# Diversity of bioprotective microbial organisms in Upper Region of Assam and its efficacy against *Meloidogyne graminicola* (#84286)



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# Diversity of bioprotective microbial organisms in Upper Region of Assam and its efficacy against *Meloidogyne graminicola*

**Rupak Jena** [Corresp., 1, 2], **Bhupendranath Choudhury** [1], **Debananda Das** [1], **Bhabesh Bhagawati** [1], **Pradip Kumar Borah** [1], **Prabhukarthipat Kartikeyan** [2], **Swoyom Singh** [3], **Manaswini Mahapatra** [4], **Milan Kumar Lal** [5], **Rahul Kumar Tiwari** [5], **Ravinder Kumar** [Corresp. 5]

[1] Department of Nematology, Assam Agricultural University, Jorhat 785013, Assam, India., Jorhat, Assam, India

[2] Division of Crop Protection, National Rice Research Institute, Cuttack, 753006, Odisha, India, Cuttack, Odisha, India

[3] Department of Entomology, Siksha O Anusandhan, Bhubaneswar, 751003, Odisha, India, Bhubaneswar, Odisha, India

[4] Department of Agriculture and Allied Sciences (Plant Pathology),, C.V. Raman Global University, Bhubaneswar, 752054, Odisha, India, Bhubaneswar, Odisha, India

[5] ICAR-CPRI, Shimla, ICAR-Central Potato Research Institute, Shimla, Shiml, India

Corresponding Authors: Rupak Jena, Ravinder Kumar
Email address: rupu.jena27@gmail.com, chauhanravinder97@gmail.com

*Meloidogyne graminicola* has a well-established negative impact on rice yield, resulting in yield losses of up to 20 to 90 percent. Studies were undertaken to isolate native strains of potential bio-control agents to manage RRKN. Eighteen bacterial strains and eleven fungal strains were isolated from the rhizosphere of crops like rice, okra, ash gourd, chili, beans and cucumber, enveloping diverse soil types of the Upper Brahmaputra Valley region of Assam. Morphological findings stated that six bacterial strains were gram-positive while twelve stained negatively, fifteen bacteria were rod-shaped, two were coccus and one diplococcus in shape and all the bacterial isolates exhibited motility. All the bacterial strains exhibited positivity for gelatin hydrolysis and catalase test. Seven bacteria showed positive while eleven showed negative reactions to the citrate test. The study of the *in vitro* efficacy of the twenty-nine bacterial and fungal isolates tested against second stage juveniles ($J_2$) of *Meloidogyne graminicola* revealed that all the bacterial and fungal isolates potentially inhibited the test organism and caused significant mortality over sterile water treatment. The promising bacterial and fungal isolates that exhibited mortality above 50% were identified as BSH8, BTS4, BTS5, BJA15, FJB 11 and FSH5. The strain BSH8 exhibited the best result of mortality with 80.79% mortality against $J_2$ of *M. graminicola.* The effective and promising bio agents were identified using the 16 S rRNA sequencing and the organisms were identified as *Bacillis subtilis* (BSH8), *Bacillus velezensis* (BTS4), *Alcaligenes faecalis* (BTS5), *Rhizobium pusense* (BJA15), *Talaromyces allahabadensis* (FSH5) and *Trichoderma asperellum* (FJB11). These results indicated the microorganism's potential

against *M. graminicola* and its potential for successful biological implementation. Further the native strains could be tested against various nematode pests of rice in field conditions. Its compatibility with various pesticides and implication of the potential strains in IPM can be assessed.

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1. Department of Nematology, Assam Agricultural University, Jorhat 785013, Assam, India.
2. Division of Crop Protection, National Rice Research Institute, Cuttack, 753006, Odisha, India
3. Department of Entomology, Siksha O Anusandhan, Bhubaneswar, 751003, Odisha, India
4. Department of Agriculture and Allied Sciences (Plant Pathology), C.V. Raman Global University, Bhubaneswar, 752054, Odisha, India
5. Division of Plant Protection, ICAR-Central Potato Research Institute, Shimla, Himachal Pradesh, India.

*Corresponding author email id: rupu.jena27@gmail.com; chauhanravinder97@gmail.com

## Abstract:

*Meloidogyne graminicola* has a well-established negative impact on rice yield, resulting in yield losses of up to 20 to 90 percent. Studies were undertaken to isolate native strains of potential bio-control agents to manage RRKN. Eighteen bacterial strains and eleven fungal strains were isolated from the rhizosphere of crops like rice, okra, ash gourd, chili, beans and cucumber, enveloping diverse soil types of the Upper Brahmaputra Valley region of Assam. Morphological findings stated that six bacterial strains were gram-positive while twelve stained negatively, fifteen bacteria were rod-shaped, two were coccus and one diplococcus in shape and all the bacterial isolates exhibited motility. All the bacterial strains exhibited positivity for gelatin hydrolysis and catalase test. Seven bacteria showed positive while eleven showed negative reactions to the citrate test. The study of the *in vitro* efficacy of the twenty-nine bacterial and fungal isolates tested against second stage juveniles ($J_2$) of *Meloidogyne graminicola* revealed that all the bacterial and fungal isolates potentially inhibited the test organism and caused significant mortality over sterile water treatment. The promising bacterial and fungal isolates that exhibited mortality above 50% were identified as BSH8, BTS4, BTS5, BJA15, FJB 11 and FSH5. The strain BSH8 exhibited the best result of mortality with 80.79% mortality against $J_2$ of *M. graminicola.* The effective and promising bio agents were identified using the 16 S rRNA sequencing and the organisms were identified as *Bacillis subtilis* (BSH8), *Bacillus velezensis* (BTS4), *Alcaligenes faecalis* (BTS5), *Rhizobium pusense* (BJA15), *Talaromyces allahabadensis* (FSH5) and *Trichoderma asperellum* (FJB11). These results indicated the microorganism's potential against *M. graminicola* and its potential for successful biological implementation. Further the native strains could be tested against various nematode pests of rice in field conditions. Its compatibility with various pesticides and implication of the potential strains in IPM can be assessed.


**Key words**: Rhizospheric, *Bacillus. velezensis*, *Meloidogyne graminicola*, mortality

## 1. Introduction

Rice (*Oryza sativa*) is a crucial food crop that plays a significant role in the world's socioeconomic scenario. It provides nourishment to around four billion people, and spreads over 160 million hectares. The significance of this crop can be measured by the fact that it contributes to 20% of the total calorie intake globally (Le, 2010). Rice is widely recognized as an essential dietary cereal crop, ranking second in terms of the area it is grown (Devi and Ponnarasi, 2009).The crops delivers approximately 67% and 34% of the energy input for nearly 3 billion people in eastern Asia and 1500 million people in Latin America and Africa respectively. In India rice covers a major share of the food plate for about 800 million people, fulfilling the 43% calorie requirement and contributing nearly 40% and 55% to the country's food grain and cereal production respectively (Pathak et al, 2020).India ranks 9$^{th}$in productivity ratio with average yield across India estimated to be approximately 2.7 thousand kg/ha (https://www.statista.com/statistics/764299/india-yield-of-rice/). Lagging productivity in rice cultivation can be attributed to various biotic and abiotic factors. Biotic factors, such as the resurgence of insects and pests, including nematodes, pose a persistent threat to rice crops. Plant parasitic nematodes alone result in a staggering 21.3% crop loss, equivalent to approximately 102,039.79 million rupees per year (Kumar et al., 2020). Over 210 species of plant parasitic nematodes have been reported to be associated with rice crop (Prot, 1994). The economically important nematodes associated with the crop are *Meloidogyne graminicola, Dictylenchus angustus, Heterodera oryzicola, Aphelenchoides besseyi, Hirschmanniella* spp. *Dictylenchus angustus* and *Aphelenchoides besseyi* feed on the aboveground parts. Rice root-knot nematode (*M. graminicola*), stem nematode (*Ditylenchus angustus*), white tip nematode (*Aphelenchoides besseyi*), and cyst nematode (*Heterodera oryzicola*) were collectively projected to cause yield losses of 10.50 percent and losses of 779.30 million rupees, respectively (Jain et al., 2007).

