developed at Liverpool University (http://hompage.ntlword.com/john.easterby/hyper32.html).

The turnover number ($k_{cat}$) was calculated by the following equation:

$$K_{cat} = \frac{V_{max}}{[E]}$$

where $[E]$ refers to the enzyme concentration.

Effect of temperature on chitinase activity and stability

The relative activity of the enzyme at different temperatures was estimated by incubating equal volumes of enzyme with 50 mM phosphate buffer (pH 7.0) containing 1% colloidal chitin as substrate for 1 h at different temperatures ranging from 30-90°C at every 5°C interval. The thermal stability was estimated by incubating the enzyme at 30, 40, 50 and 60°C for 2 h. Aliquots were drawn at every 15 min from each temperature and were analyzed for the residual chitinase activity as described earlier. All experiments were performed independently in triplicate.

Effect of pH on chitinase activity and stability

The effect of pH on chitinase activity was assessed over a pH range of 3–10 with different buffers containing 1% colloidal chitin as substrate. The buffer systems used are as follows: 50 mM citrate buffer (pH 3, 4, 5, and 6), 50 mM phosphate buffer (pH 6, 7, and 8), and 50 mM borate buffer (pH 8, 9, and 10). To determine the effect of pH on enzyme stability, the enzyme was incubated at different pH values using respective buffers at 4°C for overnight. Residual enzyme activity was measured as described. All experiments were performed independently in triplicate.

Determination of antagonistic activity against plant pathogenic fungi (PPF)

Under in vitro conditions

The antagonistic activity of bacterial isolate B. licheniformis strain UKCH17 was evaluated against plant pathogenic fungi (PPF), Rhizoctonia solani, Fusarium solani and Sclerotium rolfsii. The PPF cultures were isolated from local infected fields, purified, identified according to standard protocols and were maintained on potato dextrose agar plates. The growth inhibition of PPF was evaluated on a basal agar medium plates containing Luria-Bertani agar (LBA) and potato dextrose agar (PDA) at their half strengths. The experimental setup consists of bacterium streaked at the middle of 9 cm diameter Petri plate containing basal agar medium and the disc inoculated PPF (actively growing mycelia i.e. 3-4 days old culture) at both the corners of plate in parallel to streak. The dual inoculated plates were incubated at 28±2°C and the controls were devoid of bacteria. After 10 days of incubation, when the growth of PPF in control plates joins in the middle, the treatment plates were observed for growth inhibition. Bacteria seeded medium23 was also used to analyze the antifungal activity of the bacterium. In brief, sterile basal medium was mixed with 10% of overnight grown bacterial culture just before solidification and plated in 9 mm petri plates. An actively growing targeted fungal disc (0.5 cm diameter) was placed at the centre of plate and allowed to grow at 27°C. The diameter of fungal growth was measured when it reaches borders in control plates with no bacteria. The S. rolfsii plates were further incubated to evaluate the inhibition on sclerotia formation.

Under pot culture conditions

The antagonistic potential of B. licheniformis strain UKCH17 against R. solani and S. rolfsii was determined under pot culture conditions, using field pea (Pisum sativum sub sp. partanse). The fungal cultures for soil inoculation were obtained by growing them individually on sterilized maize seeds at 28±1°C for 7 days. This seed based fungal mycelium was mixed with sterile soil at the rate of 10 g/Kg of soil along with water to its water holding capacity and held at room temperature (30°C). After 3 days, soil was thoroughly mixed and filled in to 15 cm diameter plastic pots. Seed bacterization was done by soaking surface sterilized seeds of pea (VL 42) in bacterial suspension (10^8 cfu/mL in 0.5% carboxy methyl cellulose) for 15 min. The treated seeds were sown at the rate of 5 seeds/pot and placed in a temperature controlled glass house. Observations on percent germination and other plant growth parameters (shoot length, root length and dry biomass) were recorded on 15th and 30th day, respectively. Seeds treated with 0.5% carboxy methyl cellulose and grown under similar conditions were served as control.

