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Isolation and characterization of adult gut microflora of *Xenocatantops humilis* (Orthoptera: Catantopidae)

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Abstract

Grasshoppers are an integral part of food chain, food web and ecosystem balance. They are consumed in many parts of the world by humans as a protein source. Some species are a serious threat to agriculture and vegetation. Gut microflora plays an essential role in the metabolic activities of insects including reproduction and behavior. Identification and Characterization of gut microbial taxa can lead to better understanding of insect-microbe interactions and development of advanced, more efficient biological methods of pest control. Microbiological and genomic analyses of gut isolates of adult *Xenocatantops* showed the occurrence of *Bacillus paramycooides* of the *Bacillus cereus* group along with other diverse microbial genera.

Keywords: *Bacillus*, grasshoppers, gut microbiome, 16SrRNA, phylogeny, orthoptera, insects

Introduction

Orthopterans which include both short-horned and long-horned grasshoppers, are the key herbivores in grassland ecosystems across the world and are considered as one of the most important insect groups both ecologically and economically. Grasshoppers form an essential part of grassland ecosystems and serve as a food source for birds and also affect the soil enzymatic activity significantly [1]. *Xenocatantops humilis* is a grasshopper species belonging to family Acrididae and subfamily Catantopidae [2]. It is mainly found in temperate Asian regions of India, China, Malaysia and Papua New Guinea and it is generally found in forests [3]. Heavy infestation of *X. humilis* has been found on hill slopes adjoining cultivated fields of paddy, maize, oat cow pea. Adults and nymphs are found in groups under the leaves and also near the edges of the rice fields near aquatic sources [4, 5].

It is mainly consumed as a protein source in the South-East Asian regions like Singapore and are generally bred for the same [6]. According to several surveys, *Xenocatantops humilis* was found to be a predominant pest on tea plants and paddy fields [7][8]. Out of total 56 species of *Xenocatantops*, *X. humilis* is a pest on pepper leaves thereby reducing its production [9].

Insect microbiomes play an important role in the health and fitness of insect hosts by contributing to nutrient absorption, immune health, and overall ecological fitness [1]. They are also involved in multitrophic interactions between the host and other biological factors, including enhancement of the host's immune response. During evolution mutualism between gut microbes and insect host has been established, and the gut microbes developed strategies for adapting to the environment of the insect gut [10]. Insects, which generally harbor a lower microbial diversity than vertebrates and have recently emerged as potential model systems to study these interactions [11]. Recently microbiomes of economically important insects such as Honeybee, Fruit fly and Beetles are widely studied [12-16] but grasshoppers being the pest insects for many crops are still not in forefront with respect to the microbiome studies [1, 17-19].

Study of microflora may play a role in the development of new methods of biological pest control and for studying unknown microbes influencing insect physiology and ecology. Insect microbiome studies may contribute in increasing the protein value of edible insects such as *Xenocatantops humilis*. Gene transcriptomics data of *Xenocatantops brachycerus* will provide a useful molecular resource for gene prediction, molecular marker development, studies on

cellular signaling pathways and will be a reference for the efficient use of other grasshoppers [21]. Similar genomics approach can be used for *X. humilis* which will lead to better understanding of biology of grasshoppers and their uses as proteinaceous food source and development of next generation pesticides. In this paper we have isolated and characterized the microflora of *Xenocatantops humilis* which is one of the major pests involved in crop destruction as well as a source of food.

Materials and Methods

Xenocatantops. humilis adults were collected from Vetil Tekdi, Kothrud, Pune (Fig.1). Genus and species were confirmed by using relevant references and expert guidance (S.K. Tandon, 1972).

X. humilis were subjected to euthanasia (cold treatment). After euthanasia induction, the organisms were surface sterilized using 70% EtOH. Decapitation was performed, body appendages were removed and the entire abdomen was transferred ice-cold 0.1 M 1X PBS (pH 7.4). Abdomen was homogenized in Teflon-coated Potter Elvehjem homogenizer. Homogenate was serially diluted from 10⁻¹ to 10⁻⁴ for obtaining pure colonies. 100 µL of sample was plated on Luria Agar (LA) plates by Spread plate method after performing serial dilutions. Colony characterization and CFU/ml estimation was done post inoculation (1 -2 days). Sub-culturing was done on differential and selective media by streak plate method to obtain pure isolates. Gram staining [22] and biochemical characterization was performed for identification and characterization of pure isolates.

