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ReviewArticle

Different Chitinolytic *Bacillus* Species to Minimize Termites Attack on Agroforestry: A Review

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Abstract: Termites are known to be the most damaging pest around the world specifically in the tropical region. Pakistan is also suffering from this issue. The chemical pesticides and insecticides are broadly used for the control of insects and pests. These chemicals are diversely affecting the environment and beneficial organisms including humans. We aim to find an effective, harmless, and helpful strategy to control the termite's attack on plants and agroforestry. In this study, we use the online databases of PUBMED, NCBI, Google Scholar, and PMC for the collection of data. In this scenario, we select the microbiological control method which has economic importance as well. Our focus point is to determine chitinolytic bacterial species that have the potential for microbiological control of termites in agroforestry. In this study, we select four (4) Chitinase-producing *Bacillus* species which include *Brevibacillus laterosporus*, *Bacillus licheniformis, Paenibacillus,* and *Bacillus thuringiensis* which are known for Chitinase production. A comparative study will be done between these species to find the best termiticidal activity. We also identified the genes responsible for Chitinase production. If we overexpress these genes by using CRISPR Cas-9 technology, we will get the maximum amount of Chitinase enzymes which will be sprayed on plants. Additionally, another possibility is if we transfer these genes to plants' genome, it will produce Chitinase enzymes for their self-defense against termites.

Keywords: Termites, Chitinase, *Bacillus* species, Chi gene, Agroforestry.

1. INTRODUCTION

Termites are belonging to the group of insects (Isopteran) consisting of 2500 species out of which 300 were considered as pests that have damaging properties to plants. Termites are one of the tropics' most harmful pests and can cause significant issues in agriculture, forestry, and housing, having several families and sub-families exist. Some have subterranean nests, others in wood, such as hollow trees and some are building mounds. Fungus-growing termites are the most troublesome form of termites in agriculture. They feed on dead organic products, like crop residues, mulches, and organic soil (Humus). However, if these foods are not accessible, they will consume live plants such as nuts millets, and maize [1].

The use of different boron compounds for MDF panels' termite resistance was previously evaluated. Borax (BX), boric acid (BA), are either more efficient as sodium perborate tetrahydrate (SPT), or zinc borate (ZB). Chemical pesticides like Malathion, imidacloprid 25 WP @ 250 g/acre, chlorpyrifos 40 EC @ 1000 ml/acre, monomehypo 5 G @ 9 kg/acre and DDT are commonly used to control pests. Chemical insecticides namely Krisban, Aincoban, Chlorguared, Neptine, Greater 48Ec, Luciban, Chlorban, Bismark, Vifos, Imidagold, and Lorshban are also very effective to control termite's [2]. But these chemicals are very toxic and harmful to the environment and human health. It may distress ecological balance by disrupting the valuable biota and infect the fertile soil [3]. The negative aspects of chemical pesticides and insecticides on the environment and living organisms caught the attention of scientists to find a safe and environmentally friendly strategy to control pest attacks on plants and agroforestry [4]. The economy of Pakistan is dependent on

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cash-crops which are damaging by termites mostly. These termites feed on organic materials that they have taken from plants [5]. There are a lot of methods to control termite's attack which includes chemical, physical and botanical methods but the most efficient is the microbiological method because it is safe, environmentally friendly, and has renewable properties [3]. The exoskeleton, trachea, and gut epithelium of termites were made from chitin like other insects [6]. Most of the bacterial species synthesize and secret chitinase enzymes among which *Bacillus licheniformis, Bravibacillus laterosporus*, and *Bacillus thuringiensis* are some of them. These *Bacillus* species can produce a large sum of natural compounds by fixing nitrogen in soil and phosphate solubilizeation [7, 8, 9]. The bacillus species works as a biofertilizer as well as insecticidal activity. Thus, it is more efficient to use for termites' control which may protect humans from the side effects of toxic synthetic chemicals. It is a naturally renewable source and has economic importance.

In this study, the chitinases production by different *Bacillus* species and their termiticidal activity will be highlighted. This is a new approach to check the termiticidal activity of *Bacillus* species.

2. METHODOLOGY

We use the online databases of PUBMED, NCBI, Google Scholar, PMC, and Gene Bank for the collection of data. The data from 2000 to 2020 were reviewed, and only the relevant research article was selected for the study. Furthermore, the field survey was done and multiple formers were interviewed in the rural areas of KP, Pakistan.