Bioassay against insect pests

For insect bioassay, cell culture of the strain UKCH17 was obtained by growing the bacterium in nutrient broth at its half strength supplemented with 1% colloidal chitin as shake flask cultures (150 rpm).
in 20 mL of medium at 30°C for 4 days. The culture was directly used for bioassays after adjusting the cell strength. The entomotoxicity was estimated against 4 days old larvae of *Helicoverpa armigera*, (Approximate weight of 15 mg) by diet surface contamination technique. The preliminary bioassays showed only growth inhibition even at highest concentration tested with no mortality. So, the bioassays were done to study the growth inhibition. The artificial diet mix of *H. armigera* was poured in 12 well tissue culture plates and allowed to solidify. The diet surface of each well was spread equally with 200 µL of test concentration of bacterial suspension. After air drying, individual larva was placed on each well and allowed to feed for 4 days. Thereafter, the larvae were maintained on fresh untreated diet and allowed to grow with subsequent changing of diet at every 4 days interval. The Larval weight was measured at 7 and 11 days after treatment along with pupal weight and adult emergence. The data was analyzed by one-way ANOVA/MANOVA. Mean values were compared using the Duncan multiple range test at \( P \leq 0.05 \).

Results and Discussion

The agricultural importance of bacterial genus, *Bacillus* comes from its beneficial applications as plant growth promoting (PGP) bacteria and biocontrol agent against a variety of insect pests and plant pathogens. Particularly, chitinolytic property of different members of this bacterial genus with potential applications in biological control of pestiferous organisms by either direct utilization of bacterium as bioagent or by use of associated chitinase genes in transgenic production is well documented.

Considering that, the present study reports biocontrol potential and partial characterization of associated chitinases from a native bacterial strain, *B. licheniformis* UKCH17 isolated from Uttarakhand Himalayas, India. The microscopic studies of UKCH17 revealed its motility, endospore-formation and rod shaped vegetative cells. Further, gram positive nature, aerobic growth and catalase positivity strongly confirm the *Bacillus* nature of the bacterium. In order to support further characterization and species level identity, 16S rRNA gene fragment was sequenced (Accession No. KX113464). The GenBank BLAST search of the obtained nucleotide sequences showed representative homology with *Bacillus* species with maximum homology of 100% with *B. licheniformis* strain HT-W30-B1 (Accession No. KJ526838). A homology of 99% was also found with a number of *B. licheniformis* accessions. Further alignment and phylogenetic analysis of 16S rRNA sequences of different *Bacillus* species strongly suggests species status of the bacterial strain UKCH17 as *B. licheniformis* (Fig. 1).

The study on chitinase production by UKCH17 revealed progressive increase in enzyme activity and cell count up to 4 days after inoculation (Fig. 2) with a maximum enzyme activity of 99.5 U/mL. Electrophoresis and subsequent identification of chitinases from the supernatant proteins revealed existence of 70 kDa enzyme active band (Fig. 3). Besides, the nucleotide and deduced amino acid sequence homology of chitinase from the isolate showed it is also from *B. licheniformis* [Suppl. Figs 2 & 3 Available only online at repository (http://nopr.niscair.res.in) along with respective paper]. However, nucleotide comparison between *B. licheniformis* strains showed pyrimidines based
transition/transversion rate (Table 1). But, exceptional conservation of amino acid composition showed differential codon usage between strains as well as other related Bacillus spp. (Fig. 4). Despite of these molecular variations, reports also showed presence of unique chitinase bands in B. licheniformis viz., 61.9 kDa in strain AT6 [30], 65 kDa in LHH100 [27], 67 kDa in MB-2 [26], 72 kDa in SK1 [10] as like in the present study (70 kDa). These minor differences of 2-8 kDa may be explained by the association of signal peptides as reported from chitinases of B. thuringiensis [9]. In addition, this also supports the monomeric protein status of B. licheniformis chitinases.