A single isolate was selected for identification, biochemical characterization, genomic, and phylogenetic analyses using the gold standard 16S rRNA gene sequencing method. 1200 bp sequence length was used for finding out extent of homology with the closest neighbor in the database (EzBioCloud 16S db). Phylogenetic Tree Construction was done using MEGA X 10.2.5 version and ClustalX. Neighbour Joining method (N-J) was used for the same.

Results



Fig 1: *Xenocatantops humilis* collection site (Occurrence and habitat)



Fig 2: Adapted from <https://singapore.biodiversity.online/species/Arth-Hexa-Orthoptera>



Fig 3: Adult Abdomen: Undiluted Homogenate on LA plate (Spread Plate method)

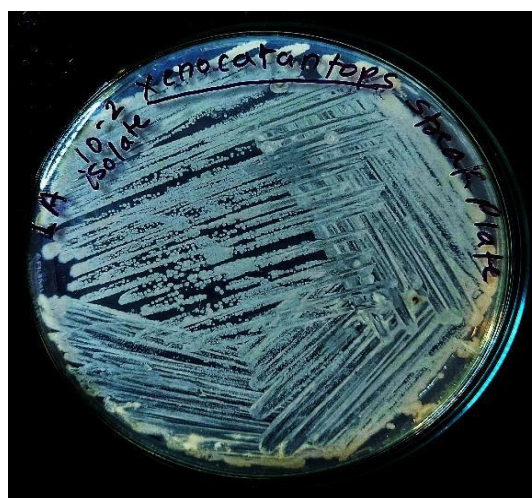


Fig 4: Adult Abdomen: Pure Isolate on LA plate (Streak Plate method)