3. MECHANISM OF CHITINOLYTIC ENZYMES

Chitinolytic enzymes (CHIs) are the hydrolysis of glycoside that catalyzes chitin decomposition. Most of the microorganisms, insects, plants, fungus, and some animals are their producers [10]. These enzymes hydrolyze the b-1-4-glycoside linkages between the residues of N-Acetyl-D-glucosamine in a chitin chain [11]. A chitinolytic system composed of a diversified group of enzymes that catalyze hydrolytic polymerization of chitin is conducted by complete enzyme hydrolysis of chitin to free N-acetylglucosamine [10, 12, 13]. CHIs (EC 3.2.1.14) can be split into two classes because of the place of a hydrolyzed bond. Endo-chitinases randomly cleave chitin chains, creating low molecular weight oligomers like diacetylochitobiose, chitotrioses, and chitototetriosis. Exo-chitinases extract chitobiosis from the chitin chain's reduced or non-reducing end. Previously two other classes of these enzymes were found; chitobiases are accountable for chitobiosis hydrolysis and b-N-acetyl-glucosaminidases generate b-N-acetyl-D-glucosamine monomers as shown in (Fig-1). Chitobiosis and b-N-acetyl-glucoseaminidases (EC.3.2.1.52) following the nomenclature developed by the international committee of the union for biochemistry and molecular biology (NCB) [14, 15]. CHIs can be split into three families 18, 19, and 20 classified by the resemblance of amino acid sequences. Family 18 involves CHIs based primarily on fungi but also on viruses, bacteria, insects, and plants. Family 19 involves plant CHIs (category 1, 2, and 4) and several obtained from fungi, e.g. *Streptomyces griseus*. Family 20 includes N-acetylglucosaminidase from Vibrio herveyi and N-acetyl hexosaminidases from Dictyostelium discoideum and humans [16, 17, 11, 13]. Several bacteria like *B. laterosporus*, *B. Licheniformis, B. thuringiensis, Paenibacillus strains*, and many pseudomonas species can produce several distinct CHIs [18, 19]. Microbial CHIs weigh between 20 and 120 kDa and most between 20 and 60 kDa [20, 21]. The CHIs optimum pH and temperature are 5.0-8.0 and 25-70 °C, each. The existence of multiple metal ions can inhibit or stabilize its activity, depending on the origin of the CHI. A strong CHI inhibitor is allosamidin, first reported as a competitive insect CHI inhibitor. Allosamidin has a comparable structure that can be created between the carbohydrate oxygen in the N-acetyl group and C-1 in the course of hydrolysis, which is an intermediate substrate, an Oxazoline ring [22].

4. CHITINASE PRODUCING *BACILLUS* SPECIES

4.1 The Chitinase producing *Brevibacillus laterosporus* and their insecticidal activity

Brevibacillus laterosporus Laubach is a rod-shaped, endospore-forming bacterium defined

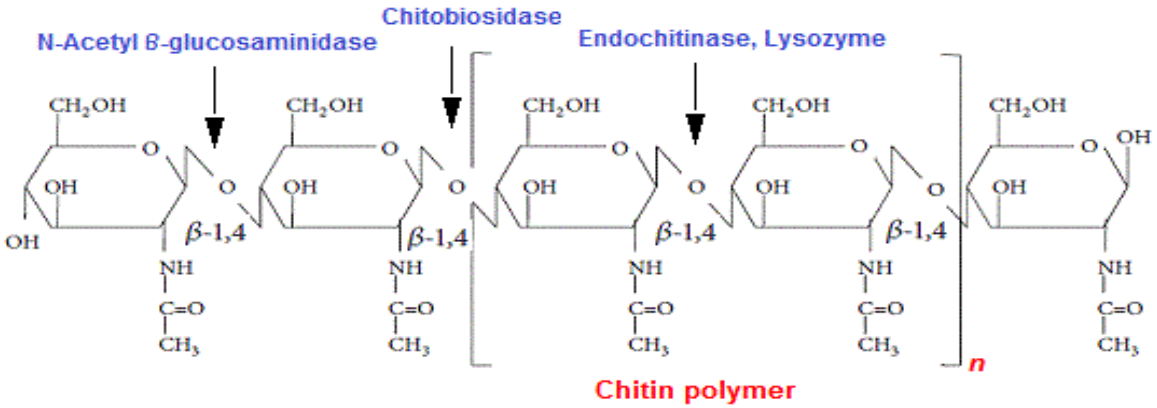

Fig 1. Bonds between chitin and the activity of Chitinase in the hydrolysis of that bonds

