Plant Archives Vol. 25, Supplement 2, 2025 pp. 1364-1374 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2025.v25.supplement-2.169

CULTURAL, BIOCHEMICAL AND PHYSIOLOGICAL CHARACTERIZATION OF INDIGENOUS ISOLATES OF *BACILLUS SUBTILIS* FROM CHHATTISGARH, INDIA

Pragya Thakur*, R. K. S. Tiwari, V. K. Nirmalkar, Varsha Awasthi and Shanta Sahu
Department of Plant Pathology, B.T.C. CARS, Bilaspur, IGKV Raipur, CG, India - 495001
*Corresponding author E-mail: tpragya665@gmail.com
(Date of Receiving : 22-03-2025; Date of Acceptance : 02-06-2025)

ABSTRACT

The experiment was conducted under *in vitro* condition at BTC, College of Agriculture Research Station Sarkanda, Bilaspur (IGKV) Chhattisgarh, India, in the year 2021-2022. In the present study, six isolates of *Bacillus subtilis* were collected from SBCL, Chorbhatti, Bilaspur, while five isolates of *B. subtilis* were isolated from different soils of rice fields representing different locations of Chhattisgarh. All the eleven isolates were characterized based on their cultural and morphological, physiological and biochemical characteristics. All the isolates were biochemically characterized and found that most of the isolates positive to gram staining, gelatin hydrolysis, KOH test and starch hydrolysis. Among all isolates, BS_8 showed negative reaction to starch hydrolysis. Isolates i.e. BS_1, BS_4, BS_5, BS_9, BS_{10}, BS_{11} had produce siderophore whereas other isolates failed to produce siderophore and showed negative reaction to the siderophore test. Amongst all, isolate BS_{10} showing positive reaction for siderophore production, BS_{10} produces higher amount of siderophore in CAS agar medium (halo-17.16mm). Physiological characteristics of all the isolates of *B. subtilis* indicated temperature of 30℃ was found to be optimum for growth and development of most of the isolates and represented in the form of higher number of CFUs as well as higher optical density (OD600nm). Isolates BS_6 showed maximum growth and development at 30℃ whereas, isolates BS_8, BS_6 showing their ability to grow at 15℃ and 40℃, respectively. All the isolates had have satisfactory growth at different pH levels however pH 7 was found to be optimum followed by pH 6 and had higher number of CFUs and optical density. However, BS_1 found to be more effective and grow at all pH. There were variations to salt tolerance among different isolates of *B. subtilis*. The growth of the all isolates was affected while different levels of NaCl compared to unamended NaCl. Moreover, BS_1 showed maximum tolerance to be different level of salts concentration followed by isolate BS_7. Different isolates were grown on PEG amended medium along with unamended PEG medium and it was found that BS_7 showed satisfactory growth on 5% PEG amended medium and showing maximum tolerance to drought.

Keywords: *Bacillus subtilis*, Colony Forming Units (CFUs), Optical density (OD).

Introduction

Plant protection scientists face a significant challenge in ensuring food and nutritional security for a growing population in the face of rising environmental concerns and the use of chemical pesticides in excess. The issue has necessitated the development and validation of alternate disease management strategies for reducing crop losses owing to biotic stressors. Management of diseases of crop plants is difficult due to absence of resistance in the host and unavailability of resistant cultivars of rice therefore chemical control is only effective methods for the management of disease show ever it also causes soil and water pollution. Biological control of plant diseases is one such technique, which employs effective biocontrol organisms such as *Bacillus subtilis*, *Pseudomonas fluorescens* and *Trichoderma* spp. As biocontrol agents, some natural antagonistic micro-organisms have shown to be effective against various pathogens (Spadaro *et al.*, 2002, Kim *et al.*, 2004; Chen *et al.*, 2008; Nirmalkar *et al.*, 2018).