The chitinolytic Bacillus strain UKCH17 exhibited a substantial enzyme activity (>70%) over a temperature range of 30-55°C with a peak activity of 9869 U/mg at 50°C (Fig. 5A). Further increase in temperature resulted in drastic reduction in activity, which might be the result of heat denaturation of protein. The thermal stability study revealed the same trend with recorded 26.8% and 46.8% loss in activity at the end of one-hour incubation at 50°C and 60°C, respectively. Enzyme incubation at 30°C and 40°C recorded relative possession of more than 80% activity even after two hours (Fig. 5B). This substantial low temperature activity (around 80% activity at 30°C) and rapid reduction in enzyme activity at high temperature can be a function of existing native environment where, the Himalayan hills are subjected to frequent freezing and thawing conditions with average low temperatures ranging from 0 to 15°C. The pH activity and stability analysis showed an optimum enzyme activity at pH 5 (Fig. 5 C & D). A relative pH optima was also reported from B. licheniformis strain LHH100 [27] (pH 4), B. licheniformis strain DSM13 [26] (pH 6) and B. licheniformis strain SK-1 [10] (pH 6-8). The high stability coupled with activity at acidic pH also make the B. licheniformis chitinases, a well suitable candidate for biotechnological applications in bioconversion of chitin waste [29].

The enzyme kinetics of strain UKCH17 was studied using natural substrate, colloidal chitin at 50°C. The Lineweaver–Burk plot of enzyme activity at different substrate concentrations (Fig. 6) revealed a maximum activity (V_max) of 3801 U/mg with K_m of 0.387 mg/mL. The turn over number (k_cat) and apparent second-order rate constant (k_cat/K_m) were found to be 6335 s^-1 and 16386 mL mg s^-1, respectively.

The antifungal activity of UKCH17 was manifested in the form of direct inhibition and degradation of

<table>
<thead>
<tr>
<th>Chitinase Gene</th>
<th>A</th>
<th>T</th>
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<th>C</th>
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<tbody>
<tr>
<td>A</td>
<td>-</td>
<td>0.89</td>
<td>0.88</td>
<td>1.44</td>
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<tr>
<td>T</td>
<td>1.53</td>
<td>-</td>
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<td>C</td>
<td>1.71</td>
<td>0.89</td>
<td>0.88</td>
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</table>

[Only entries within a row should be compared. Transitional substitutions were shown in bold and those of transversions were shown in italics]
Fig. 4—Deduced amino acid sequence alignment of partial chitinase gene from strain UKCH17 (Accession number-KX235873), ACF40833 (B. licheniformis strain MY75), AHN92007 (B. licheniformis strain LHH100), BAL45516 (B. licheniformis strain SVD1), AEQ55312 (B. licheniformis strain KNUC213), AKI30028 (B. licheniformis strain UTM104), AAF23368 (B. circulans), ALX35002 (B. pumilus), ABG57262 (B. subtilis). [Numbers written indicates the position of nucleotides. The two unconserved amino acids within B. licheniformis strains were given in boxes]

Fig. 5—Effect of temperature (A & B); and pH (C & D) on activity and stability, respectively of chitnases produced by UKCH17 fungal mycelia in all the tested PPF in dual culture plate. The plates showed distinctive rapid growth and motility of test bacterium that result in mere coverage of plate followed by visual enzymatic degradation of
fungal mycelia (Fig. 7 A & B). The amputation of fungal mycelium is progressive with time. The bacteria seeded medium showed cent percent growth inhibition from the inoculated disc of test fungi. The microphotography of contact point between growing periphery of bacteria and test fungi revealed uneven thickenings (Fig. 7C) and leakage of protoplasm from bent points of mycelia (Fig. 7D). The antagonistic potential of UKCH17 under pot culture conditions also showed a significant protection of pea seeds and plants against deleterious effects of both \textit{R. solani} and \textit{S. rolfsii}. It was observed that seed bacterization registered superior values of all the recorded parameters over control (Table 2). Significantly higher germination levels, increased root and shoot lengths along with dry biomass showed the potential of UKCH17 as biocontrol agent against the tested PPF. However, the bacterial effect was significant at the pre-germination stage in both \textit{R. solani} and \textit{S. rolfsii}. Besides chitinases, \textit{B. licheniformis} strains are well known producer of variety of secondary metabolites viz., cellulases\textsuperscript{33,34}, amylase\textsuperscript{35}, keratinase, chitosanases, proteases, lipases, surfactins\textsuperscript{36-39}, etc. All these enzymes exert either pathogenic activity or synergize the pathogenicity against pestiferous organisms either directly or indirectly by increasing plant growth. A little increase in all the plant growth parameters observed in UKCH17 bacterization alone over control also indicates production of these plant growth promoters.