morphologically by the manufacturing of a typical canoe-shaped parasporal body (CSPB) strongly connected to one side of spore, determining its central place in the sporangium, it is an omnipresent species separated from various substrates including soil [23], freshwater [24], marine water [25], mosquito bodies [26], surfaces of leaves [27], food that contains starch [28], animal hide and wool [29]. Insects of various order including *Coleoptera, Lepidoptera, Diptera, Nematode* and *Mollusks* have been revealed to have a biocontrol potential for this entomopathogen species [30]. Apart from its invertebrate's pathogenicity, distain B types, the wide-spectrum antimicrobial activity shows *laterosporus*, particularly against fungi, bacteria, and insects. The observed pathogenicity and mode of action have also related to a broad range of molecules, including proteins and antibiotics. The latest complete genome sequence of the species has disclosed that it can produce polyketides, toxins, and non-ribosomal peptides [31, 32].

A novel strain of *Brevibacillus* was found in one of the studies reported in India. Laterosporus *(Lak1210)* capable of producing 25 to 90 kDa chitinase masses of family 18 at optimum temperature 70°C and pH 6.0 to 8.0. Mass spectrometric evaluation of tryptic fragments revealed these to be part of two separate chitinases that are nearly identical to two putative chitinases, a 4-domain 89.6 kDa chitodextrinase and a 2-domain enzyme called chiA1, 69.4 kDa [8]. *Brevibacillus laterosporus* (strain; LMG 15441) can produce chitinase up to the maximum amount.

Some strains of *B. laterosporus* were later reported during sporulation to generated insecticides crystal proteins (ICPS) [33], which shows high levels of toxicity to mosquito larvae, *Culex quinquefasciatus*, and *Aedes aegypti*. Extracellular protease with important nematocidal activity was also revealed to generate laterosporus [34]. To the best of our understanding, *B. laterosporus* strain has not yet defined the existence of chitinase and their feasible roles in biocontrol. Insecticidal proteins (ISPS) produced by *B. laterosporus* are poisonous for other species of Coleopteran, such as Leptinotarsa spp. New ISPs have been identified as ISP1A and ISP2A and DNA sequences have been determined to encrypt them [35]. Besides, MIS and RAR toxins are also reported as insecticidal which is produced by these species [36].

In the various phases of the bacterial development process, vegetative to sporulation, the poisonous impacts were noted after aftertouch or consumption of various fractions. Chitinase and specific proteins toxins have been recognized and their mode of action investigated. However, there are still many elements that need clarification and further research.

4.2 The Chitinase producing *Bacillus licheniformis* and their insecticidal activity

The gram-positive endospore formation of the *Bacillus licheniformis* is a widely spread and

isolated micro-organism soil, animals, and plant materials [37, 38, 39]. It is widely exploited in industrial procedures [40]. In specific, *B. licheniformis* outstanding protein secretion capacity has made it an ideal host for the large-scale production of commercially used enzymes (e.g. Chitinases, amylases, and proteases) [41, 42]. It is usually considered secure, and some of these species strains are declared probiotics [43].

One isolate strain, called S213, displayed a powerful chitinolytic activity among nine Chitinase producing strains isolated from soil. Maximum activity of Chitinase achieved in late stationary phase with optimum temperature 50-60°C and pH 6.0, respectively. A significant Chitinase about 65 kDa molecular weight was produced by the S213 strain [44]. Additionally, *B. licheniformis* strain NM120-17 had the highest chitinolytic activity at 40°C temperature and 7.0 to 8.0 pH respectively. The activity of chitinase produced by these strains was enhanced by Na^{+}, Cu^{2+}, Mg^{2+}, and Ca^{2+} and was inhibited by Zn^{2+}, Ag^{2+}, and Hg^{2+} [45]. Most *B. licheniformis* generate a large number of enzymes that have the potential to degrade the chitin wall and intestinal epithelium of termites. They can remain at optimum pH and temperature on their surface; further research is needed to demonstrate their termiticidal activities. *B.licheniformis* (strain ATCC 14580; DSM 13) can produce the chitinases in great amounts and have efficiency in microbes controlling strategies.