*M. graminicola* is a sedentary obligate endo-parasitic nematode and its female reproduces by meiotic parthenogenesis and amphimixis. It lays eggs (200-500) inside the root in a gelatinous matrix. First-stage juveniles (IJs) are produced after 4-7 days, and they molt into second-stage juveniles in next 2-3 days ($J_2$). Under favorable environmental conditions, the infectious $J_2$ is poised to hatch out of the egg. The $J_2$ is the infective stage and it enters through the elongation zone and induces the formation of syncytium and gall (Abad et al. 2003). The loss caused by *M.*

*graminicola* alone was reported at a range of 16–32% in irrigated and 11–74% in flooded and submerged paddy in India (Soriano et al., 2000). Overall, *M. graminicola* has a well-established negative impact on rice yield, resulting in yield losses of up to 20 to 90 percent. The pathogenic nematodes in soil not only act as primary invaders against agricultural crops but also supplement the secondary pathogens like bacteria, fungi, viruses, etc., in establishing disease quadrangle. The use of chemical pesticides has been a quick solution for managing nematodes, but the recent focus on sustainability and the harmful effects of pesticides has led to the need for a safer alternative. The shift towards more sustainable practices has made it imperative to find a safer solution for managing pests.

Bio-control agents (BCA) are the potentially efficient microorganisms in the rhizosphere that can be harnessed and successfully implicated against detrimental microorganisms (Rayemakers, 2020). Studies reported the successful application and identification of bioagents for the control of nematode plant diseases (Nagendranet al., 2013), however recently efficient bio-control agents like *Pasteuria penetrans*, *Bacillus* sp., *Verticillium chlamydosporium, Paecilomyces lilacinus, Trichoderma* spp. etc. have shown promising results against various plant parasitic nematodes like *Meloidogyne* spp., *Aphelenchus avenae, Globodera* spp., *Pratylenchus* spp., *Heterodera* spp., *Rotylenchulus* spp (Tothne Bogdyani et al., 2021). Assam is an important state in India under the BGRIE (Bringing Green Revolution to Eastern India) with a total area of 2.28 million hectares under rice with production rate of 4.86 million tons (Agriculture statistics at a glance, 2019) and parallel in fronting the menace of *M. graminicola* which threats the crop sustainability. Therefore a study was undertaken to isolate and identify the promising native bioprotective rhizospheric microorganisms from Upper Brahmaputra Valley region of Assam (UBVA) and in succession the *invitro* efficacy of the isolated strains against the disease causing second stage juveniles of rice root knot nematode *M. graminicola.*

## 2. Material and Methodology

### 2.1. Maintenance of inoculum (*Meloidogyne graminicola*)

The pure culture of the rice root-knot nematode *Meloidogyne graminicola* was maintained in the susceptible rice variety Luit in pots, in the Department of Nematology, Assam

Agricultural University, Jorhat for its requisite during the course of *in-vitro* evaluation. The seeds were obtained from ICR Farm, seedlings were germinated and then transplanted into 5kg pots for inoculum maintenance.

### 2.2. Collection of soil samples

A roving survey was conducted in the month of August 2021from areas of UBVA like Alengmora, Sibsagar, Titabor, Jorhat, Golaghat and around 110 soil samples (100gms each) were collected from the rhizosphere of various crops like ash gourd, chili, okra, rice, beans, and cucumber (**Table 1**).The soil samples were labeled with various information like date of collection, age of the crop, type of soil, and variety of crop and were refrigerated at 5-10°C until further use.

### 2.3. Isolation of bacterial and fungal isolates

The soil samples collected from the crop's rhizosphere were thoroughly mixed individually and about 1 gm of the soil was weighed and taken for isolation of bacterial bioagents. The isolation of bacterial bioagents from soil involved the use of serial dilution procedures. Sterile dilution blanks were marked sequentially, starting from stock and$10^{-1}$ to $10^{-6}$. A fresh sterile pipette transferred one ml from the stock to the $10^{-1}$ dilution blank. From a dilution tube of $10^{-7}$, 0.1ml of dilution fluid was transferred into nutrient agar culture media. The plates with soil fluid were sealed using paraffin wax and incubated at 28±2°C for 24 hours in order to recover the maximum possible colonies of bacteria. After 24 hrs., the morphologically different bacterial colonies were selected and were repeatedly streaked to achieve pure bacterial colonies (**Table 1**). The same protocol was followed for the isolation of fungal bioagents, but instead of nutrient agar, PDA was used as a culture media, and the plates were incubated for 3-4 days. The morphologically different fungal colonies were selected and repeatedly streaked to achieve pure fungal colonies (**Table 1**).

### 2.4. Morphological and biochemical characterization of bacterial isolates

Colony characters like size, shape, color of the colonies, and gram stain were recorded as cultural and morphological characters of the bacteria. A few biochemical tests such as KOH solubility test, starch hydrolysis test, citrate test, catalase test, and gelatin hydrolysis tests were

also conducted for identification of the bacteria following protocols given in The Bergey's Manual of Determinative Bacteriology (De Ley and Frateur, 1974) (S. Figure 7).

### 2.5. Preparation of culture filtrates

Pure bacterial isolates were seeded to 100 ml of nutrient broth media and were incubated at 28°C for 48 hours. The liquid suspension was passed through Whatman No. 1 filter paper and once through a bacterial filter (Aalten and Gowen, 1998) and then centrifuged for 15 minutes at 6000 rpm. The suspended residues were discarded once the supernatant was collected for testing. The same procedure was repeated for fungal bioagents, but instead of nutrient broth, potato dextrose broth was used as a seeding agent for pure fungal isolates.