Among them, *Bacillus subtilis* is an effective bacterial biocontrol agent, for management of soil-borne pathogens like *Rhizoctonia, Fusarium*, *Sclerotium*, etc. This microbe can antagonize pathogens by competing for niche and nutrients, by stimulating the defensive capacities of the host plant and more directly by producing low molecular weight fungal toxic compounds (Compant *et al.,* 2005, Chen *et al.,* 2008; Thakur *et al.,* 2022). *Bacillus subtilis* showed antibiotic resistance to penicillin, amoxicillin, and ampicillin. *B. subtilis* strains are known to produce antibiotics such as subtilin, bacillin, subtenolin, and bacillomycin. According to extensive research work, the effects of *B. subtilis* on plant disease suppression are responsible due to antibiosis, lysis of pathogen hyphae, competition for space and nutrients as well as there are also known to create induced systemic resistance (ISR) in the plants (Yu *et al.,* 2011, Cao *et al.,* 2011; Li *et al.,* 2013). It has been reported that *B. subtilis* can increase plant tolerance to both drought (Patel *et al.,* 2017, Khan *et al.,* 2019; Thakur *et al.,* 2023) and salt stresses (Woo *et al.,* 2020, Jadhav *et al.,* 2010; Kumar *et al.,* 2014). Recently reported that inoculation with *B. subtilis* GOT9 confers enhanced tolerance to drought and salt stress in Thale cress (*Arabidopsis thaliana*) and mustard (*Brassica campestris)* through modulation of plant gene expression, including upregulation of biosynthesis genes for abscisic acid (ABA), which is one of the main plant hormones for stress regulation (Kumar *et al.,* 2024).

In addition, *B. subtilis* instantly promoting plant growth and ability to reduce abiotic stresses otherwise limit the agricultural productivity (Blake *et al.,* 2020). Thus, these descriptive characteristics may be instrumental in identification of the isolates with higher biocontrol and plant growth promotion activity. Furthermore, the local isolates of the *Bacillus subtilis* may have higher ecological adaptability and exhibit higher biocontrol potential. Therefore, the present study was investigated to isolation and characterization of indigenous isolates of *Bacillus subtilis* from the rhizospheres of different rice growing regions of Chhattisgarh, India.

Materials and Methods

Bacterial strains

Eleven isolates of *Bacillus subtilis* were used for investigation. Six isolates of *Bacillus subtilis* were procured from State Bio Control Laboratory (SBCL), Chorbhatti, Bilaspur, Chhattisgarh and five isolates were obtained from the rhizosphere soil samples of different rice growing regions of Chhattisgarh. Procured isolates designated as BS_1 to BS_6 and rhizospheric isolates designated as BS_7 to BS_{11}.

Collection of Soil Samples, Isolation and Identification

Collection of different soil samples from rice field of different location of Chhattisgarh. One g of soil sample was suspended in 9 ml sterile water and subjected to serial dilution (10^{-1} -10^{-8}). An aliquot of 0.1 ml/ 100µl of each dilution was spread on LB agar medium by pour plate method (Janisiewicz, 1988; Pramer and Schmidt, 1965). The inoculated plates were incubated at 28℃ for 24 h. After incubation, the individual colonies were selected based on the their colour, shape, edges further sub culture to obtained pure culture. The isolated pure colonies were examined to their morphological characteristics and gram staining for the identification of isolated strains as *Bacillus subtilis* (Rangaswami and Mahadevan, 2008; Kumar *et al.,* 2023).

Cultural characterization

The isolated bacteria were identified and the confirmation of the *Bacillus subtilis* isolates were studied with the following colony characteristics. Pure culture of all bacterial isolates were grown on Luria Bertani agar medium for colony development. The individual colonies of each isolate were observed for their colony colour, colony shape/form and colony elevation (Killani *et al.,* 2011).

Biochemical characterization

Gram's staining

For the identification of isolates Gram's staining technique were carried out and study the morphological characters of the isolates of *Bacillus subtilis* (Buchananand Gibbons,1974; Kumar *et al.,* 2023).

KOH test

This test was performed to identified *Bacillus subtilis* isolates. One to two drops of 3% KOH potassium hydroxide were placed on a clean glass slide. A loopful bacterial colony was picked up from 24 hours old culture with the help of sterilize inoculating loop and mixed with KOH solution on glass slide for10 seconds. Raised the inoculating loop from the slide for 0.5 to 1 cm. Appearance of mucoid thread which was treated as positive test and if watery suspension is visible which was denoted as negative reaction (Suslow *et al.,* 1982; Kumar *et al.,* 2023).

Gelatin hydrolysis

Bacterial cultures were inoculated through stab of a gelatin Luria Bertani (LB) broth tube and incubated at 28±2 ℃ for 4 days, uninoculated tubes served as control and observed for liquefaction. After incubation, culture tubes were placed in 5℃ or ice bucket for 15 minutes before determining of liquefaction. The positive reaction for liquefaction of gelatin was recorded. If the tubes showed tilted, indicate negative result for gelatin hydrolysis (Harrigen and Margeret, 1966; Kumar *et al.,* 2023).