4.3 The Chitinase producing *Bacillus thuringiensis* and their insecticidal activity

Bacillus thuringiensis is effective for insect control as a specific, safe, and efficient tool. It is a gram-positive bacterium that generates protein inclusions during sporulation [46]. These inclusions can be differentiated by phase-contrast microscopy as distinctive crystals. These inclusions include proteins known as crystal proteins, Cry proteins, and S-endotoxins which are extremely poisonous to a broad range of significant insect pests, both agriculture and health-related, and other invertebrates. Crystal proteins are a good option to chemical pesticides for the control of insect pests for agriculture, forestry, and in the home due to their evaluation specificity and their environmental security. To control insects and overcomes the insect's pesticide issue, it has been suggested that the rational use of Bt toxins should provide various options [56]. BUPM255 is one of the *B. thuringiensis* chitinase producing strains with its elevated chitinolytic and antifungal effects. A 2031 base pair open reading framework encoding 676 residual amino acids proteins was present in the cloning and sequence of the respective gene called Chi255, similarity analyzes of nucleotides and amino acids have shown that Chi255 is a fresh Chitinase gene, showing several distinctions from the *B. thuringiensis* published Chi genes [47].

Bacillus thuringiensis NM101-19 strain is also responsible for chitinase production up to a large extent. A study reported in Egypt shows that these strains produce above 20 kDa chitinase masses at an optimum temperature of 30°C and pH 6.0 to 7.0 [45]. Additionally, *B. thuringiensis* species Colmeri 15A3 can produce 36 kDa masses at an optimum temperature of 20 to 60 °C and pH 4.0 to 8.0 respectively (Shown in table 2) [48]. *B. thuringiensis*, and *Serovar knonhukain* (str. 79-27) can also responsible for producing the chitinase enzymes. These species are known to be the best for insecticidal activities because it can produce chitinases in that condition in which most of the insects growing. Further investigation is needed to confirm its activity towards termites.

4.4 The Chitinase producing *Paenibacillus* Species and their insecticidal activity

Many *Paenibacillus* strains have been identified in a variety of environments, the majority of them were found in the soil having a strong association with plant roots. Many species being relevant to animals, plants, and the environment. According to the *Paenibacillus* functions they have been discovered in disparate habitats (from polar to tropical regions and from aquatic to the driest deserted environments). Some species of *Paenibacillus* responsible for the production of antimicrobial substances that are used as biopesticides and should produce enzymes that can be utilized for bioremediation [49]. *Paenibacillus* spp. can produce up to a large extent of chitinase enzymes can hydrolyze chitin, which is the structural polysaccharide of insect exoskeletons and gut epithelium, leading to low feeling rates and death of infected insects [50].

Some Species of *Paenibacillus* including (*Paenibacillus pabuli* strain NBRC 13638,

Table 1. The *Bacillus* strain*s* and their gene sequences that involve in Chitinase production and have insecticidal activity