### 2.6. *In vitro* efficacy of bacterial and fungal bio-agents

The extracted cell-free culture filtrates of isolated bacterial and fungal bio-agents were considered as stock solution (100% concentration). The sterile water was added to the stock solution to have S/2, S/4, and S/8 concentrations of cell-free bacterial and fungal culture filtrates.Two ml of cell-free stock culture filtrate of isolated bacterial and fungal bio-agents were poured into the sterile cavity blocks. To each cavity block 50 freshly hatched juvenile ($J_2$) of *M. graminicola* isolated overnight were added. A completely randomized block design (3 factorial CRD) with three replications was used to arrange all cavity blocks stored in the lab at room temperature. At 6, 12, 24, and 48 hours after exposure, observations on juvenile mortality were made. Apart from the treatments with different bioagent concentrations of culture filtrates, sterile distilled water (SDW) was also maintained as a control. The percent juvenile mortality was calculated using the formula given below:

$$\text{Percent mortality} = \frac{\text{Number of dead juveniles in the treatment}}{\text{Total number of juveniles in the treatment}} \times 100$$

### 2.7. Identification of efficient bio-agents

The bacterial and fungal isolates exhibiting minimum 50 % mortality in the *in vitro* assay were selected for amplification of the 16S rDNA. Genomic DNA and were extracted using the EXpure Microbial DNA extraction kit from Bogar Bio Bee stores Pvt. Ltd. Amplification of the genomic DNA were carried out using 16S rRNA based primer i.e. forward primer—27F (5′ AGAGTTTGATCTGGCTCAG 3′) and reverse primer- 1492R (5′ TACGGTACCTTGTTACGACTT 3′).The PCR product obtained from bacteria were sequenced at Triyat scientific Co. Pvt. Ltd., Maharashtra. The phylogeny analysis of query sequence with the closely related sequence of MUSCLE 3.7 was used for multiple alignments of sequences (Edgar, 2004) and the program Tree Dyn 198.3 was used for the construction of phylogenetic trees (Dereeper et al., 2008) **(Table 2).**

### 2.8. Statistical analysis

The *invitro* three factorial completely randomized design statistical programs were used to analyze data on percent mortality statistically. The main effects' isolates, concentrations, times of exposure, and their interactions were assessed for important differences at P=0.05.The percentage values were subjected to arcsin transformation and data were analyzed using SAS and IBM SPSS statistics 20.0 software. Tukey test and DMRT test wereconducted to determine the significance of treatments.The experimental data obtained were analyzed by following Fisher's method of Analysis of Variance.Thestandard error of deviation (S.Ed) between the mean of the treatment combination was calculated as:

$$\text{S.Ed} \pm = \sqrt{\frac{2 \times \text{Error Mean Square}}{\text{Number of replication}}}$$

## 3. Results

### 3.1. Isolation and characterization of bacterial bioagents from crop rhizosphere

Among the 110 soil samples taken from the Upper Brahmaputra Valley region of Assam, a total of n=18 potential spore forming bacterial microorganisms and n=11 potential fungal isolates were isolated and pure cultures were maintained based on colonial morphology, microscopy, biochemical reactions. The spore-forming bacterial strains were selected for further confirmatory

tests.The locality and village from where they were isolated, as well as their classification as bacteria (B) and fungus (F), were all taken into account while coding the bacterial and fungal isolates **(Table 1).**

### 3.1. Morphological and Biochemical characterizations of isolated bacterial strains

#### 3.1.1. Morphological characterization

Terminal and subterminal spores were segment of the 18 motile bacterial isolates out of which 15 were rod-shaped, two were coccus-shaped, and one was diplococcus-shaped. The isolated bacteria exhibited diverse colony characteristics resembling regular, irregular, slightly raised, flat,and colors, such as whitish, creamish white, creamish yellow, yellowish orange, and others.Bacterial isolates BAK2, BAB3, BTS5, BTS6, BTM7, BGA11, BGA12, BJA13, BJA14, BJA15, BJR17, and BJB 18 were gram-negative in response to the gram staining test, while strains BAK1, BTS4, BSH8, BSL9, BSL10, and BJR16 tested positively **(Table 3).**

#### 3.1.2.Biochemical Characterization

KOH supplements and replicates the gram staining results in which the 3% potassium hydroxide dissolves the cell wall of the gram-negative bacterial isolates leading to the string formation by the released viscous chromosomal material of the strain. The isolates BAK1, BTS 4, BSH8, BSL9, BSL10, and BJR 16 thick peptidoglycan deposition in the cell wall resists the dismantling of the cell wall thus confirming its gram-positive identity. Establishment of potential biocontrol strains characterizes by their ability to withstand challenging abiotic conditions. The citrate test demarks the strains with the potential to channelize citrate into its carbon and energy source. The enzyme citrate permease (citrase) facilitates the citrate into bacteria. Distinctive color variation of the media from Prussian blue to green signifies positive response to citrate test. All the bacterial isolates exhibited positivity to the production of gelatinase enzyme that hydrolyzes gelatin to amino acids via polypeptides. Amino acids are essential component required in the regulation of biochemical activities of the potential strains.The presence of gelatinase is marked with the medium transformation from semi-solid to liquid. The bacterial carbohydrate metabolism accompanies hydrogen peroxide ($H_20_2$) as the end product that is self-destructive. The presence of

catalase alleviates the impact by converting ($H_20_2$) to water and oxygen. In the present study, all the bacterial isolates showed the presence of the catalase enzyme by the production of bubbles **(Table 3) (S. Figure 7).**

### 3.2. *In vitro* Bioassay of Bacterial and Fungal isolates against Rice root knot nematode:

All the bacterial cultural filtrates exhibited a marked and significant degree of larvicidal activity against the nematode compared to control. Four bacterial isolates BSH8, BTS4, BTS5 and BJA15 exhibited mortality greater than 50 % but the culture filtrate of BSH8 (isolated from rhizosphere of okra crop) showed average mortality of 80.79% against the infective juveniles of *M. graminicola*. After 48 hours of exposure, the strain BSH8 triggered 100%, 96.67%, 95.33%, and 87.33% death at descending concentrations. The isolate BTS4 with average mortality of 71.29% expressed mortality of 97.33% at S concentration and 91.33% mortality at S/2 concentration after 2 days of scrimmage. The culture filtrate of the strain BTS5 and BJA15 exhibited average mortality of 68.75% and 68.33% overall after 2 days of treatment against *Meloidogyne graminicola* (**Graph1**). The strains of BSH8, BTS4, BTS5, and BJA15 were at par for annihilation of juveniles. The strain BGA11 isolated from rice showed the lowest potential for causing mortality with 11.69% overall and 24% at S concentration after 48 hrs of exposure. Neglecting the cultural strain and the time of exposure, the mortality rate of the juveniles of *M. graminicola* at the concentration S/8 was 28.93%, while at the concentration S, the mortality was exhibited to be a maximum of 52.78%. Similarly, irrespective of the cultural strain and concentration of the culture filtrate, the mortality was highest at 48hrs after exposure at 50.61% and the minimal mortality was 25.96% six hrs after exposure time (**Table 4**) (**Fig. 1**)

The effectiveness of the isolated fungal strains were tested against *M. graminicola*, it became apparent that the fungal microorganisms substantially displayed mortality regardless of the different cultural filtrate concentrations and exposure times as compared to controls (sterile water). In consonance with*in vitro* tests, FJB11 and FSH5 were the two most promising and efficient fungal strains, and statistically significant. The fungal strain FJB11 isolated from the rhizosphere of chilli exhibited a mortality mean of 78.83% against the juveniles of *M. graminicola* at all tested concentrations (**Fig. 1**). The strain FJB11 demonstrated alleviation of nematode mortality in various concentrations (S/8, S/4, S/2, and S) after 48 hours of exposure. The results

manifested 77.33%, 82.67%, 90.67%, and 100% mortality, with ascending concentrations respectively. The strain FSH5, isolated from the rice rhizosphere, indicated promising results with an average nematode snuffing of 66.25%. The highest mortality was observed at the S concentration (90%) and S/2 concentration (82%) after 48 hours of exposure. On average, the concentration effect showed the highest mortality at the S concentration (52.37%) and the lowest at the S/8 concentration (34.29%). The average effect of exposure time for the fungal isolates was highest at 48 hours (50.73%) and lowest at 6 hours (32.07%) **(Table 4)**.