Starch hydrolysis

Starch is a complex carbohydrate (polysaccharide) composed of two constituents of amylase and amylopectine. This test was carried out for identification of bacteria that can hydrolyze a complex carbohydrate starch by using extra cellular enzymes (Cowan, 1974; Kumar *et al.,* 2023).

The test isolates were single streaked on the starch agar plates and incubated at 28±2℃ for 72 hours. After incubation, the plates were flooded with Lugol's iodine solution for 30 seconds. Starch hydrolysis reaction was identified by appearance of clear zone surrounding the streaking growth of each test isolates.

Siderophore production

Chrome azurol S (CAS) agar plate method (Schwyn and Neilands 1987) was used for qualitative assay was performed as siderophore production.

All tested isolates of *Bacillus subtilis* (24 hours old) was randomly spotted with the help of inoculation loop on CAS blue agar plates. Plates were incubated at 28℃ for 4 days. All the experiments were carried out in triplicate. Formation of an orange halo around the bacterial colonies which indicate the ability of the bacterial strains to produce siderophore and it has been considered as a positive indication for siderophore production.

Physiological Characterization

Isolates were screened for their effect in different temperatures, pH and ability to tolerate salinity and drought using LB broth (Luria Bertani) medium. Optical density was recorded using spectrophotometer at 600 nm and CFUs were also counted by using serial dilution technique (10^{-8} dilution).

Screening at different Temperature

The effect of temperature on optical density (OD 600nm) and Colony Forming Units (CFUs ml^{-1}) of each isolate of *Bacillus subtilis* at different temperature ranging from 15℃, 20℃, 25℃, 30℃, 35℃ and 40℃ in Luria Bertani (LB) broth medium.

Ten ml of LB broth in 30 ml capacity of test tube was inoculated with 1×10^{8} ml^{-1} population of test isolates were used. Inoculated tubes were incubated at temperature ranges from 15℃, 20℃, 25℃, 30℃, 35℃ and 40℃ for 96 hours under shaking incubator, uninoculated tube served as control. The optical density (Kumar *et al.,* 2014) was measured at 600nm with spectrophotometer while CFUsml^{-1} were also counted by using serial dilution technique.

Serial dilution method: One ml bacterial suspension through micropipette was suspended in 9ml sterilize water and further serially diluted from 10^{-1}-10^{-8} dilution. 0.1 ml or 100µl diluted bacterial suspension were used and spread by pour plate method on LB agar medium. The plates were incubated at 28±2℃ for 48hours. After 48 h of incubation bacterial colony of each isolate were counted. The entire experiment conducted in three replications using CRD design (Killani *et al.,* 2011).

Screening at different pH

All the isolates were tested for pH tolerance in LB broth medium at different pH ranges at 4.0, 6.0, 8.0 and7.0 (control) to study the effect of pH on growth of *Bacillus subtilis* isolates.

Ten ml of LB broth with different pH levels were used. The pH was adjusted using 0.1 N NaOH and diluted HCl by using plastic dropper and was detected with the help of pH meter. Tubes were inoculated with1×10^{8} /ml population of test isolates. The pH of LB broth medium was detected by pH meter as 7 and used as control. Incubation was carried out at 28℃ for 96 hours. Optical density was measured at 600nm using a spectrophotometer (Kumar *et al.,* 2014). Furthermore, CFUs were counted by using serial dilution technique (Killani *et al.,* 2011; Kumar *et al.,* 2023).

Screening for salinity tolerance

The effect of salt on growth of *Bacillus subtilis* isolates were studied *in vitro* with different NaCl concentration in LB broth medium.

Ten ml of Luria Bertani (LB) broth medium amended with various concentrations of NaCl viz 0.25M (1.4%), 0.5M (2.9%) and 0.75M (4.3%) was taken in 30 ml capacity of test tubes for each isolate and autoclaved. Initial population fall isolates were 1×10^{8}/ml were used in this study. Inoculated tubes with amended of different NaCl concentration were incubated at 28℃ for 96 h. unamended NaCl tubes

served as control. The optical density (OD600nm) was recorded using spectrophotometer (Kumar *et al.,* 2014). CFUs were also counted using serial dilution technique in LB agar medium (Killani *et al.,* 2011).