S. No.	Gene ID	Gene	Protein	Bacteria name	Strain	Locus tag and Gene Symbol	Sequence
1	2854537	ChiA	Chitinase	*Bacillus thuringiensis*	Serovar konkukian str. 97-27	BT9727_3469	NC_005957.1 (3554763..3555845)
2	2858240	ChiA	Chitinase	*Bacillus thuringiensis*	Serovar konkukian str. 97-27	BT9727_0362	NC_005957.1 (429452..431476, complement)
3	34043470	ChiA	Chitinase	*Bacillus clausii* KSM-K16	Strain: KSM-K16	ABC_RS03410	NC_006582.1 (703829..705625)
4	3028169	ChiA	Chitinase	*Bacillus licheniformis*	Strain: ATCC 14580; DSM 13	TRNA_RS23180	NC_006270.3 (314685..316766)
5	29259029	ChiA	Chitinase	*Brevibacillus brevis*	Strain: NBRC 100599 (= 47)	BBR47_RS08865	NC_012491.1 (1765162.1767342)
6	32033700	ChiA	Chitinase	*Paenibacillus pabuli* NBRC 13638	Strain: NBRC 13638, culture-collection: NBRC:13638	PPA03S_RS11730	NZ_BCNM01000007
7	29545074	ChiA	Chitinase	*Paenibacillus borealis*	Strain: DSM 13188, culture-collection: DSM:13188	PBOR_RS28075	NZ_CP009285.1
8	2828079	chiB	Chitinase	*Bacillus cereus var. thuringiensis*	Unspecified	NEW ENTRY	Unspecified
9	34875106	ChiC	Chitinase	*Brevibacillus laterosporus LMG 15441*	Strain: LMG 15441	BRLA_RS04215	NZ_CP007806.1
10	1205584	ChiA	Chitinase	Bacillus cereus ATCC 14579	Strain: ATCC 14579	BC3237	NC_004722.1 (3216495..3216770, complement)
11	40819973	ChiC	Chitinase	*Paenibacillus tianmuensis*	Strain: CGMCC 1.8946	BLO51_RS24325	NZ_FMTT01000052.
12	32919819	ChiC	Chitinase	*Paenibacillus glucanolyticus*	Strain: 5162, culture-collection: DSM:5162	A3958_RS13055	NZ_CP015286.1
13	31573373	ChiC	Chitinase	*Paenibacillus odorifer*	Strain: DSM 15391, culture-collection: DSM:15391, type-material: type strain of *Paenibacillus odorifer*	PODO_RS24240	NZ_CP009428.1
14	29413597	ChiC	Chitinase	*Bacillus simplex*	Strain: SH-B26	UP17_RS24620	NZ_CP011008.1

Table 2. The chemical properties of chitinase produced by various *Bacillus species*.

S. No.	*Bacillus* spp.	Type of Enzyme	Mol. weight (kDa)	Temperature (°C)	pH	References
1	*Bacillus sp. 13.26*	Exo-chitinase	60	60	7 to 8	[52]
2	*Bacillus licheniformis*	Exo-chitinase	Maximum	50 to 70	5 to 6	[53]
3	*Bacillus subtilis NPU 001*	Endo-chitinase	31	50	6	[54]
4	*Bacillus brevis*	Endo-chitinase	85	60	8	[55]
5	*Bacillus thuringiensis spp. colmeri 15A3*	Exo-chitinase	36	20 to 60	4 to 8	[48]
6	*Brevibacillus laterosporus (Lak1210)*	_	25 to 90	70	6.0 to 8.0	[8]
7	*B. licheniformis strain NM120-17*	–	Maximum	40	7.0 to 8.0	[45]
8	*B. licheniformis strain S213*	–	65	50 to 60	6.0	[44]
9	*Bacillus thuringiensis NM101-19 strain*	_	20	30	6.0 to 7.0	[45]

Paenibacillus boreals strain DSM: 13188, *Paenibacillus tianmuensis* strain CGMCC1.8946, *Paenibacillus glucanolyticus* strain DSM:5162, and *Paenibacillus oderifer* strain DSM 15391) are responsible for chitinase enzymes and can degrade the chitin composite surface layer and gut lining of many insects and pests (Shown in table 1).

One of the species *Paenibacillus* spp. D1, its isolated chitinases shows to cause concentration-dependent mortality of cotton bollworm (*Helicoverpa armigera*). Indicating the potential of the organism or its enzyme to be used as an insecticide in the field [50, 51].

5. CONCLUSION AND FUTURE RECOMMENDATIONS

The use of these *Bacillus* species is more effective because they are safe, harmless, and non-pathogenic to humans and animals. These bacterial species are found in soil and are isolate easily. Due to their activity of producing a large amount of chitinases enzyme we can use it for the control of termites as insecticides. The Chitinase genes can be over expressed in bacterial species by using the CRISPR Cas-9 system or other technologies. Due to overexpression of the Chitinase gene, we can get Chitinase enzyme in bulk amount. We can store it and can spray it on plants or directly on termites.

In the future, experimental work on these species is needed. Another opinion is to isolate the Chitinase gene from these species and to be transformed into the plant cells. By transformation of that gene to plant, it will express there and shows the chitinase producing activity. It is more effective because the plant will resist termites and will kill those termites which consume that plant residue.

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