Fig 5: Streak plate method : Pure Isolate

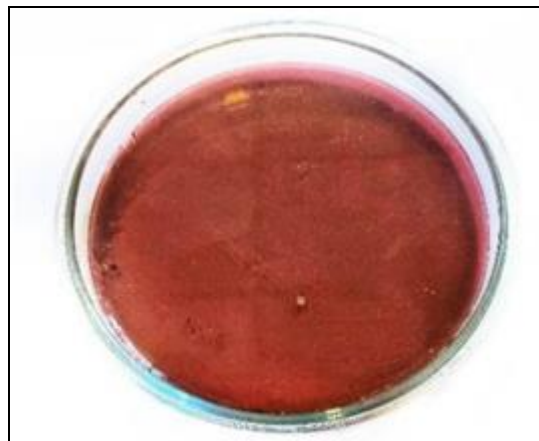


Fig 6: EMB Agar plate : No growth

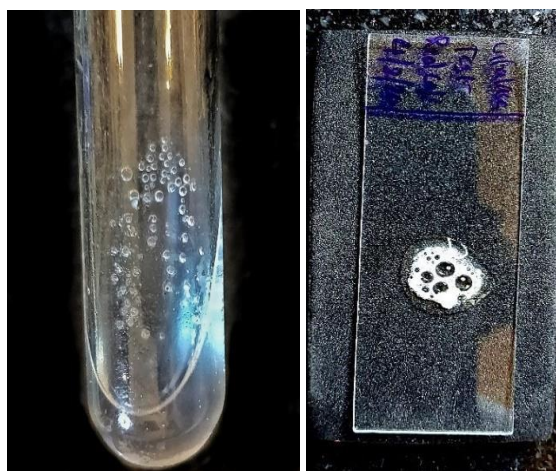


Fig 7: Catalase Test: +Ve

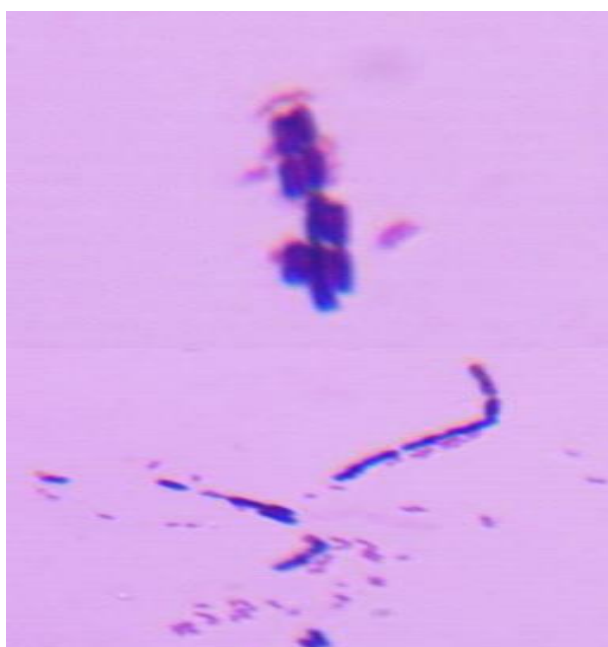


Fig 8: Gram Staining of Isolate Gram +ve rods (Bacilli)

Table 1: Colony Characterisation

Sr. no.	Colony Characteristics	Isolate 1
1	Grams Nature	+ve (Fig. 8)
2	Shape	Rods
3	Size	Pin point to pin head size 1 mm
4	Margin	Entire
5	Colour of Colony	White
6	Opacity	Opaque
7	Elevation	Flat
8	Surface	Smooth
9	Consistency	Muroid
10	Catalase Test	+ve (Fig. 7)
11	Motility Test	-ve

The isolate showed no growth on EMB agar (Fig.6).

Bioinformatics

Using standard genomic techniques of RNA isolation, purification and PCR, the following 16SrRNA sequence was used for *in silico analysis* involving NCBI BLAST (Fig. 9, 10).

>16S rRNA sequence of *Xenocatantops* isolate B_FEB_21_131

GATTAAGAGCTTGCTCTTATGAAGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCATAAGACTGGG
 ATAACTCCGGGAAACCGGGGCTAATACCGGATAACATTTTGAACCGCATGGTTTCGAAATTGAAAGGCGGCTTCGG
 CTGTCACTTATGGATGGACCCGCGTCGATTAGCTAGTTGGTGAAGTAACGGCTCACCAAGGCAACGATGCGTAG
 CCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCAGACTCCTACGGGAGGCAGCAGTAGGGA
 ATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTTCGTA AAAACTCTG
 TTGTTAGGGAAGAACAAGTGTAGTTGAATAAGCTGGCACCTTACGGTACCTAACCAGAAAGCCACGGCTAACT
 ACGTGCCAGCAGCCGCGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTG
 GTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTTCATTGGAAACTGGGAGACTTGAGTGCAGA
 AGAGGAAAGTGGAAATCCATGTGTAGCGGTGAAATGCGTAGAGATATGGAGGAAACACCAGTGCGGAAGGCGACT
 TTCTGGTCTGTA ACTGACTGAGGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGC
 CGTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTATGTGCTGAAGTTAACGCATTAAGCACTCCGCCTG
 GGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCGCACAAGCGGTGGAGCATGTGGTTTAA
 TTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAACCCTAGAGATAGGGCTTCTCCTTCGGGA
 GCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTGAGATGTTGGGTAAAGTCCC GCAACGAGCGCA
 ACCTTGATCTTAGTTGCCATCATTTAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGG
 ATGACGTCAAATCATCATGCCCTTATGACCTGGGCTACACACGTGCTACAATGGACGGTACAAAGAGCTGCAAG
 ACCGCGAGGTGGAGCTAATCTCATAAAACCGTTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGCTGG
 AATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGACACACCCGCCGTCA

NCBI-BLAST Multiple Sequence Alignment (MSA) for 16SrRNA sequence of *Xenocatantops* sp. abdominal Isolate