### 3.3. Sequencing and Phylogenetic analysis of bacterial and fungal isolates

The fingerprints of bacterial and fungal communities were generated by separation of 16S rRNA gene fragments.The amplified PCR fragments of the efficient bacterial and fungal isolates containing 16S rRNA were subjected to analysis for homology using the n BLAST. The bacterial isolate BSH8 (OQ216891) was identified as *Bacillus subtilis* and was isolated from the okra rhizosphere (**S. Figure 1**). The strains BTS4 (OQ216889), BTS5 (OQ216890), and BJA15 (OQ216892) were identified as *Bacillus velezensis*, *Alcaligenes faecalis*, and *Rhizobium pusense*, respectively, and were isolated from the rice rhizosphere (**Fig. 2**) (**S. Figure 2, Figure 3, Figure 4**). Similarly, the fungal strains were also subjected to 16S rRNA identification. The bioagents FSH5 and FJB11 were reported to be *Talaromyces allahabadensis* (OQ244365)and *Trichoderma asperellum* (OQ244366)that, were isolated from okra and chilli rhizosphere (**S. Figure 5, Figure 6**). The isolate FSH5 was also overlapping with the sequence of accession OM372948.1 and FJB11 was found to be 100% identical to the accession MN872484.1(**Fig. 3**).

## 4. Discussion

Prevention of nematode disease progression requires feasible approaches supportable to environment. Varying indiscriminate chemical applications in both quality and quantity to manage nematodes impairs soil health. Promising native microbial strains are becoming increasingly significant in nematode management.The existence of countless and diverse advantageous microbial species in the earth's rhizosphere is enormous, and the studies undertaken are simply the tip of the iceberg Ciancioa et al. (2019).The plant holobiome hibernates majority of microbes and

we extricated eighteen bacterial and eleven fungal culturable promising microbes from 110 rhizospheric soil samples of agronomic crops such as rice, okra, cucumber, beans, ash gourd, and chilli. An aggregate of 60 bacterial strains were identified and characterized based on morphological, biochemical, and 16S rRNA gene sequencingfrom the submerged rice rhizosphere Amruta et al. (2016). Microbes are positively correlated withthin water films for proliferation and active soil metabolics. The gram-positive bacteria's dominated the colonies of various PGPR isolated from chilli rhizosphere and efficiently resisted the noxious wilt disease of chili Yanti et al. (2017)**.** Efficient bacterial strains of fluorescent *Pseudomonads* habitat rhizospheric soils of chilli, field bean, green gram, brinjal, sunflower, red gram, groundnut, tomato, bermuda grass, beans, sorghum, paddy and sesame Manjunatha et al. (2012).Furthermore, bacterial strains were reported in the rhizosphere of soils with nematode outbreaks (AbdelRazek, G.M. and Yaseen, R., 2020).The efficient bacterial and fungal microbial strains, later *to invitro* tests, through [illegible] RNAwere identified to be *B. subtilis* (OQ216891)*, Bacillus velezensis* (OQ216889)*, Alcaligenes faecalis*(OQ216890)*, Rhizobium pusense*(OQ216892), *Trichoderma asperellum*(OQ244366) and *T. allahabadensis* (OQ244365)

Microbial-rich Indian soils nurture ubiquitous spp. of *Bacillus* and *Pseudomonads* and varied reports support its efficient resistance to varied biotic stresses including economically salient nematodes Pandey et al., (2011), Khan (2018), Saikia et al., (2018). The extended viability and aggressive exertions of *Bacillus subtilis* against nematodes and their eggs facilitates its importance. *Invitro* results exhibited an average mortality of 80.79 % against infecting juveniles of *M. graminicola*. The crude cultural filtrate of *B. subtilis* releasing diffusible and volatile antibiotics, siderophores, cellulase, glucanase, protease, and chitinase substantiates the mortality of *M. graminicola. M. incognita, M. oryzae* Kumar et al. (2020), Amruta et al. (2016). *Bacillus* spp. being an aggressive colonizer synthesizes metabolites that inhibit membrane protein and enzymes **Schippers et al. 1987**.The various antibiotics, proteolytic enzymes, high surfactin, and iturin activity supplemented the mortality of the juveniles *in vitro*. The strain'sability to deliver lytic enzymes such as chitinase, glucanase, and protease affects the nematode cuticle and survival Chen et al., (2015), Kavitha et al. (2012). The phytonemtaodes are eliminated from the plant's rhizosphere by production of antimicrobial peptides, secreting lytic enzymes, vying for nutrients and space, and causing systemic resistance Kang et al. (2015).

Promising bacterial strain synonymized with *Bacillus amyloliquefacians* is now reclassified as *B. velezensis* Dunlap et al. (2016), Castro et al., (2020),proliferates in a diverse ecology including oilseed plants Asaturova et al., (2021) and black pepper Tran et al., (2020). The gram positive, aerobic and endospore forming beneficial bacterium was first reported from the mouth of river Velez in Malaga province of Spain Ruiz et al. (2005).The salt-tolerant biocontrol strain *B. velezensis* diminished 71.29% of the juveniles of *M. graminicola* within 48 hrs. Antagonistic activity of *Bacillus velezensis* BZR 277 was reported against the devastating root knot nematodes *M. incognita* in lab conditions Asaturova et al. (2021)**.** The resistance imparted against juveniles sourced from production of antibiotics and enzymes. Antibodies increase the chitinase activity of *Bacillus velezensis*, making it a promising bio-agent for controlling nematodes such as *Meloidogyne* species Tran et al., (2020). Increased chitinase activity, or an antagonistic effect depending upon infestation pressure facilitates the mortality of infective juveniles Lee and Klim**.,** (2016). *B. velezensis* synthesizes cyclic lipopeptides, such as surfactin, bacillomycin-D, fengycin, and bacillibactin, as well as polypeptides like macrolactin, bacillaene, and difficidin, that are be harmful to nematodes Rabbee et al., (2019).