Screening for drought tolerance

Polyethylene glycol-6000 was amended in 10 ml LB broth medium at different concentrations 5% and 10%. Five per cent concentration of PEG @ 5 ml/100 ml and 10% @10 ml PEG/100ml water in 10 ml LB broth taken in 30 ml capacity and autoclaved. PEG unamended medium served as control. 1×10^8 per ml population of test isolates were used as initial inoculum. Inoculated tubes were incubated at 28℃ for 96 hours under shaking incubator. The optical density was measured at 600nm and CFUs were also counted using serial dilution technique (Killani *et al.,* 2011, Kumar *et al.,* 2014).

Statistical analysis

All the experimental data were statistically analyzed using CRD design with desired transformation as applicable.

Results and Discussion

Cultural and Morphological characterizations of *Bacillus subtilis* isolates

All the eleven isolates of *Bacillus subtilis* were showed creamy white colony colour and circular in shape with flat colony elevation except BS_5 with showed circular to irregular in shape with raised colony elevation. Isolates BS_1, BS_2, BS_3, BS_4, BS_6, BS_7, BS_8, BS_9, BS_{10}, BS_{11} showed entire colony margin whereas the isolate BS_5 was appears to be lobate as well as entire colony margin.

Observations presented in the (Table 1) revealed that, all the isolates of *B. subtilis* were circular to irregular shape, flat colony elevation with creamy white colonies. Similarly, Satapute *et al.* (2012) isolated twenty-five *B. subtilis* isolates from rhizosphere soil of groundnut, from the fields of university of agricultural sciences, Dharwad. Amongst twenty-five isolates, two isolates were identified as gram-positive bacilli. Isolated colonies were creamy off white colour, rough colony and umbonate edge. Wafula *et al.* (2014) isolated ten isolates of *Bacillus subtilis* from soil of Ngeretea catchment area muranga's country, Kenya using dilute nutrient agar medium and studied the morphological character as identified as gram-positive rod-shaped bacteria. The colony morphology of *Bacillus subtilis* isolates varies from flat to filamentous or branching, with it her smooth or rough colony, irregular form with white to cream in colour. Huang *et al.* (2017) isolated *Bacillus subtilis* SL-44 strain from rhizosphere soil of cotton and the morphological character as identified as gram-positive rod shape bacteria. The colony morphology of the isolates were ivory-white colour, wrinkle, rough, opaque colony.

Biochemical Characterizations of *Bacillus subtilis* isolates

Results presented in the (Table 2) indicates that the positive and negative reaction of *B. subtilis* isolates. All the eleven isolates of *B. subtilis* showed positive reaction towards gram staining, gelatin hydrolysis. All the isolates indicated negative reaction to KOH test. Amongst all isolates, positive reaction to starch hydrolysis while BS_8 showed negative reaction to starch hydrolysis other isolates showed positive reaction to the test means these isolates produce amylase enzymes that use for commercial purpose. Among different isolates i.e. BS_1, BS_4, BS_5, BS_9, BS_{10}, BS_{11} showed positive reaction to siderophores production whereas, BS_2, BS_3, BS_6, BS_7, B S_8 showed negative reaction. However, BS_{10} was seems to be more effective to producing higher amount of siderophore which indicate the applicability of this isolate as biofertilizers as well as biocontrol agents.

The above similar findings of Jamali *et al.* (2019) who reported the biochemical characterization of *Bacillus subtilis* strain RH5 showed positive reaction to gram reaction. Nagendran *et al.,* (2019) found that *B. subtilis* isolates showed positive reaction to gram staining. Morin *et al.,* (2000) who reported that all isolates of *B. subtilis* showed positive reaction to KOH test. The results correlated with the findings of Huang *et al.* (2017) who reported that *Bacillus subtilis* (SL-44) strain tested positive to gelatin hydrolysis. Similarly, Satapute *et al.* (2012) also reported that the biochemical tests of *Bacillus subtilis* strain (AS-4) showed positive reaction to gelatin hydrolysis. Jadhav *et al.* (2010) found that the gelatine hydrolysis ability and positive reaction of starch hydrolysis by the two isolates of *B. subtilis*. Similarly, Khan *et al.* (2011) and Karimi *et al.* (2012) they reported that the starch hydrolysis ability of isolates of *B. subtilis*. Jabborova *et al.* (2021) reported *Bacillus subtilis* (L2) strain showed positive for siderophore production on the chrome azurol S medium. Patel *et al.* (2017) isolated *B. subtilis* isolate (S65) showed positive for siderophore production.