Organism	Blast Name	Score	Number of Hits	Description
Bacillaceae	firmicutes		100	
. Bacillus	firmicutes		77	
. . Bacillus cereus group	firmicutes		31	
. . . Bacillus paramycooides	firmicutes	2473	1	Bacillus paramycooides hits
. . . Bacillus tropicus	firmicutes	2468	1	Bacillus tropicus hits
. . . Bacillus nitratireducens	firmicutes	2468	1	Bacillus nitratireducens hits
. . . Bacillus luti	firmicutes	2468	1	Bacillus luti hits
. . . Bacillus albus	firmicutes	2468	1	Bacillus albus hits
. . . Bacillus cereus ATCC 14579	firmicutes	2462	2	Bacillus cereus ATCC 14579 hits
. . . Bacillus cereus	firmicutes	2462	4	Bacillus cereus hits
. . . Bacillus wiedmannii	firmicutes	2457	1	Bacillus wiedmannii hits
. . . Bacillus proteolyticus	firmicutes	2457	1	Bacillus proteolyticus hits
. . . Bacillus pacificus	firmicutes	2446	1	Bacillus pacificus hits
. . . Bacillus paranthracis	firmicutes	2446	1	Bacillus paranthracis hits
. . . Bacillus toyonensis	firmicutes	2440	1	Bacillus toyonensis hits
. . . Bacillus pseudomycooides	firmicutes	2440	2	Bacillus pseudomycooides hits
. . . Bacillus thuringiensis	firmicutes	2440	3	Bacillus thuringiensis hits
. . . Bacillus mobilis	firmicutes	2435	1	Bacillus mobilis hits