*B. velezensis* produces lipopeptides identified through PCR amplification Azabou et al., (2020) and variety of extracellular metabolites, such as lipopeptides and biosurfactant molecules, have antimicrobial properties against many plant pathogens Ryu et al., (2004)**.** The presence of two chitinase genes chiA and chiB respectively exhibited increased hydrolytic activity against colloidal chitin of nematode eggs Tran *et al*. (2022). Promising multifaceted biocontrol strain *A. faecalis* habitats in diverse and challenging conditions including oil spill soils Yadav et al., (2020), *Mimosa calodendron* Felestrino et al., (2020),*C.forskohlii* (Mastan, 2020)and okra crop (Ray et al. 2016)**.**This represents the earliest instance of *A. faecalis* being observed in an Assamese rice crop rhizosphere. RRKN juveniles population were alleviated and growth inhibited prior to exposure of *A. faecalis* in *invitro* c litions. Strains of *A.faecalis* reduced the hatching rates and improved mortality of the juveniles of *Pratylenchus* spp. and *Meloidogyne* spp **(**Felestrino et al. (2020)**.** VOC's and antibiotic diffusates caused mortality of *M. incognita* (Lu et al. 2014).Inhibtion of nematodes against the strain encompasses an array of antimicrobial substances including identified Espprotein, an extracellular serine protease that affected well over 85% of *M. incognita* juveniles' intestines Ju et al., (2016).The BCA is reported adaptive to harsh environmental conditions and

encodes HCN, siderophore, and phenolic compound synthesis, exhibiting mortality against nematodes Felestrino et al., (2020**).** The dominance of chitin in nematode eggs and breakthrough for genes encoding chitinolytic, esterase activity, proteases and peptides N. Annamalai et al., (2011)**,** and production of dimethyl sulfate recognizes the strain as a future promising antinemic microorganism Xu et al., (2015).

*Rhizobium* a genus dominant in pulses as PGPR was identified in submerged rice rhizosphere. Avowed as efficient strain for dissemination of potassium from mica *R. pusense* besides 21 phosphate-solubilizing bacteria was isolated from Egyptian agricultural soils Hauka et al. (2017). Indian ecology dominant food crops such as potato, pigeon pea, maize, banana, sugarcane, tobacco shares *R.pusense* (Phospate solubilizing rhizobacteria) rich microbiome that exhibits potassium solubilizing ability Meena et al. (2015**).** The application of potassium (K) demonstrates that the K treatment can lower the occurrence of detrimental nematode infections in rice and boost crop output Liu et al., (2022**).** *Rhizobium pusense* exhibited 68.33% mortality in *invitro* conditions. The dynamic impact of cultutral filtrate of *R. pusense* resists the hatching of nematode eggs and antibiotics impairs juvenile proliferation Khan et al., (2018).The released nematicidal compounds resists *Meloidogyne javanica* at varying degrees (Parveen et al., 2019).Toxin identified as rhizobitoxine synthesized by *R. japonicum* aggragavtes the nematode pathogenesis (Siddique et al., 2007).

Antagonizing microbe,*Trichoderma spp*. are becoming widely recognized as a means of restraining plant diseases (Dukare et al., 2019).*T. asperellum* isolated from chilli rhizosphere encompasses all ecological niches signifying itself as ubiquitous. Fungal isolates including *T. asperellum* dwells in rhizosphere of cocoyam, cacao and banana crop (Tondje et al.,2007),sugar beet rhizosphere Egypt (Gueye et al., 2020) and mango orchard, Mexico (dos Santos et al. 2021).Undistrubed ecosystem reserves unexplored and potential microbes. The northeastern region harbours *Trichoderma sp.* in partially explored in pockets of Manipur (Kamala and Devi., 2012), the tea gardens of Assam (Naglot et al., 2015),and sorghum plants of Uttrakhand (Manzar et al., 2021)**.** *Meloidogyne spp*. is effectively parasitized by *T asperellum* through inhibited egg hatching and direct larval mortality (Kumari et al. 2020). MISC antibodies released from the fungal strain, in bioassay conditions, regulates improved juvenile death Sharon (2009)**.** Arsenal of antagonistic activities against the nematode by *Trichoderma* species were reported to contribute to the biological defiance, including nutrient and space competition, antibiosis, mycoparasitism,

366 and induced systemic resistance of plants (Lombardi et al., 2018). *Trichoderma* spp. excrete
367 several lytic enzymes (glucanases, chitinases, proteases and lipases) to degrade cell wall
368 components of other pathogens. The transcriptional activity of chi18-5 (chit42) and chi18-12
369 (chit33) of *Trichoderma* regulates chitinolytic enzyme systems during egg-parasitism(Szabo et
370 al., 2012). *Trichoderma* spp. produces nematicidal compounds such as trichodermin and trypsin-
371 like protease which exhibits larvicidal and ovicidal activities against the nematodes (Yang et al.,
372 2012). The mortality *invitro* could be facilitated due to conidial attachment to the nematode body
373 thus immobilizing the host, antifungal compounds (Sharon et al. 2009). The native strains
374 considered safe to mammals promises faster growth rate (31-47cm), finer condial arrangement and
375 heat resistant at 30°C as compared to *T. viride* with 11-33 cm growth radius and suitable growth
376 at 11°C (Samuels et al., 1999).

377 *Talaromyces allahabadensis* parasitsed the nematode juveniles and restricted its movement
378 leading to mortality of 66.24%. Seventy three fungal isolates collected from different regions
379 including *Talaromyces assiutensis* parasitized all the juveniles of *M. javanica* in *in vitro* predation
380 test and killed 30-50% of the juveniles (Hamza et al. 2017). The exhibited potential mortality is
381 due to the presence of a novel potent nematicidal thermolides (Guo et al., 2012). Varying genus of
382 economically phytonematodes including *Pratylenchus zeae* falls prey to *Talaromyces* sp.
383 (Kisaakye et al. 2014).

384

385 ## 6. Conclusion

386 The rhizospheric soils are rich in micro organisms and in our study eighteen bacterial
387 strains and eleven fungal strains were isolated from soil samples collected from rhizosphere of
388 crops like rice, okra, ash gourd, chili, beans, cucumber from diverse conditions and soil types in
389 Upper bhramaputra valley region of Assam. The isolated native strains efficiently resisted the
390 growth and population dynamics of infecting RRKN in bio assay conditions. The native strains
391 can be further explored as potential bio-control agents against economical nematode pests of rice
392 in field conditions either alone or in combination. Molecular insights into activation of genes for
393 production of antibiotics and trapping mechanisms of nematodes are to be deepened. The
394 multidimentional faecets of promising identified microbes enhanches the changes of successful
395 establishment of the BCA's in the soil which is of primary concern. *Alcaligenes faecalis* being slat

tolerant can be a viable option in the present soil deteorating and abiotic challenging conditions. *T. asperellum* ability to proliferate rapidly in harsh conditions improves its chances to be successful. The VOC's and antibiotics released against *M.graminicola* are to be studied for further insight into mechanism. Further the native strains provides encouraging insight into integrated pest management with synergistic impact on environmental sustainability.