Physiological characterization of *Bacillus subtilis* isolates

Screening at different temperature

The results indicate in the (Table 3) that the optimum temperature for the growth and CFUs for

almost of the isolates of *B. subtilis* was 30℃ and growth (OD) and CFUs was hampered at low and high temperatures of 15℃ and 40℃. At 30℃, the highest optical density and maximum number of cfus/ml was recorded from isolate BS_6 (1.70×10^{10}cfu /ml, OD 0.632) followed by BS_5 (1.52×10^{10} cfu/ml, OD 0.445), BS_3 (1.50×10^{10} cfu/ml, OD 0.431). Data on optical density as well as colony forming units (CFUs/ml) of different isolates of *B. subtilis* recorded at different temperature ranges from 15℃, 20℃, 25℃, 30℃, 35℃ and 40℃. indicate that temperature i.e. 30℃ was found to be optimum and favourable for maximum growth of all isolates. Whereas, growth of most of the isolates were adversely affected at low temp (15℃ and 20℃) as well as high temperature (40℃). Moreover, *B. subtilis* isolates i.e. BS_8 (4.53×10^{9}cfu/ml, OD 0.256) and BS_4 (4.02×10^{9}cfu/ml, OD 0.248) showed satisfactory growth at low temperature. Similarly, *B. subtilis* isolates i.e. BS_{10} (1.22×10^{9}cfu/ml, OD 0.129), BS_1 (1.93×10^{9}cfu/ml, OD 0.150), were perform greater and have had optimum number of colonies and optical density. These findings indicates that isolates which perform better at low temperature should be explore for their application in those area where temperature remains low within crop severity and other hand the isolates which had satisfactory growth at high temperature can be explore for their application as biocontrol agent in those area where temperature ranges 35℃ to 40℃.

All above of Similar findings of Satapute *et al.* (2012) who reported that the growth of *B. subtilis* strains (AS-4) at very low and high temperature. Whereas the maximum optical density was recorded at 27℃ as optimum temperature and the growth of *B. subtilis* strain (AS-4) was affected at 4℃ and 55℃. The optical density (660nm) was observed 0.02, 0.11, 0.39, 0.28, 0.03 and 0.01, at 4℃ 17℃ 27℃ 37℃ 45℃ and 55℃. Delgadillo *et al.,* (2018) who reported that the temperature effect on growth rate of *Bacillus subtilis* isolates at temperature ranges15℃, 28℃ and 37℃. Here reported that *B. subtilis* (PY-79) strain significantly higher growth rate at 28℃ between 2.19 to 2.36×10^{5} cfu/ml/h. Whereas, the same strain had decline growth 0.77×10^{5} cfu/ml/h at 37℃. Similar growth pattern of Kodiak strain, BEB-8b (1.34 to 1.62×10^{6} cfu/ml/h, 0.74×10^{6} cfu/ml/h) and (3.30 to 3.37×10^{8} cfu/ml/h, 3.41 to 3.98×10^{8} cfu/ml/h) was also reported at 28℃ and 37℃. Similarly, Gauvry *et al.,* (2020) reported that optimal growth rate (μopt) of *B. subtilis* strain (BSBI) at 46.9℃ i.e. 4.04/h. Moreover, the maximum colony forming unit's ability of the strain observed (8.0×10^{9} cfu /ml) at 25℃. Whereas, very low CFUs was reported at 37℃ i.e. (1.2×10^{4} cfu/ml).

Screening at different pH

The results illustrated in the (Table 4) indicate that the growth of different isolates of *Bacillus subtilis* at different pH level showed the variation in optical density as well as colony forming units. The optimum pH for the higher optical density and maximum cfus/ml was at 7 pH. The growth and colony forming units was hampered at low and high pH of 4 pH and 8 pH (Alkaline pH).

