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Characterization and Virulence of an Indigenous Soil *Bacillus* sp. Prospecting for Mosquito Control

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SC and TKD designed the study, wrote the protocol, interpreted the results and wrote the first draft of the manuscript. Author TSG conducted the experimental study and field study analyzed the data and contributed to write the manuscript. All the authors read and approved the final manuscript.

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ABSTRACT

Aims: Aquatic habitat of the rice fields is the effective breeding site of the mosquitoes whose overcrowding would promote their pathogens. But no attention has been paid to these habitats for isolation and identification of the bacterial biocides. Therefore, the study was envisaged to isolate and identify the mosquitocidal bacteria from the unexplored rice field soil of the Burdwan district (a premier rice producer), West Bengal, India. It was also aimed to evaluate the virulence of the potent pathogenic organisms in the laboratory and field against the mosquitoes.
Study Design: Laboratory and field study.
Place and Duration of the Study: Parasitology and Microbiology Research Laboratory,

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Department of Zoology, The University of Burdwan, Burdwan 713104, West Bengal, India and Microbiology Laboratory, Crop Production Division, Central Rice Research Institute, Cuttack 753 006, Orissa, India. The study was conducted between June, 2011 to July 2012.

Methodology: The soils were collected from the rice-fields, serially diluted up to 10^{-3} level, 100 µl suspension was plated on NA medium and incubated at 30±0.1ºC in the BOD incubator for 72 h. The colonies were checked under a phase-contrast microscope and those having spores were purified by dilution plating on NA plates. Phenotypic, biochemical and molecular characters of the bacteria were studied following standard methods. The mosquitocidal activity of the selected organism was assessed using different mosquito species both in the laboratory and field.

Results: On the basis of phenotypic, biochemical and 16S rDNA (Acc. no. GU190368) analysis, the selected bacteria (Ts 116) was identified as *Bacillus* sp. In the laboratory, the LC_{50}s of the *Bacillus* sp. Ts116 against late third instar larvae of *An. subpictus*, *Ar. subalbatus* and *Cx. quinquefasciatus* were (2.37, 2.2 and 9.6) X 10^6 bacteria/ml, respectively. After 7d, treatment with 100 ml suspension (containing 2.7x10^6 bacteria/ml) of bacteria/m^3 breeding site effected 97.35, 95.65 and 100% mortality of *An. subpictus*, *Ar. subalbatus* and *Cx. quinquefasciatus* larvae, respectively.

Conclusion: Indiscriminate use of chemical insecticides causes vector-resistance resulting in serious health and environmental hazards. The *Bacillus* sp. Ts116 (GU190368) of the rice fields had potential to be exploited in mosquito control programms.

Keywords: Bacillus sp; An. subpictus; Ar. subalbatus; Cx. quinquefasciatus; mosquitocidal activity.

1. INTRODUCTION

The mosquitoes viz. *Anopheles*, *Culex*, *Aedes, Mansonia* and *Armigeres* spp. transmit the pathogens of malaria, filaria, dengue, some arboviruses etc. all over the world [1,2,3]. The *Cx. quinquefasciatus* and *An. subpictus* (sibling species A) are the vectors of malaria and filaria in India, respectively [4,5]. Indiscriminate use of chemical insecticides viz. DDT, gammexane, malathion, chlordane etc. caused resistance development and resulting in health and environment problems [1]. Therefore, biocontrol would be the best choice for safer mosquito management. Several bacterial, fungal and protozoan pathogens have been recorded and formulated for mosquito control [6,7,8]. Nevertheless, in India, neither indigenous microbial mosquitocides have been commercialized nor exotic formulations are readily available which debarred their extensive use [9]. Bacteria, especially *Bacillus thuringiensis* (Bt) and *B. sphaericus* (Bs), are the most successful mosquitocides but no native pathogen of *Cx. quinquefasciatus* and *An. subpictus* is known to date [1,10]. On the other hand, flooded rice ecology being the effective breeding site of the mosquitoes, it would be a potential source of the pathogens too which remained almost unexplored. In India, research on microbial control of mosquitoes is negligible and probably limited within one vector control research centre and only a few biocide formulations i.e. Bt var. *israelensis* (Deltafix, RK Biovecta of Bti AS) and Bs have been developed [11]. Unwarrantedly, *B. sphaericus* has resulted in resistance development in *Cx. quinquefasciatus* [12] and resistance inheritance was recorded in a selected strain of *An. stephensi* which was sensitive to Bti [13]. Therefore, the present investigation was envisaged to isolate and characterize the resident mosquitocidal *Bacillus* spp. from the rice fields of Burdwan (a premier year-round rice producer), West Bengal, India and to assess their virulence against different mosquito larvae in the laboratory and field conditions with the aim to exploit the potent pathogens for vector control in the end.