Fig 9: NCBI BLAST Significant hits

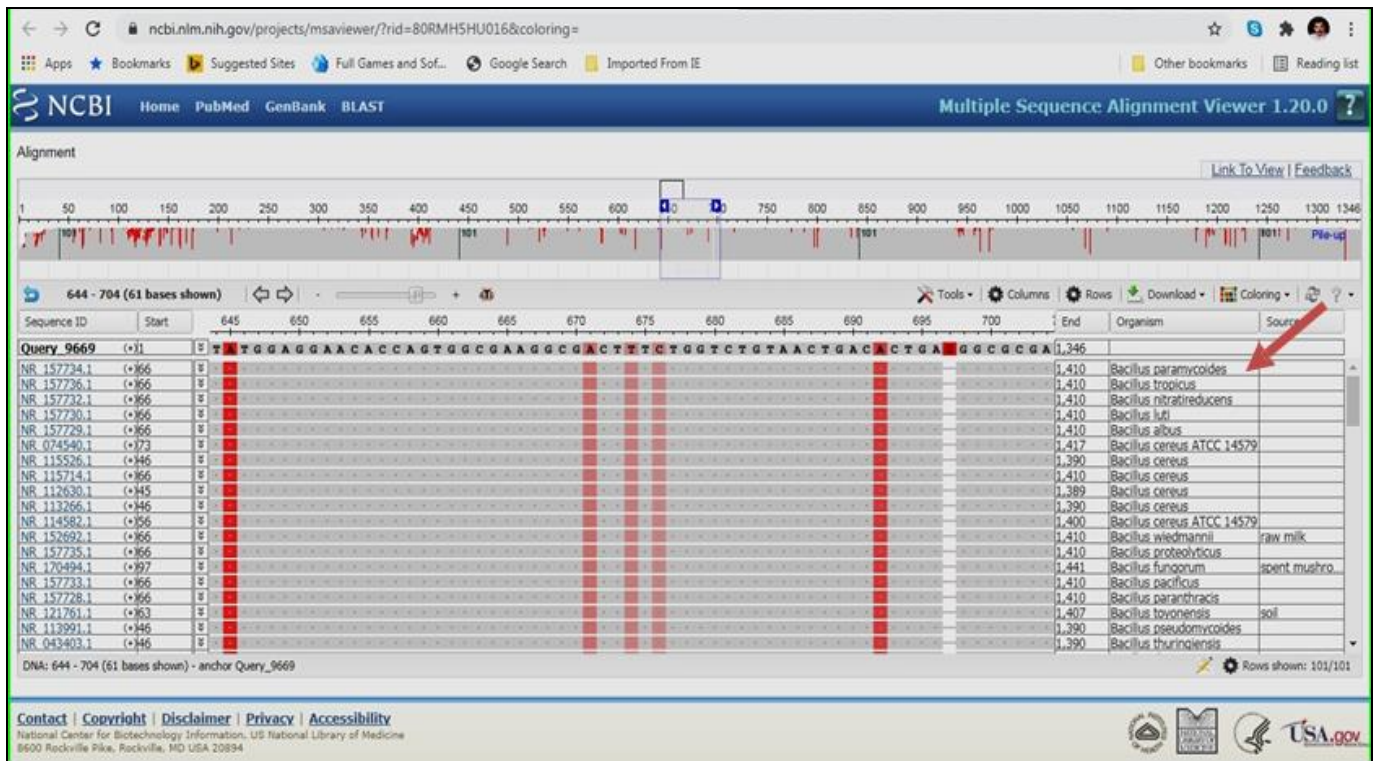


Fig 10: 16SrRNA sequencing followed by *in silico* analysis using Ez Bio Cloud DB showed the following results

PRN	Strain No.	Closest Neighbour*		% Similarity
		Taxonomic Designation	Accession No.	
B_FEB_21_131	1	<i>Bacillus paramycoides</i> NH24A2(T)	MAOIO1000012	99.85

Fig 11: Closest Neighbour*

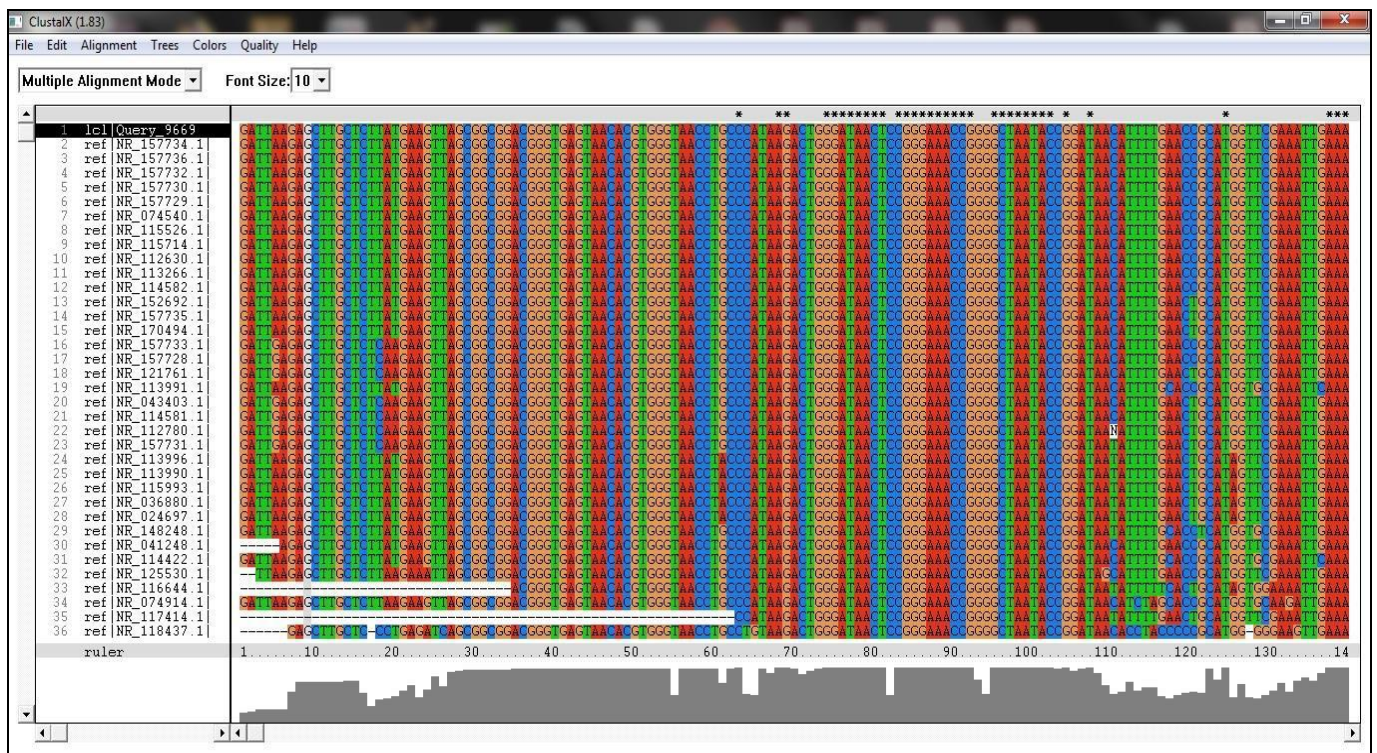


Fig 12: Clustal X Multiple Sequence Alignment (MSA) of BLAST hits for 16S rRNA sequence of *Xenocatantops humilis* abdominal isolate