At 7 pH, maximum number of CFUs and OD was recorded from isolate BS_1 (1.15×10^{10}cfu/ml, OD 0.401) followed by BS_4 (1.06×10^{10} cfu/ml, OD 0.396) and least CFUs and OD was recorded from BS_{11} (6.66×10^{8} cfu/ml, OD 0.290) followed by BS_8 (7.66×10^{8} cfu/ml, OD 0.301). At pH 4, growth was affected at low pH and isolate BS_3 (7.33×10^{8} cfu/ml, OD 0.201) least affected and showed maximum number of CFUs and OD followed by BS_8 (5.66×10^{8} cfu/ml, OD 0.110). Whereas, the least number of CFUs and OD was recorded from isolate BS_1 (1.01×10^{8} cfu/ml, OD 0.038). At pH 8, the maximum CFUs and OD was recorded from isolate BS_1 (7.56×10^{9} cfu/ml, OD 0.321) followed by BS_4 (2.26×10^{9} cfu/ml, OD 0.273). Whereas, the least CFUs and OD was observed from isolate BS_8 (3.90×10^{8} cfu/ml, OD 0.073). The results indicate that growth of different isolates of *B. subtilis* was affected at very acidic (4.0) and alkaline pH conditions (8.0). However, the growth was more affected at acidic $pHBS_1$ (1.01×10^{8} cfu/ml, OD 0.038) and alkaline $pHBS_8$ (3.90×10^{8} cfu/ml, OD 0.073). Moreover, the isolates had optimum number of CFUs of all pH ranges i.e. 4.0, 6.0, 7.0, 8.0. Among different pH Concentration pH 7 was found to be optimum for the growth of the isolates BS_1 (1.15×10^{10} cfu/ml, OD 0.401) followed by pH 6.

Similar findings of Satapute *et al.* (2012) who reported the low optical density of *B. subtilis* strains (AS-4) at pH level 4.0 pH (0.05), 5.0 pH (0.07), 6.0 pH (0.18),7.0 pH (0.35), 8.0 pH (0.26), 9.0 pH (0.05) and 10.0 pH (0.02). Delgadillo *et al.,* (2018) who also reported the growth of *B. subtilis* (PY-79) strain was significantly affected at pH5 (2.19×10^{5} cfu/ml/h) and pH 8 (2.36×10^{5} cfu/ml/h). Further, he also reported the higher growth rate of Kodiak strain at 5 pH (1.62×10^{6} cfu/ml/h) and minimum growth rate at pH 7 (1.34×10^{6} cfu/ml/h). He also reported BEB-ib was strongly affected by different pH (5.0 and 8.0) with maximum growth ranging from 3.5×10^{8} to 4×10^{8} cfu/mL/h. Similarly, Gauvry *et al.,* (2020) reported the effects of

pH and sporulation abilities on growth of *Bacillus subtilis* BSBI. The strain found that able to grow at pH level from 4.9 to 9.1.

Screening for salinity tolerance

Results presented in the (Table 5) indicate that the growth of *Bacillus subtilis* isolates at different NaCl concentration showed that CFUs of all isolates drastically reduced by increase of salt concentration.

The data on CFUs of *B. subtilis* isolates recorded at low concentration of salt (0.25M) indicate that most of the isolate had have satisfactory colony forming units ranging from 6.33×10^8cfu/ml to 5.36×10^9 cfu/ml. The maximum colony forming units and optical density was recorded in *B. subtilis* isolate i.e. BS_7 (5.36×10^9 cfu/ml, OD 0.390) followed by BS_4 (4.96×10^9 cfu/ml, OD 0.388), BS_2 (4.76×10^9 cfu/ml, 0.375) and BS_1 (4.56×10^9 cfu/ml, OD 0.372). However, the growth of *B. subtilis* isolate i.e. BS_3 (3.26×10^9 cfu/ml), BS_5 (2.90×10^9cfu/ml), BS_9 (2.16×10^9 cfu/ml) and BS_6 (1.56×10^9 cfu/ml) was least affected by 0.25M NaCl. Whereas, the minimum colony forming units and OD 600nm was recorded in *B. subtilis* isolate BS_8 (6.33×10^8 cfu/ml, OD 0.290) as compared to unamended NaCl. At 0.5M NaCl concentration, colony forming units (CFUs) of all the isolates was decrease in increasing the NaCl concentration as compared to 0.25M NaCl. The maximum CFUs was recorded from *B. subtilis* isolate i.e. BS_4 (4.56×10^9cfu/ml, OD 0.374) followed by BS_5 (2.83×10^9 cfu/ml, OD 0.351), BS_1 (2.76×10^9 cfu/ml, OD 0.348) and BS_3 (2.46×10^9 cfu/ml, OD 0.341). Whereas, the minimum CFUs was recorded from *B. subtilis* isolate i.e. BS8 (4.33×10^8 cfu/ml, OD 0.183) which was greatly affected by 0.5M concentration of NaCl. At high salt concentration colony forming units was ranging from 2.00×10^8 cfu/ml to 3.63×10^9 cfu/ml. The CFUs and optical density was decreased when increasing the NaCl concentration as compared to unamended NaCl including 0.25M, 0.5M NaCl. The maximum colony forming units and optical density was recorded from isolate i.e. BS_1 (3.63×10^9 cfu/ml, OD 0.250) followed by BS_3 (1.96×10^9 cfu/ml, OD 0.211), BS_2 (1.86×10^9 cfu/ml, OD 0.201), BS_7 (1.73×10^9 cfu/ml, OD 0.190) and BS_9 (1.03×10^9 cfu/ml, OD 0.130). Whereas, the least growth (CFUs) and OD 600nm was recorded in isolate BS_6 (2.00×10^8cfu/ml, OD 0.030) strongly affected in 0.75M NaCl. The colony forming units of different isolates of *B. subtilis* on salt concentration indicate that the growth of most of the isolates of *B. subtilis* was drastically decrease with increasing in the concentration of NaCl (0.25M, 0.5M, 0.75M) as compared to unamended (without NaCl-control) which had maximum colony forming units of almost all isolates. However, some of the isolates i.e.BS_1 had tolerance towards higher concentration of salt and had have higher colony forming units (3.63×10^9 cfu/ml, OD 0.250) which can be successfully used in dessert region in saline soil.