2. MATERIALS AND METHODS

2.1 Isolation of Bacteria

Soil samples were collected from the rice-fields. A portion of soil was blotted to optimum dryness within the sterile filter papers. One g soil was suspended in 9 ml sterile water and diluted serially up to 10^{-3} level. A 100 µl portion was mixed with 100 ml nutrient agar (NA) (g/l: peptone 5, beef extract 3, agar 2, pH7), distributed in five plates and incubated at 30±0.1ºC in the BOD incubator for 24 h. The colonies were checked under a phase-contrast microscope and those having spores were purified on NA plates and maintained at 4±0.1ºC on NA slants.

2.2 Characterization of Bacterial Isolate

Morphological, physiological and biochemical characters of the bacteria were studied following standard methods [14,15,16,17]. Antibiotic sensitivity was tested using different antibiotic discs viz. kanamycin (30 µg/disc), nalidixic acid (30 µg/disc), rifampicin (5 µg/disc), doxycycline (30 µg/disc), gatifloxacin (10 µg/disc), vancomycin (30 µg/disc), gentamycin (10 µg/disc), ampicillin (10 µg/disc), ofloxacin (5 µg/disc), levofloxacin (5 µg/disc), streptomycin (10 µg/disc) following Brown [18]. The bacterium was phenotyped according to Logan and de Vos [17].

2.3 Scanning Electron Microscopy of Bacterial Isolate

Bacteria were grown for 3 d on NA plates, smears were prepared on cover glasses, heat fixed over a flame for 1-2 sec followed by 2.5% glutaraldehyde (aqueous) for 45 min. The slides were then dehydrated passing through 50, 70, 90 and 100% alcohol for 5 min each. The specimens were gold coated and observed under a SEM.

2.4 Extraction and Electrophoresis of Genomic DNA

Genomic DNA from the bacteria was isolated following Janssen [19]. The organisms were grown for 6-8 h at 30±0.1℃ in 2 ml of nutrient broth (NB). The cultures were centrifuged at 10,000 rpm for 10 min at 4±0.1ºC; pellet was washed with 8.5% (w/v) NaCl solution followed by sterile water, the supernatant was discarded and the pellet was suspended in 576 µl TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) by repeated pipetting. To it, 30 µl of 10% SDS and 3 µl of proteinase K (20 mg/ml in 0.5% SDS) were mixed and incubated for 1 h at 37±0.1ºC. To the reaction mixture, 100 µl of 5M NaCl and 80 µl of CTAB/NaCl solution (10% CTAB in 0.7M NaCl) were mixed sequentially, incubated for 10 min at 65±0.1ºC. To the reaction mixture 0.7-0.8 ml chloroform-isoamyl alcohol (24:1 v/v) was mixed, centrifuged at 5,000 rpm for 4-5 min at 4±0.1ºC. The aqueous and viscous upper phase was removed in a fresh microcentrifuge tube, equal volume of phenol-chloroform-isoamyl alcohol (25:24:1, v/v/v) was added, mixed by gently inverting the tubes and spun for 5 min at 4±0.1ºC and 7000 rpm. The supernatant was taken in a fresh tube, added 0.6 volume of isopropyl alcohol, inverted back and forth until a stringy white precipitate of nucleic acid was clearly visible. The precipitate was pelleted by spinning for 20-30 sec. at 7000 rpm at 4±0.1ºC. The DNA spool was washed with 70% ethanol to remove residual CTAB, spun for 5 min at 7000 rpm at 30±0.1ºC. The supernatant was removed and the pellet was dried in a lyophilizer. The DNA samples were revived in sterile water and electrophorsed through 0.8% agarose gel at a constant 5V for 30 min and subsequently 5V/cm for the required time according to the gel size. The DNA profile was visualized under a UV transilluminator (312 nm), documented and analyzed using Photocapt software.