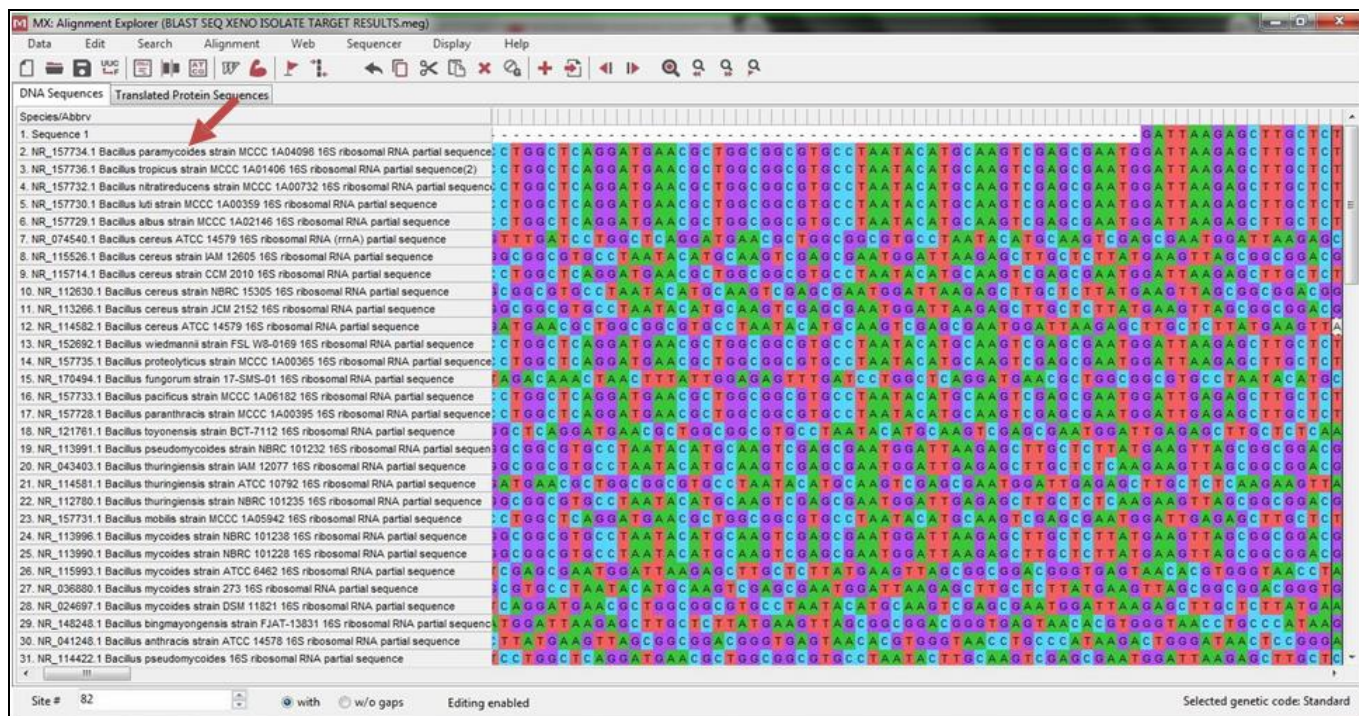


Fig 13: MSA of 16SrRNA sequence of the isolate yielded significant hits for Genus *Bacillus* with highest similarity to *Bacillus paramycoides*