The present results confirmed finding of Satapute *et al.* (2012) who reported the adverse effect of different concentration of NaCl (10%, 15%) on growth and development, the effect of salt on growth of soil isolates *Bacillus subtilis* strain AS-4 on different concentration of NaCl at 10% and 15%. The optical density (660nm) was observed 0.35, 0.55, 0.70, 0.70, 0.55 and 0.45 at 10% NaCl on different time period (5, 10, 15, 20, 25 and 30 hours). Furthermore, 0.08, 0.30, 0.40, 0.61, 0.70 and 0.70 optical density was reported at 15% NaCl on same time intervals. The strain AS-4 showed ability to tolerate high salt concentrations by growth in medium containing 10% and 15% NaCl. Similar, finding of Khan *et al.,* (2017) who reported that *Bacillus subtilis* (Y16) showed adaptation to salt stress at 5% and 10% NaCl concentration. The population count showed 27.83 CFU mL^{-1} and 18.73 CFU mL^{-1} under NaCl stress at 5% and 10%. Patel *et al.,* (2017) reported that isolates of *Bacillus* spp were grow in 15g NaCl(w/v). whereas, *Bacillus subtilis* S65 isolate were able to grow in the presence of 14g NaCl (w/v). Jamali *et al.,* (2019) reported *Bacillus subtilis* strain RH5 tolerance to salt upto 12% of NaCl concentration.

Screening for drought tolerance

Results presented in the (Table 6) indicate that the growth of *Bacillus subtilis* isolates at two PEG concentration (5% and 10%) and unamended PEG (control). At 5% polyethylene glycol concentration, the colony forming units of *B. subtilis* isolates were ranging from 5.33×10^8 cfu/ml to5.30×10^9 cfu/ml. CFUs and OD 600nm was decreases when increasing the PEG concentration as compared to unamended PEG. The maximum colony forming units and OD was recorded from isolate i.e. BS_7 (5.30×10^9 cfu/ml, OD 0.375) followed by BS_6 (4.26×10^9 cfu/ml, OD 0.366), BS_3 (2.16×10^9 cfu/ml, OD 0.360), BS_4 (1.83×10^9 cfu/ml, OD 0.354) and BS_2 (1.63×10^9 cfu/ml, OD 0.351), Whereas, the least colony forming units was recorded in isolate BS_8 (5.33×10^8 cfu/ml, OD 0.251). At 10% PEG concentration, colony forming units was ranging from 2.33×10^8 cfu/ml to 2.06×10^9 cfu/ml. The maximum growth (CFUs) was recorded in isolate BS_9 (2.06×10^9cfu/ml, OD 0.339) followed by BS_4 (1.66×10^9 cfu/ml, OD 0.325) and BS_7 (1.36×10^9 cfu/ml, OD 0.319). Whereas, the least colony forming units were recorded in *B. Subtilis* isolate BS_{10} (2.33×10^8 cfu/ml, OD 0.339) followed by BS_6 (4.33×10^8 cfu/ml, OD 0.231). The data on colony forming units recorded