2.5 Amplification and Sequencing of 16SrRNA Gene, and Phylogenetic Analysis

The ~1.5 kbp rDNA fragment was amplified using high-fidelity PCR polymerase. The PCR product was sequenced bi-directionally through a genetic analyzer using the forward primer (5'-AGAGTRTGATCMTYGCTWAC-3') and reverse primer (5'-CGYTAMCTTWTTACGRCT-3'). The sequence data were aligned using the ClustalW submission form (http://www.ebi.ac.uk/clustalw) and analyzed by ClastalW software [20]. Evolutionary distances were calculated using the method of Jukes and Cantor [21] and the topology was inferred using the neighbor-joining method [22]. Phylogenetic trees were constructed following Tamura et al. [23].

2.6 Estimation of Total Cellular Protein from Bacteria

The bacteria were grown in nutrient broth medium for 12 h. The bacterial cultures were centrifuged and the pellet was taken, washed two times with 8.5% NaCl followed by once with Tris-buffer saline (TBS), pH 7.5. Then the pellet was kept at 0ºC for extraction of protein. The bacterial pellet was suspended in 1 ml of cold TBS and 200 µl of Triton X100. Cell mixture was kept at 0ºC for 15 minute, centrifuged at 4ºC for 10 min at 6000 rpm. The supernatant was transferred to a fresh Eppendorf tube. The pellet was resuspended in 1 ml of cold TBS and kept at 0ºC for 15 min, centrifuged and the supernatant was taken. To the supernatant 10% TCA was added in the ratio of 1:10, centrifuged at 6000 rpm for 5 min, supernatant was discarded and the pellet was dissolved in small volume of sterile distilled water and used for the protein analysis.

2.7 SDS-PAGE Electrophoresis of Cellular Proteins

Samples and the markers were mixed with equal volume of 1x SDS gel loading buffer, heated to 100°C for 3 min to denature the proteins and then loaded in the wells. The electrophoresis was conducted initially at 5V/cm constant voltage till the dye front moved up to the resolving gel and then the voltage was increased to 15V/cm constant voltage (150V, 20-40 mA, 10W for standard gel size) and the gel was electrophoresed until the bromophenol blue dye reached almost to the bottom (about 1 cm above the end) of resolving gel. The gel was removed from the electrophoresis apparatus and stained with coomassie brilliant blue stain composed of 0.25 g coomassie brilliant blue R250 dissolved in 40 ml of methanol followed by addition of 10 ml of glacial acetic acid and volume was made up to 100 ml. The stain was filtered before use. The gel was immersed in at least 5 volume of staining solution at least for 4 h at RT. After the stain was drained out from the gel, it was treated with methanol: acetic acid: water (30:10:60, by volume) destaining solution for 8-10 h. Proteins were observed as blue bands through a visible light transilluminator and photographed through the photodocumentation system. Molecular weight of different protein fractions was estimated by PhotoCapt SW.

2.8 Assessment of Crystal Protein Production

Besides LM visualization, crystal protein production (if any) was assessed by SDS-PAGE analysis [24] also. The bacteria were grown for 7 d on a shaker at 100 rpm at 30±0.1℃ in 50 ml nutrient broth, culture was centrifugation at 10000 g for 10 min at 4±0.1℃, the pellet was washed 3 times in 50 ml of crystal wash (1M NaCl containing 0.1% SDS) solution and finally once with sterile distilled water. The pellet was re-suspended in 3 ml sterile distilled water, mixed with equal volume of alkaline solubilization buffer (50 mM anhydrous Na2CO3 containing 10 mM dithiothreitol (DTT), pH 10) and incubated at 37±0.1℃ for 12 h on a shaker at 25 rpm. The pH of the solubilized crystal was neutralized with 0.5 M HCl, treated with 1/10 volume aqueous trypsin (200 U/gm potency) solution (1 mg/ml) and incubated for 3 – 4 h at 37±0.1℃. An equal amount of trypsin was added again and incubated for 12 h at 37±0.1℃, centrifuged at 10000 g for 15 min at 4±0.1℃ and the supernatant was taken. Protein profile of trypsinized extract was analyzed by SDS–PAGE following the cellular protein analysis mentioned elsewhere.