Abbreviations:

1. RRKN- Rice root knot nematode
2. BGRIE - Bringing Green Revolution to Eastern India
3. ICR- Institutional cum research
4. UBVA- Upper Brahmaputra Valley region of Assam
5. rpm- revolutions per minute
6. DNA- Deoxyribonucleic acid
7. RNA- Ribonucleic acid
8. PCR- Polymorphic chain reaction
9. BLAST- Basic Local Alignment Search Tool
10. VOC's- Volatile Organic Compound
11. BCA's- Biocontrol Agents
12. PGPR- Plant growth promoting rhizobium
13. HCN- Hydrogen cyanide

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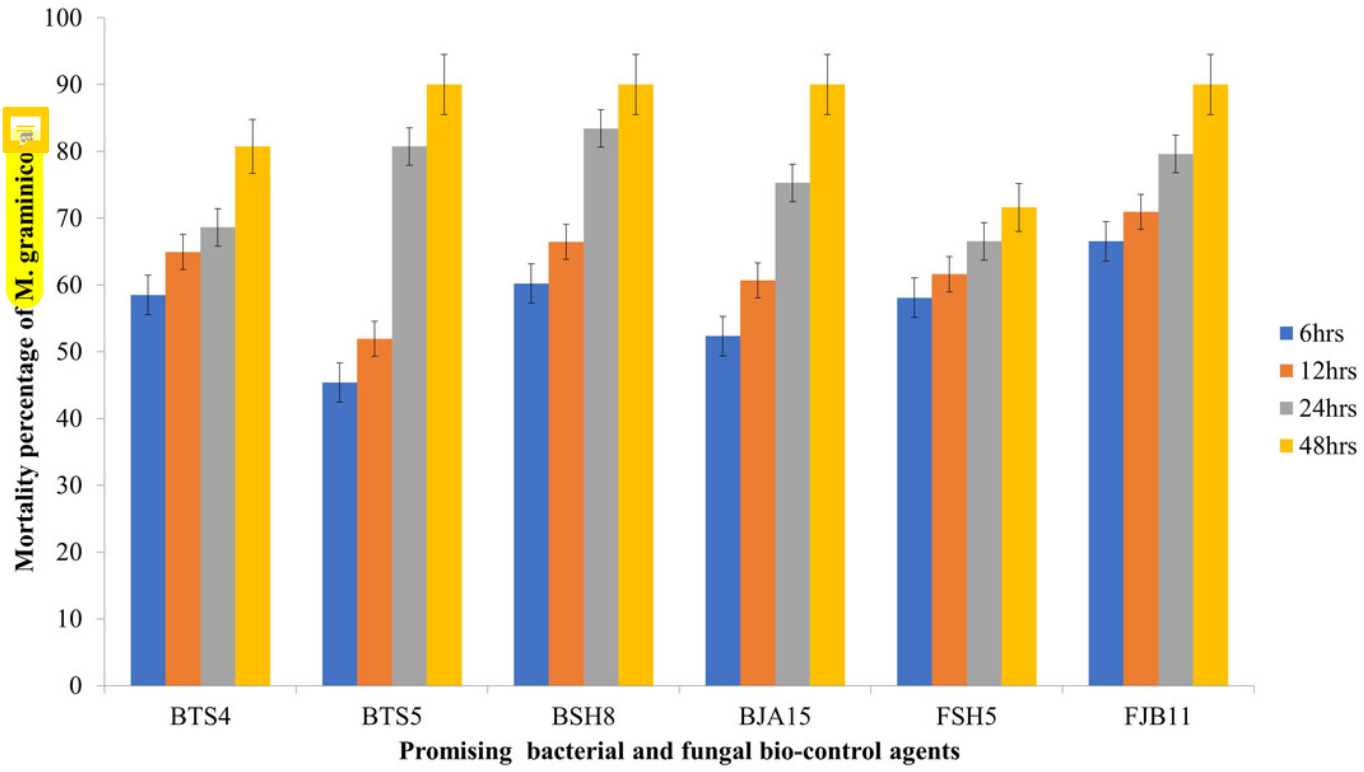
# Figure 1

Fig. 1: Mortality rate of *M. graminicola*

Fig. 1: Mortality rate of *M. graminicola* against the potential BCA after 6,12,24,48 hrs in *invitro* condition with 5% standard error. (P < 0.05)



# Figure 2

Fig 2: Phylogenetic tree of potential bacterial strains with out-group and boot strap values

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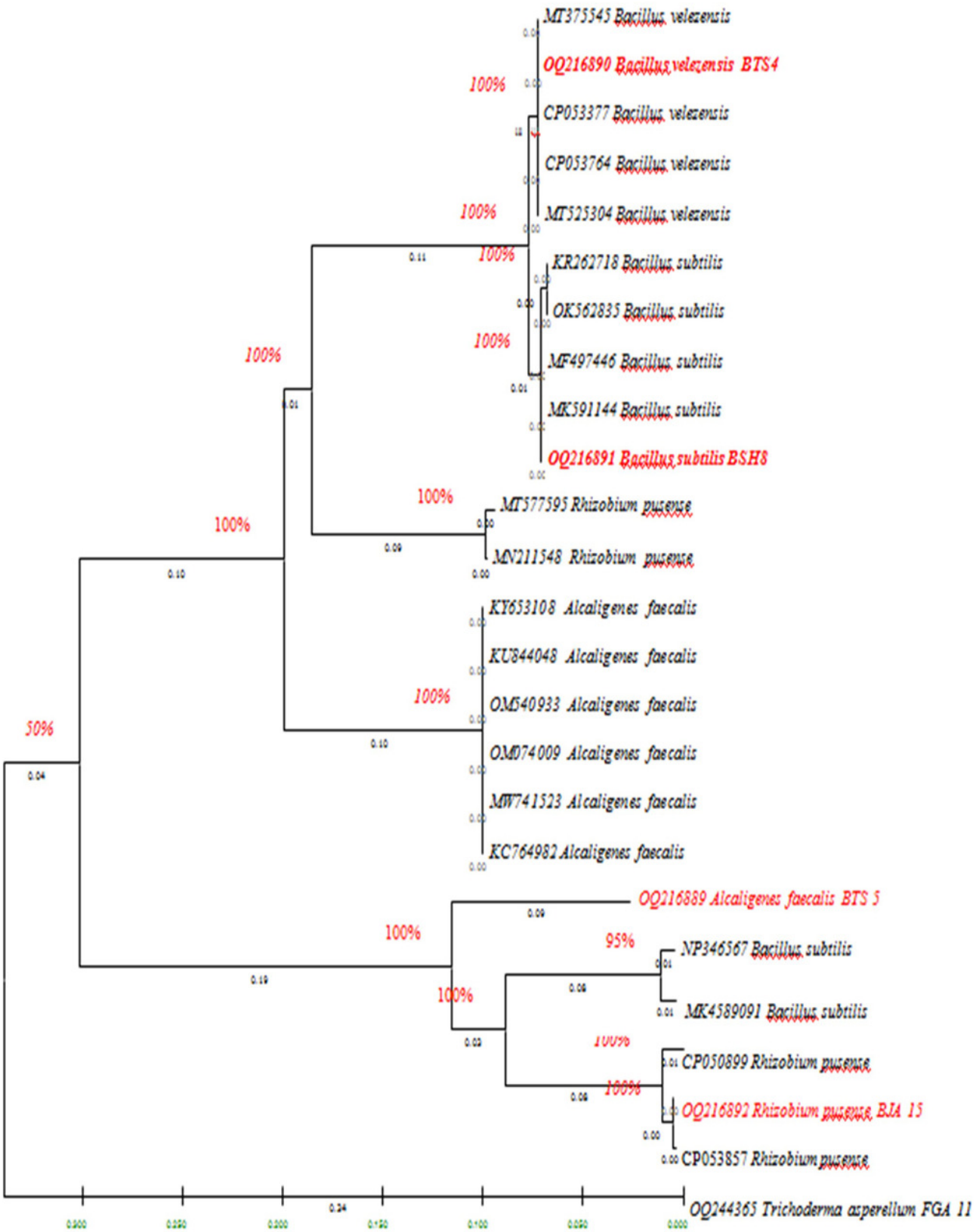
MT375545 Bacillus velezensis
OQ216890 Bacillus velezensis BTS4
100%
CP053377 Bacillus velezensis
CP053764 Bacillus velezensis
100%
MT525304 Bacillus velezensis
100%
KR262718 Bacillus subtilis
OK562835 Bacillus subtilis
100%
MF497446 Bacillus subtilis
MK591144 Bacillus subtilis
OQ216891 Bacillus subtilis BSH8
100%
MT577595 Rhizobium pusense
100%
MN211548 Rhizobium pusense
KY653108 Alcaligenes faecalis
KU844048 Alcaligenes faecalis
100%
OM540933 Alcaligenes faecalis
50%
OM074009 Alcaligenes faecalis
MW741523 Alcaligenes faecalis
KC764982 Alcaligenes faecalis
OQ216889 Alcaligenes faecalis BTS 5
100%
95%
NP346567 Bacillus subtilis
100%
MK4589091 Bacillus subtilis
100%
CP050899 Rhizobium pusense
100%
OQ216892 Rhizobium pusense BJA 15
CP053857 Rhizobium pusense
OQ244365 Trichoderma asperellum FGA 11