The direct bioassay studies of crude culture of UKCH17 against \textit{H. armigera} showed no mortality of \textit{H. armigera} even at the highest concentration (\(10^7 \text{ cfu/mL}\)) tested. However, a nominal 39.6\% reduction in larval weight was observed after 7 days of treatment. The after growth on untreated diet exhibited no such significant differences in larval weight, pupal weight and adult emergence (Table 3).

\begin{table}[h]
\centering
\begin{tabular}{|l|c|c|c|c|}
\hline
\textbf{Treatment} & \textbf{Germination} & \textbf{Root length (cm)} & \textbf{Shoot length (cm)} & \textbf{Dry biomass (g)} \\
\hline
\textit{R. solani} & 20\textsuperscript{b} & 6.2\textsuperscript{c} & 9.3\textsuperscript{c} & 0.118\textsuperscript{a} \\
\textit{R. solani} + \textit{B. licheniformis} UKCH17 & 80\textsuperscript{a} & 12.5\textsuperscript{a} & 12.8\textsuperscript{b} & 0.199\textsuperscript{a} \\
\textit{S. rolfsii} & 15\textsuperscript{b} & 3.7\textsuperscript{d} & 6.2\textsuperscript{c} & 0.108\textsuperscript{bc} \\
\textit{S. rolfsii} + \textit{B. licheniformis} UKCH17 & 75\textsuperscript{a} & 9.8\textsuperscript{b} & 10.5\textsuperscript{b} & 0.195\textsuperscript{a} \\
\textit{B. licheniformis} UKCH17 & 85\textsuperscript{a} & 13.3\textsuperscript{a} & 12.9\textsuperscript{a} & 0.203\textsuperscript{a} \\
Control & 80\textsuperscript{a} & 13.0\textsuperscript{a} & 10.8\textsuperscript{b} & 0.132\textsuperscript{b} \\
\hline
\end{tabular}
\caption{Table 2—Effects of \textit{B. licheniformis} strain UKCH17 seed bacterization on germination and growth parameters of \textit{Pisum sativum} in sick pots of \textit{Rhizoctonia solani} and \textit{Sclerotium rolfsii}}
\end{table}

\begin{table}[h]
\centering
\begin{tabular}{|l|c|c|c|c|}
\hline
\textbf{Concentration (CFU/mL)} & \textbf{Larval weight (mg)} & \textbf{Pupal weight (mg)} & \textbf{Adult emergence (%)} \\
\hline
1\times10^7 & 82.3±1.3\textsuperscript{a} & 382.8±12.8\textsuperscript{b} & 289±11.6\textsuperscript{a} & 72.9±0.6\textsuperscript{a} \\
1\times10^5 & 103.1±2.3\textsuperscript{a} & 422±18.4\textsuperscript{a} & 293.8±6.7\textsuperscript{a} & 73.4±0.8\textsuperscript{a} \\
Control & 136.2±3.2\textsuperscript{a} & 436.1±11.6\textsuperscript{a} & 312.1±5.3\textsuperscript{a} & 83.3±0.3\textsuperscript{a} \\
\hline
\end{tabular}
\caption{Table 3—Growth and development of \textit{Helicoverpa armigera} larvae fed with UKCH17 surface contaminated diet for 4 days}
\end{table}
This lack of toxicity can be attributed to predominant exo cleavage activity of bacterial chitinases with less efficiency of chitin degradation. Additionally, insects have the ability to recoup the damage done to peritrophic membrane, a chitin rich primary target to bacterial chitinases.

Mountain and hill regions present a unique ecological reserve of microbes that evolved and adapted to existing edaphic, environmental and biotic factors which offers distinctive beneficial traits having potential commercial applications. In the present study, we identified a strong antifungal *B. licheniformis* strain UKCH17 isolated from Indian Himalayan region with an extracellular enzyme, chitinase production. The isolate showed an *in vitro* restrain in growth and multiplication of *R. solani*, *F. solani* and *S. rolfsi*. Further, pot studies also showed protection against these plant pathogens indicating its great potential in successful utilization as biocontrol agent. Keeping this in view, further studies were planned to find out the management potential of the isolate against other pest species of the region.

**Conflict of Interest**

The authors declare that there is no conflict of interests.

**Acknowledgment**

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