2.9 Collection and Maintenance of Mosquito Larvae

Cx. quinquefasciatus and *Ar. subalbatus* larvae were collected with the help of plankton net from drain water in and around three village areas of Burdwan district of West Bengal, India. Larvae were reared in a mixture of drain and tap water (1:1 v/v) in white enamel trays at 27±1℃ and 80±5% relative humidity (RH) for 2-4 d. The larvae were fed on phyto- and zooplanktons during culture or experiments. Larvae of *An. subpictus* were captured from submerged rice fields of the same localities and were reared similarly in clean rice field water. The adults were maintained on honey soaked cotton pads at 27-28℃ and 80 ± 5% RH in cages (60 cm^3) in the Parasitology and Microbiology Research Laboratory, Department of Zoology of the host University.

2.10 Bioassay of the Bacteria against Mosquito Larvae

To determine the virulence of the *Bacillus* sp. against different mosquito species (*An. subpictus*, *Ar. subalbatus* and *Cx. quinquefasciatus*), the bioassay tests were carried out at 30±2℃; against 100 late third instar larvae maintained in 1000 ml sterile water in glass beakers. Mortality was recorded at 24h intervals.

An aliquot of 5 ml suspension (containing 2.7 x 10^6 bacteria/ml) of the *Bacillus* sp. was mixed with each liter of water in the beakers. Each test was replicated three times along with a control and the mortality (%) was determined following Abbott [25].

$$\text{Mortality (\%)} = \frac{\text{(\% mortality in the experiment) – (\% mortality in control)}}{\text{100 - (\% mortality in control)}} \times 100$$

The inoculum ($2.7x10^6$ bacteria/ml) was applied to 100 ml/m^3 water of the breeding sites with the help of compression pump to two shallow ponds having *An. subpictus* (average density 45.26 larvae/dip), two drains having *Ar. subalbatus* (average density 75.43 larvae/dip) and another two drains having *Cx. quinquefasciatus* (average density 63.27 larvae/dip). Two shallow ponds and two drains with an average mosquito larval density of 52.4 larvae per dip for each species were left untreated to serve as control. A 250 ml capacity dipper was used to estimate larval density taking 25 dips in each breeding habitat. Mosquito larvae were sampled from both treated and untreated sites by the dipper method at 24 h intervals after treatments up to 7 d during July 2012 to determine larval mortality. Average reduction (%) of larval density of *An. subpictus*, *Ar. subalbatus* and *Cx. quinquefasciatus* was determined before and after the treatment of the habitats with the bacterial isolate.

3. RESULTS AND DISCUSSION

3.1 Phenotypic Characterization of the Bacterial Isolate

The bacterium TS116 (GU190368) formed circular, off-white, flat and entire colonies. Bacteria were rod shaped, gram positive, motile, facultative anaerobic spore formers (Fig 1). The bacteria did not produce any crystal like structures. Dimensions of vegetative cells were (1.9-2.4) x (0.93-1.05) µm (l x dia.). The ellipsoidal spores were formed in a central or paracentral position without swelling the sporangium. Average diameter of the spores was (1.35-1.47) x (0.75- 0.80) µm (l x dia.).

3.2 Biochemical and Phylogenetic Analysis

The organism was positive for catalase, lipase, urease, protease (casein hydrolysis and gelatin hydrolysis), oxidase, amylase, lecithinase and citrate utilization and H_2S production but negative for indole production, nitrate reduction, Vogues-Proskauer and methyl red tests. The organism did not ferment xylose, mannitol and arabinose (Table 1). On the basis of morphological, physiological and biochemical characters the bacterial isolate Ts116 was identified as *Bacillus* sp. [17]. The nucleotide base composition of the 16S rDNA of the organism was determined which showed that the GC and AT contents were 53.34% and 46.66%, respectively (Table 2). The phylogenetic relationships derived from the neighbor-joining analysis of 16S rRNA gene sequence of the *Bacillus* sp. Ts116 with 20 validly described species of the genus *Bacillus* proved that the isolate branched with *Bacillus cereus* MBL13 (GenBank entry: FJ535616) having 72% bootstrap support and confirmed its identity as *Bacillus* sp. (Table 3, Fig. 2). SDS-PAGE of the total cellular proteins of the isolate Ts116 is given in Fig 3. The total protein profile of Ts116 had 14 protein components having the molecular weight (M.W.) ranging from 14.813 to 111.000 kDa (Fig. 3, Table 2) which corroborate the 20-66 kDa proteins prevailing in other *Bacillus* spp. also [26,27]. However, variations of the protein composition of the isolate Ts116 (Fig. 3, Table 2) with those of other *Bacillus* spp. [26,27] signify the species or strain level difference of the former with other *Bacillus* members. As the SDS-PAGE protein profile are second level of information which correlates with DNA-DNA hybridization and the 60-80 kDa protein range discriminates different *Bacillus* spp., the cellular protein profile of the *Bacillus* sp. Ts116 would be helpful to differentiate it from the *Bacillus* pathogens of other insects.