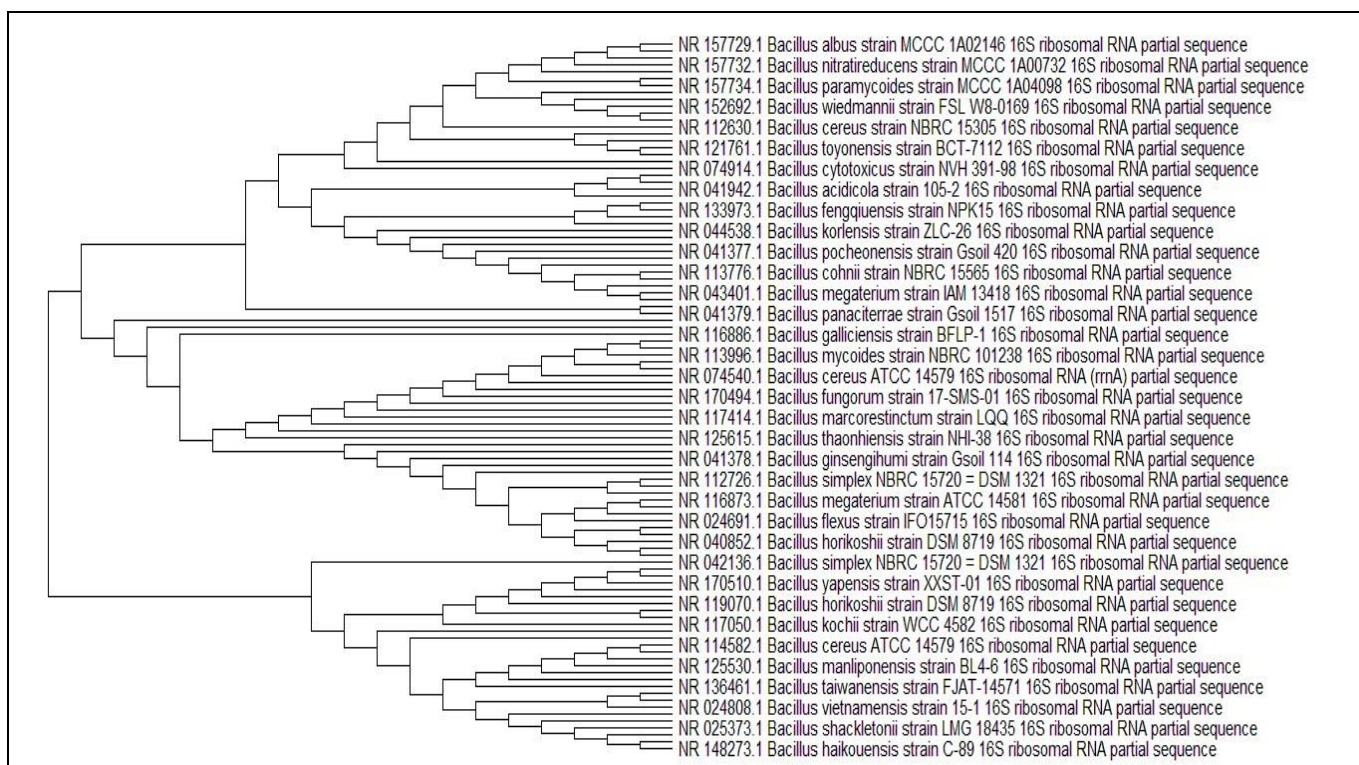


Fig 14: Phylogenetic tree construction in MEGA X using N-J method

Based on colony characterization and 16S rRNA sequencing the isolate was found to belong to *Bacillus cereus* group further it with 99.85 % confirmed similarity to *Bacillus paramycoides* (Fig.13,14).

Discussion

Most herbivorous insect intestines contain various cellulose and other biopolymer degrading symbiotic microorganisms (Mannesmann 1972). Insect diet profoundly affects gut microflora and gut microbes can adapt remarkably to changes in insect diet (Kaufman and Klug 1991; Santo Domingo *et al.*

1998). Occurrence of *Bacillus cereus* group members in the gut of insects like grasshoppers may be co-related to their herbivorous diet, since *Bacillus cereus* group is commonly found in soil, on vegetables and even processed foods [23]. *Bacillus paramycoides* hydrolyses starch, skimmed milk and casein [20]. This indicates the possible role of *B. paramycoides* in metabolism of starch and cellulose by secretion of extracellular amylases in *X. humilis* gut [20].

Conclusion

Genomic analysis of entire gut microbiome of *Xenocatantops*

humilis will definitely lead to development of novel bio-control strategies (bio-pesticides) and pest control.

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