Abstract:

The present invention provides a new strain of Brevibacillus laterosporus LAK 1210 (MTCC 5487) having an accession number MTCC 5487, as a dual producer of insecticidal proteins and inducible chitinolytic enzymes with a potential utility in agriculture and forest insect pest management, plant disease control and mosquito control programs. The multiple effects of this new insecticidal strain of Brevibacillus laterosporus LAK 1210, as a chitinase producer with the ability to degrade marine wastes finds many biotechnological applications in the areas of agriculture, forestry, medicine and environment. Furthermore, the object of the invention is also directed to a new chitinase with novel characteristics from a newer source of microorganism and its novel applications in biomass utilization and waste management. Methods and compositions of the invention utilize the vegetative cells, their derivates (spores, cellular extracts, enzyme fractions) and the polypeptides to develop a natural pesticide for control of insects and phytopathogenic fungi. Another object of this invention is to expand the synergistic effect of chitinases on insecticidal activity for an improved pesticidal activity where a wider range of pests are impacted.
AMENDED SHEET (ARTICLE 19)

WO 2013/050867 AMENDED CLAIMS PCT/IB2012/001982
received by the International Bureau on 23 October 2013 (23.10.2013)

What is claimed is:

1. A biologically pure culture of a new strain of *Brevibacillus laterosporus* designated as *Brevibacillus laterosporus* LAK 1210 having Accession number MTCC 5487.

2. The *Brevibacillus laterosporus* LAK 1210 as claimed in claim 1, wherein the strain produces chitinases and insecticidal proteins.

3. The *Brevibacillus laterosporus* LAK 1210 as claimed in claim 2, wherein the chitinases produced by the strain exhibits antifungal activity against phytopathogenic fungi.

4. The *Brevibacillus laterosporus* LAK 1210 as claimed in claim 1, wherein the strain exhibits an inhibitory effect on insects.

5. The chitinases as claimed in claim 2, wherein the enzyme is active over a broad pH range of pH 3.0 to pH 9.0, with two pH optima of pH 6.0 and 8.0.

6. The chitinases as claimed in claim 2, wherein the enzyme is active over a broad temperature range of 25°C to 75°C, highly thermostable with a temperature optimum of 70°C.

7. A process for producing a supernatant comprising chitinases and insecticidal proteins, wherein the process comprises culturing liquid culture of novel strain of *Brevibacillus laterosporus* designated as LAK 1210 having accession number MTCC 5487 in a culture medium marine shell waste to obtain cell culture, and obtaining the supernatant comprising chitinases and insecticidal proteins from the cell cultures.

8. The process as claimed in claim 7, wherein the marine shell wastes is selected from a group consisting of shrimp shell powder, crab shells, squid pen powder.

9. A composition comprising cells of the strain *Brevibacillus laterosporus* LAK 1210 having Accession number MTCC 5487 and/or chitinases obtained from the *Brevibacillus laterosporus* LAK 1210 having Accession number MTCC 5487.

10. The composition as claimed in claim 9 comprising i) chitinases from the culture supernatant of *Brevibacillus laterosporus* LAK 1210 having accession number MTCC 5487 ii) cells of the strain *Brevibacillus laterosporus* LAK 1210 having accession number MTCC 5487 and
iii) spores, parasporal inclusions and insecticidal crystal toxic proteins, chitinase enzyme fractions and cellular fractions from totally or partially lysed cells of *Brevibacillus laterosporus* LAK 1210 having accession number MTCC 5487; and a carrier.

11. The composition as claimed in claims 9 and 10, wherein the composition is formulated in the form of wettable powders, dust, pellets, granules, seed treatment products, emulsions, sprayable solutions, aqueous solutions, oil or water based dispersions, ULV formulations or microencapsulations.

12. The composition as claimed in claim 11, wherein the composition is a biopesticide, biofungicide, insecticide or nematicide.

13. A biocontrol method for protecting or treating or modulating phytopathogenic infection in plant or a part thereof, wherein the method comprises applying synergistically effective amounts of the composition as claimed in claim 12 to said plant or the part thereof, natural or artificial soil or planting media.

14. The method as claimed in claim 13, wherein the phytopathogenic infection is a plant disease caused by at least one fungus selected from the group consisting of *Fusarium, Rhizoctonia, Pythium, Phytophthora, Cercospora, Puccinia, Venturia, Alternaria, Ucinula, Ustilago, Colletotrichum, Erysiphe, Botrytis, Sclerotium and Monilinia*.

15. The method as claimed in claim 13, wherein said *Fusarium* is *Fusarium oxysporum* f. sp. *lycopersici, Fusarium moniliforme*.

16. The method as claimed in claim 13, wherein the phytopathogenic infection is caused by at least one insect belonging to Lepidoptera, Diptera, Coleoptera, Homoptera or Hymenoptera.

17. The method as claimed in claim 13, wherein the phytopathogenic infection is caused by the insects, in particular, gypsy moth, spruce budworm, corn earworm, tobacco budworm, fall armyworm and also effective against mosquitoes and mosquito larvae.

18. An improved method for protecting or treating or modulating phytopathogenic infection in plant or a part thereof, wherein the method comprises applying the composition as claimed in claim 12 concurrently with the Bacillus-based insecticides, wherein the method enhances the
insecticidal effectiveness of the Bacillus-based insecticides for insect control, wherein the Bacillus-based insecticides are insecticides comprising insecticidal strains of Bacillus.

19. The method of claim 18, wherein the said Bacillus is *Bacillus thuringiensis* or *Bacillus sphaericus*. 

AMENDED SHEET (ARTICLE 19)
International Search Authority has acknowledged the:

a) Novelty of claims 23-24
b) Industrial Applicability of claims 1-24

However, the authority has objected to the:

a) Novelty of claims 1-22
b) Inventive step of claims 1-24

In response to the International Searching Report (ISR) and the objections raised in the Written Opinion of the International Search Authority, the Applicant has amended the claims 1, 3, 7-10, 12, 15, 16-24 and cancelled claims 5-6, 11, 13-14.

Response to Item II

The priority document US61545120 was sent to WIPO by fax on 6 February 2013 (copy enclosed).

Response to Item V

Novelty and Inventive Step:

The Applicant has amended claims, the Applicant has amended the claims 1, 3, 7-10, 12, 15, 16-24 and cancelled claims 5-6, 11, 13-14. The total number of amended claims is 19.

Arguments in view of objections on novelty and inventive step:

The novel strain of Brevibacillus laterosporus designated as LAK1210 (MTCC 5487) is a dual producer of insecticidal proteins and chitinases. The novelty of the strain concerns the characteristics and exhibition of both insecticidal activity and chitinolytic activity. The Bacillus laterosporus strain reported by Shanmugiah et al (D1) has chitinolytic activity and no insecticidal activity has been reported. Sakia et al (D7) reported a strain of with antibacterial and antifungal compounds and not chitinolytic activity. WO2008/031887A2 (D6) discloses an invention that uses a non-chitinolytic, insecticidal strain of Brevibacillus laterosporus effective against dipeterans (mosquitoes and mosquito larvae) and this strain also has been reported to produce only insecticidal proteins, with no chitinase activity. The same applies to US5045314A (D5) in which the invention discloses the use of insecticidal strain of Bacillus laterosporus against nematode ova/larvae. The strain Brevibacillus laterosporus designated as LAK1210 (MTCC 5487) is a chitinolytic, insecticidal strain of Brevibacillus laterosporus has not been documented till it was reported by Prasanna et al (D3). The claims in US61545120 and claims 1-4 in the PCT/IB20 12/001982 concerns both the chitinolytic activity and insecticidal activity of the strain.