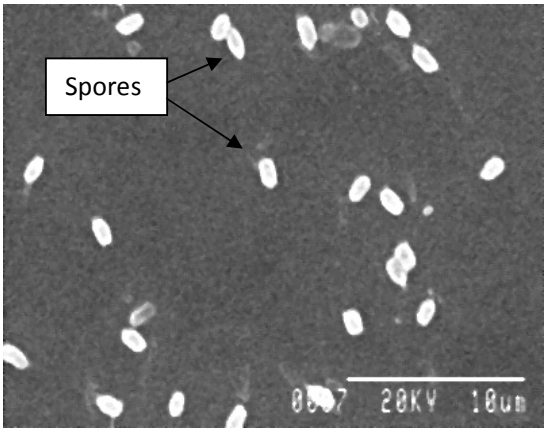

Fig. 1. Scanning electron micrograph of spores of *Bacillus* sp. Ts116

The SDS-PAGE profile of the protein extract through crystal protein extraction protocol did not produce any protein band (data not given) comparable to any cryastal toxin of *Bacilus thuringiensis* or *B. sphaericus* (Porter et al. [1]; Dangar et al. [24]). Absence of crystals under LM and absence of crystal toxin band on SDS-PAGE electrophoresis proved that the *Bacillus* sp. Ts116 is not crystal toxin producer and favour that it would not be *Bacillus thuringiensis* or *B. sphaericus.*

Table 1. Phenotypic and biochemical characterization of the bacterial isolate Ts116

Characters	Observation
Colony spherical, off-white, flat, entire	
Bacterium rod shaped, Gram (+)ve, 1.9-2.4 µm in length; 0.5-1.3 µm in diameter, spore oval shaped, 0.93-1.05 µm in average diameter	+
Growth on nutrient, NaCl (up to 8%), catalase, urease, oxidase, amylase, lipase, protease (casein and gelatin hydrolysis), H_2S production; citrate utilization	+
Indole, Vogues-Proskauer, methyl red test, acid production (fructose, glucose, galactose, mannose, xylose); nitrate reductase	-
gentamycin (10 µg/disc); doxycycline (30 µg/disc), kanamycin (30 µg/disc), nalidixic acid (30 µg/disc); rifampicin (5 µg/disc); streptomycin (10 µg/disc), vancomycin (30 µg/disc), ofloxacin (5 µg/disc), levofloxacin (5 µg/disc), gatifloxacin (10 µg/disc)	Sensitive
ampicillin (10 µg/disc)	Resistant