# Figure 3

Fig 3: Phylogenetic tree of potential fungal strains with out-group and boot strap values

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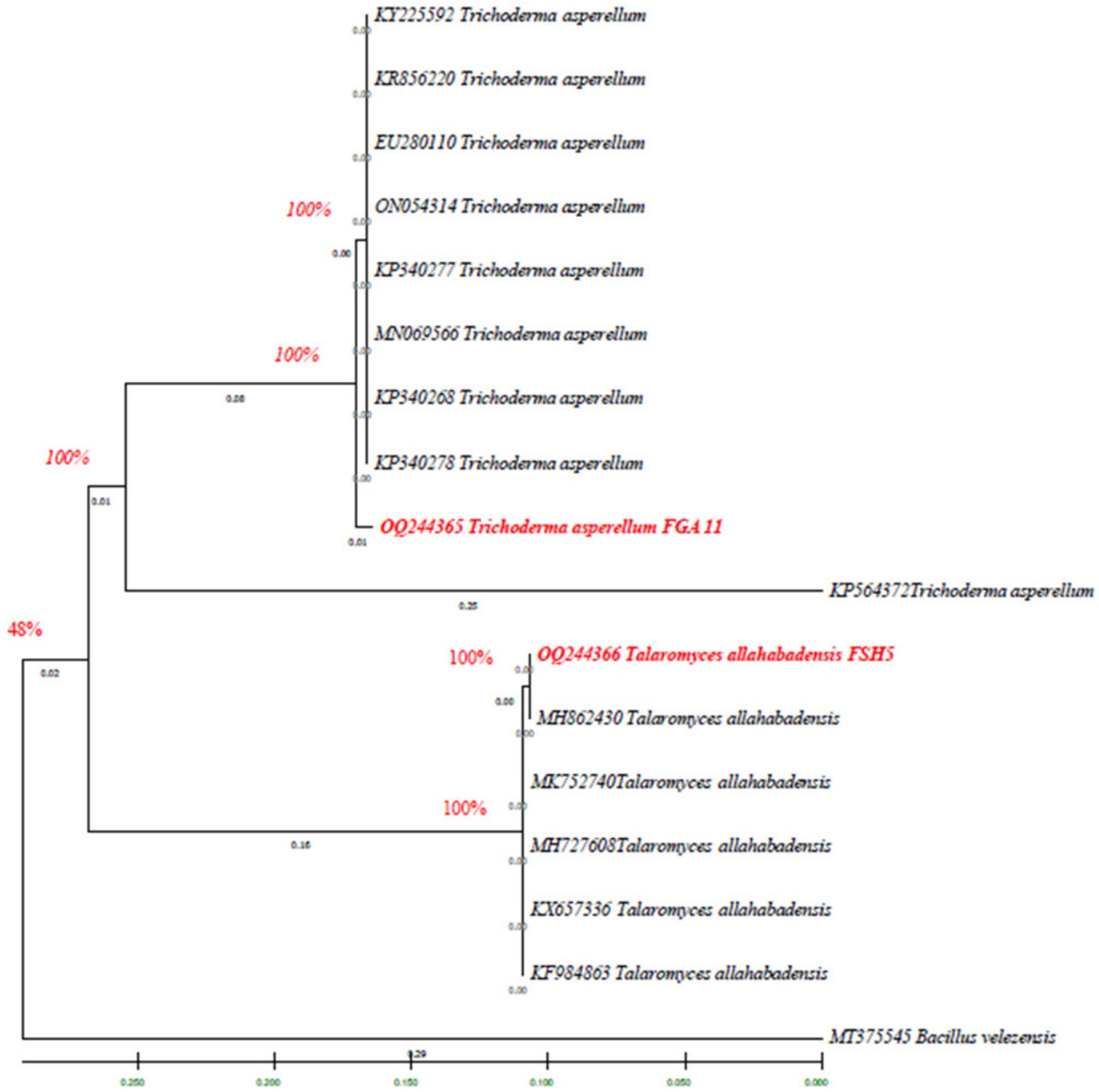

## Table 1(on next page)

Table 1: Documentation on collection of rhizospheric soil samples from various crops in Upper areas of Assam.

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| Sl.no | Code | Name of Place | Village name | Code | Name of Place | Village name |
|---|---|---|---|---|---|---|
| 1. | BAK1 | Alengmora | Kahargaon | FAK1 | Alengmora | Kahargaon |
| 2. | BAK2 | Alengmora | Kahargaon | FTS2 | Titabor | ShyamGaon |
| 3. | BAB3 | Alengmora | Bahphola | FTS3 | Titabor | ShyamGaon |
| 4. | BTS4 | Titabor | ShyamGaon | FTK4 | Titabor | KarsoliGaon |
| 5. | BTS5 | Titabor | ShyamGaon | FSH5 | Sibsagar | HulalKalita |
| 6. | BTS6 | Titabor | ShyamGaon | FSS6 | Sibsagar | Salaguri |
| 7. | BTM7 | Titabor | Madhavpur | FSS7 | Sibsagar | Salaguri |
| 8. | BSH8 | Sibsagar | HulalKalita | FGM8 | Golaghat | Merapani |
| 9. | BSL9 | Sibsagar | LahonGaon | FJA9 | Jorhat | AAU |
| 10. | BSL10 | Sibsagar | LahonGaon | FJA10 | Jorhat | AAU |
| 11. | BGA11 | Golaghat | Amguri | FJB11 | Jorhat | Barbheta |
| 12. | BGA12 | Golaghat | Amguri | | | |
| 13. | BJA13 | Jorhat | AAU | | | |
| 14. | BJA14 | Jorhat | AAU | | | |
| 15. | BJA15 | Jorhat | AAU | | | |
| 16. | BJR16 | Jorhat | Rowriah | | | |
| 17. | BJR17 | Jorhat | Rowriah | | | |
| 18. | BJB18 | Jorhat | Barbheta | | | |

## Table 2(on next page)

Table 2: Data on potential bacterial and fungal strains isolated from the crop rhizosphere and the crop characteristics:

**Table 2**: **Data on potential bacterial and fungal strains isolated from the crop rhizosphere and the crop characteristics:**

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| Code | Name of Place | Village name | Crop | Variety | Type of soil | Stage of the crop |
|---|---|---|---|---|---|---|
| **BTS4** | Titabor | Shyam Gaon | Rice | Ranjit | Sandy loam (Submerged) | Flowering |
| **BTS5** | Titabor | Shyam Gaon | Rice | Ranjit | Sandy loam (Submerged) | Flowering |
| **BSH8** | Sibsagar | Hulal Kalita | Okra | Kranti | Loamy | Fruiting |
| **BJA15** | Jorhat | Jorhat | Rice | Luit | Sandy Loam | Flowering |
| **FSH5** | Sibsagar | HulalKalita | Okra | Kranti | Loamy | Fruiting |
| **FJB11** | Jorhat | Barbheta | Chili | Baljuri | Loamy | Fruiting |

## Table 3(on next page)

Table 3. Morphological and Biochemical characteristics of the isolated strains of Bacteria

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2 **Table 3. Morphological and Biochemical characteristics of the isolated strains of Bacteria**

| Isolated strain | Colour | Gram staining | Shape | Motility | KOH | Citrate utilization | Gelatin hydrolysis | Catalase test |
|---|---|---|---|---|---|---|---|---|
| **BAK1** | White | +ve | Rod | + | -ve | -ve | +ve | +ve |
| **BAK2** | Creamish yellow | -ve | Coccus | + | +ve | +ve | +ve | +ve |
| **BAB3** | Creamish yellow | -ve | Rod | + | +ve | +ve | +ve | +ve |
| **BTS4** | White | +ve | Rod | + | -ve | +ve | +ve | +ve |
| **BTS5** | Yellow | -ve | Rod | + | +ve | -ve | +ve | +ve |
| **BTS6** | Whitish | -ve | Coccus | + | +ve | +ve | +ve | +ve |
| **BTM7** | Whitish | -ve | Rod | + | +ve | +ve | +ve | +ve |
| **BSH8** | Yellow | +ve | Rod | + | -ve | +ve | +ve | +ve |
| **BSL9** | Yellow | +ve | Rod | + | -ve | -ve | +ve | +ve |
| **BSL10** | White | +ve | Rod | + | -ve | -ve | +ve | +ve |
| **BGA11** | White | -ve | Rod | + | +ve | +ve | +ve | +ve |
| **BGA12** | Yellow | -ve | Rod | + | +ve | +ve | +ve | +ve |
| **BJA13** | White | -ve | Rod | + | +ve | +ve | +ve | +ve |
| **BJA14** | White | -ve | Rod | + | +ve | +ve | +ve | +ve |
| **BJA15** | White | -ve | Rod | + | +ve | -ve | +ve | +ve |
| **BJR16** | White | +ve | Diplococcus | + | -ve | +ve | +ve | +ve |
| **BJR17** | Yellow | -ve | Rod | + | +ve | -ve | +ve | +ve |
| **BJB18** | Creamy white | -ve | Rod | + | +ve | -ve | +ve | -ve |

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## Table 4(on next page)

Table 4: Effect of culture filtrate of the isolated promising bacterial and fungal strains on the juvenile mortality of RRKN (*Meloidogyne graminicola).*

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**Table 4: Effect of culture filtrate of the isolated promising bacterial and fungal strains on the juvenile mortality of RRKN (*Meloidogyne graminicola).***

| Treatments | Culture filtrate concentrate | Period of exposure | | | | Treatment (T) |
|---|---|---|---|---|---|---|
| | | 6hr | 12 hrs | 24hrs | 48hrs | |
| **BTS4** *(Bacillus velezensis)* | S/8 | 39.33 (38.83) | 53.33 (46.92) | 64.00 (53.17) | 78.67 (62.51) | 71.29 (59.71) |
| | S/4 | 46.00 (42.70) | 58.00 (49.61) | 69.33 (56.41) | 83.33 (66.03) | |
| | S/2 | 60.67 (51.17) | 72.67 (58.50) | 85.33 (67.56) | 91.33 (73.26) | |
| | S | 72.67 (58.50) | 82.00 (64.92) | 86.67 (68.63) | 97.33 (80.73) | |
| **BTS5** (*Alcaligenes faecalis*) | S/8 | 36.67 (37.27) | 47.33 (43.48) | 62.00 (51.95) | 78.67 (62.50) | 68.75 (58.79) |
| | S/4 | 41.33 (40.00) | 55.33 (48.07) | 70.00 (56.80) | 84.67 (66.96) | |
| | S/2 | 48.67 (44.24) | 62.00 (51.94) | 94.00 (75.95) | 99.33 (87.29) | |
| | S | 50.67 (45.38) | 72.00 (58.06) | 97.33 (80.73) | 100 (90.00) | |
| **BSH8** (*Bacillus subtilis*) | S/8 | 51.33 (45.76) | 63.33 (52.73) | 75.33 (60.23) | 87.33 (69.17) | 80.79 (66.12) |
| | S/4 | 2.00 (51.95) | 70.67 (57.21) | 81.33 (64.40) | 95.33 (77.58) | |
| | S/2 | 76.67 (59.78) | 83.33 (65.91) | 92.00 (73.65) | 96.67 (79.60) | |
| | S | 75.33 (60.22) | 84.00 (66.44) | 98.00 (83.44) | 100 (90.00) | |
| **BJA15** | S/8 | 36.67 (37.26) | 49.33 (44.62) | 64.00 (53.13) | 75.33 (60.22) | 68.33 (58.07) |

| | | | | | | |
|---|---|---|---|---|---|---|
| (*Rhizobium pusense*) | S/4 | 42.00 (40.40) | 53.33 (46.91) | 66.67 (54.73) | 80.00 (63.45) | |
| | S/2 | 50.67 (45.39) | 60.00 (50.78) | 84.00 (66.52) | 99.33 (87.29) | |
| | S | 62.67 (52.34) | 76.00 (60.68) | 93.33 (75.28) | 100.00 (90.00) | |
| **FSH5** (*Talaromyces allahabadensis*) | S/8 | 39.33 (38.83) | 48 (43.85) | 54.67 (47.68) | 64 (53.13) | 66.24 (54.97) |
| | S/4 | 50.67 (45.38) | 59.33 (50.42) | 65.33 (53.99) | 72 (58.15) | |
| | S/2 | 60 (50.79) | 67.33 (55.17) | 74 (59.41) | 82 (64.98) | |
| | S | 72 (58.09) | 77.33 (61.60) | 84 (66.53) | 90 (71.62) | |
| **FJA11** (*Trichoderma asperellum*) | S/8 | 53.33 (46.91) | 62.67 (52.34) | 72 (58.06) | 77.33 (61.57) | 78.83 (64.18) |
| | S/4 | 63.33 (52.74) | 74.67 (59.81) | 77.33 (61.60) | 82.67 (65.45) | |
| | S/2 | 72.67 (58.52) | 79.33 (63.00) | 85.33 (67.55) | 90.67 (72.37) | |
| | S | 84 (66.53) | 89.33 (70.95) | 96.67 (79.60) | 100 (90) | |

* Figures in parenthesis are arc signed values.