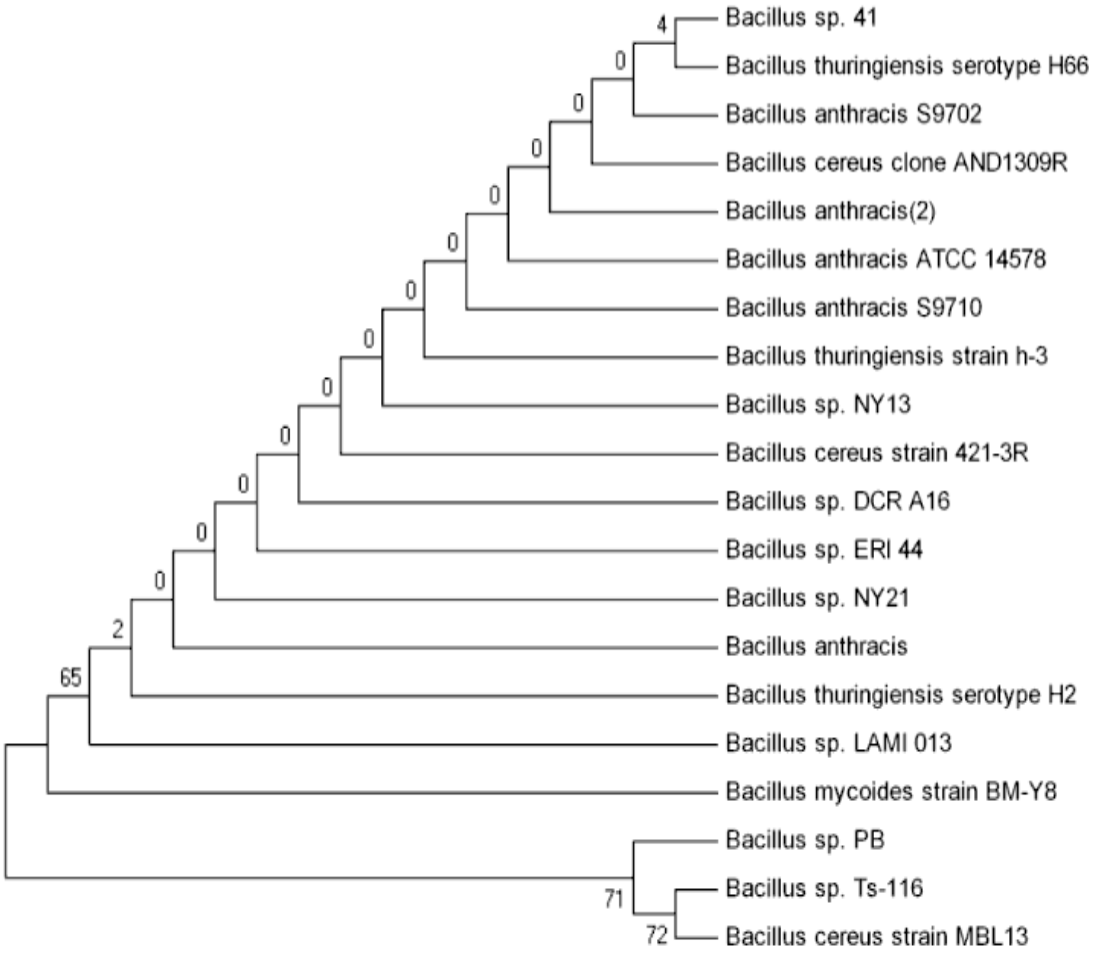

Fig. 2. Neighbor-joining tree constructed based on partial 16S rRNA genes sequences of *Bacillus sp* Ts-116 strain along with few other 16S rRNA genes retrieved from NCBI and RDP

Table 2. Nucleotide base composition of the 16SrDNA sequence and SDS-PAGE protein profile of the total cellular proteins of bacterial isolate Ts116

Nucleotide profile		AT and GC content		Protein profile	
				Bands	M.W
Nucleotide	Number (Mol %)	AT content (Mol %)	GC content (Mol %)	1.	111.000
				2.	90.706
				3.	82.765
				4.	79.676
A	370 (25.50)			5.	72.176
				6.	65.528
C	329 (22.67)	46.66	53.34	7.	63.175
				8.	57.662
G	445 (30.67)			9.	51.000
				10.	48.348
T	307 (21.16)			11.	44.530
				12.	38.119
				13.	18.016
				14.	14.813

Table 3. Alignment view and distance matrix table taking *Bacillus* sp. Ts116 as reference sequence

Organisms	NCBI accession number	S_ab score*
Bacillus anthracis; ATCC 14578	AB 190217	0.996
Bacillus anthracis; S9702	AB190221	0.996
Bacillus anthracis; S9710	AB190222	0.996
Bacillus anthracis; S9713	AB 190223	0.996
Bacillus anthracis	AY643481	0.997
Bacillus thuringiensis	DQ286301	0.998
Bacillus thuringiensis	DQ286354	0.997
Bacillus cereus; AND1309R;	DQ289992	0.996
Bacillus cereus; 421-3R	DQ328633	0.998
Bacillus sp. NY13	EF458316	0.997
Bacillus sp.NY21	EF458317	0.997
Bacillus sp. PB	EU363722	0.999
Bacillus sp. PD	EU363723	0.996
Bacillus sp. 41;	EU779995	0.998
Bacillus thuringiensis; h-3;	EU862321	0.998
Bacillus mycoides; BM-Y8;	EU924505	0.998
Bacillus sp. ERI44;	EU984074	0.998
Bacillus sp. LAMI013;	FJ413053	0.995
Bacillus sp. DCR_A16;	FJ535616	0.997
Bacillus cereus; MBL13;	FJ535616	1.000

**S_ab: Sequence match score*

The bacterium was sensitive to recommended doses of gentamycin (10 µg/ml), doxycycline (30 µg/ml), kanamycin (30 µg/ml), nalidixic acid (30 µg/ml), rifampicin (5 µg/ml), streptomycin (10 µg/ml), vancomycin (30 µg/ml), ofloxacin (5 µg/ml) and levofloxacin (5 µg/ml, gatifloxacin (10 µg/ml) but resistant to ampicillin (10 µg/ml) (Table 1). The results confirmed the reports that strains of *Bacillus* spp. of agricultural ecologies are generally resistant to the penicillin group of antibiotics which would have some relation with use of agrochemicals [9,24].

Table 4. Efficacy of the *Bacillus* sp. Ts116 against different mosquito larvae at different breeding habitats

Mosquito species	Breeding habitat	Pre-treated larvae/dip*	Average reduction (%) in larval density (days)		
			1st	3rd	7th
Anopheles subpictus	Shallow ponds	45.26±0.34	65.24	84.28	97.35
Armigeres subalbatus	Drains	75.43±0.64	54.52	95.65	95.65
Culex quinquefasciatus	Drains	63.27±0.60	74.84	100	100
Mixed population of different species	Control habitat	52.4±0.34	0.00	0.00	0.00

** (Average ± SE) of 25 replications of 250 ml dipper*

3.3 Bioassay of Mosquitocidal Activity of *Bacillus* sp. Ts 116

Laboratory evaluation of bacterial suspension against the mosquito larvae of different species is shown in Table 4 above. In the laboratory, the LC_{50}s (bacteria/ml) of *Bacillus* sp. Ts116 (GU190368) against *An. subpictus*, *Ar. subalbatus* and *Cx. quinquefasciatus* late third instar larvae were (2.37, 2.2 and 9.6) X 10^6 bacteria/ml, respectively which conformed with the lethal doses of the biocides viz. *Bacillus* spp., *B. thuringiensis*, *B. sphaericus* etc. against different mosquito larvae viz. *An. subpictus Cx. quinquefasciatus, Ar. subalbatus*. Field evaluation of the *Bacillus* sp. Ts116 revealed that treatment of 100 ml bacterial suspension (10^6 bacteria/ml) per cubic meter area of aquatic habitat harbouring larvae of different mosquito species at different breeding habitats reduced the vector population (Table 4). Average larval density of *An. subpictus* in shallow ponds was 45.26/dip before treatment but after treatment the density drastically reduced by 97.35% within 7d (Table 4). Pre-treatment average per-dip larval density of *Ar. subalbatus* and *Cx. quinquefasciatus* in drains was 75.43 and 63.27/dip, respectively (Table 4) but 7d post-treatment reduced the number of *Ar. subalbatus* and *Cx. quinquefasciatus* larvae by 95.65% and 100%, respectively (Table 4). The observations revealed that the suspension of *Bacillus* sp. Ts116 was more effective against *Cx. quinquefasciatus* than *An. subpictus* and *Ar. subalbatus* larvae which corroborated that the bacterium is a potent pathogen of the mosquitoes [1,7,8,10,28]. There are various reports of resistance to different strains and formulations of *B. sphaericus* in mosquitoes from different countries [3,29,30,31,32,33,34,35]. Therefore, this bacterial isolate was adjudged as a potent biocontrol agent of mosquito larvae and would be helpful in public health programmes to control the vector borne diseases.

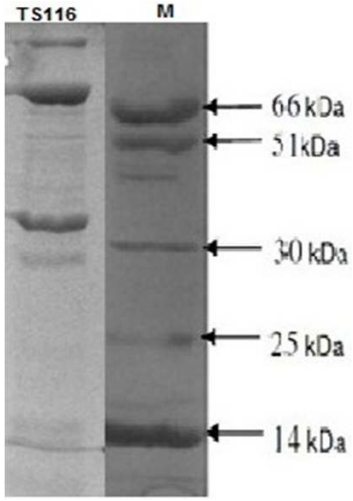

Fig. 3. SDS-PAGE profile of total cellular protein of Bacillus sp. Ts116 (LaneM- Protein Marker, Lane-1- Protein sample)

4. CONCLUSION

Bacillus sp. Ts116 (GU190368) would be a potential bio-control agent of mosquito larvae and could be exploited for public health programmes to control the vector borne diseases in West Bengal or elsewhere in India. The investigation also proved that agricultural lands would be rich sources of native biocides like *Bacillus* spp., which have bioprospect potential to control of the mosquitoes of the rice ecologies.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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