from different isolates of *B. subtilis* on PEG concentration indicate that the growth of most of the isolates of *B. subtilis* was drastically decrease with increasing in the PEG concentration (5% and 10%) as compared to unamended (without PEG- control) which had maximum CFUs and tolerance to drought of almost all isolates. However, some of the isolates i.e. BS_9 (2.06×10^9 cfu/ml) and isolate BS_4 (1.66×10^9 cfu/ml), had tolerance towards higher concentration of PEG (10%) and have had maximum colony forming units. Overall, amongst all the isolates, BS_7 (5.30×10^9 cfu/ml and BS_9 (2.06×10^9 cfu/ml) showed maximum tolerance to drought at 5% and 10% (PEG) concentrations, respectively.

The present results confirmed finding of Patel *et al.,* (2017) who reported that out of 67 bacterial isolates, 32 (47.8%) isolates of *Bacillus* spp. were shown drought tolerance at 13g/100ml polyethylene glycol (PEG) concentration. Similarly, *Bacillus subtilis* S65 showed maximum tolerance to drought at 13g/100ml (13% PEG) concentration of polyethylene glycol.

Table 1: Morphological characterizations of *Bacillus subtilis* isolates

Sr. No.	Isolates code	Place of collection	Colony color	Colony form /Shape	Colony elevation	Colony margin
1	BS_1	SBCL	Creamy white	Circular	Flat	Entire (smooth)
2	BS_2	SBCL	Creamy white	Circular	Flat	Entire (smooth)
3	BS_3	SBCL	Creamy white	Circular	Flat	Entire (smooth)
4	BS_4	SBCL	Creamy white	Circular	Flat	Entire (smooth)
5	BS_5	SBCL	Creamy white	Circular to irregular	Raised	Lobate, Entire
6	BS_6	SBCL	Creamy white	Circular	Flat	Entire (smooth)
7	BS_7	Mohla	Creamy white	Circular	Flat	Entire (smooth)
8	BS_8	Bemetara	Creamy white	Circular	Flat	Entire (smooth)
9	BS_9	Bemetara	Creamy white	Circular	Flat	Entire (smooth)
10	BS_{10}	Rajnandgaon	Creamy white	Circular	Flat	Entire (smooth)
11	BS_{11}	Kawardha	Creamy white	Circular	Flat	Entire (smooth)

Table 2: Biochemical Characterization of *Bacillus subtilis* isolates

Isolates of *Bacillus subtilis*	Gram reaction	KOH Test	Gelatin hydrolysis	Starch hydrolysis	Siderophore production
BS_1	+	-	+	+	+
BS_2	+	-	+	+	-
BS_3	+	-	+	+	-
BS_4	+	-	+	+	+
BS_5	+	-	+	+	+
BS_6	+	-	+	+	-
BS_7	+	-	+	+	-
BS_8	+	-	+	-	-
BS_9	+	-	+	+	+
BS_{10}	+	-	+	+	+
BS_{11}	+	-	+	+	+

+ positive reaction, - negative reaction

Table 3: Effect of different temperatures on colony forming units (CFUs/ml) and optical density (600nm) of *Bacillus subtilis* isolates

Isolate	15℃		20℃		25℃		30℃		35℃		40℃	
	CFUs/ml	OD	CFUs/ml	OD	CFUs/ml	OD	CFUs/ml	OD	CFUs/ml	OD	CFUs/ml	OD
BS_1	1.93×10^9	0.150	5.90×10^9	0.313	4.46×10^9	0.334	4.51×10^9	0.220	6.20×10^9	0.332	6.63×10^9	0.320
BS_2	1.06×10^9	0.118	2.63×10^9	0.209	1.50×10^9	0.198	4.93×10^9	0.234	1.50×10^{10}	0.440	1.27×10^{10}	0.413
BS_3	2.93×10^9	0.209	1.16×10^9	0.194	1.66×10^9	0.208	1.50×10^{10}	0.431	1.63×10^{10}	0.534	1.55×10^{10}	0.519
BS_4	4.02×10^9	0.248	2.23×10^9	0.205	1.86×10^9	0.220	9.10×10^9	0.350	8.50×10^9	0.347	5.76×10^9	0.339
BS_5	2.23×10^9	0.173	9.13×10^8	0.189	2.36×10^9	0.305	1.52×10^{10}	0.445	4.96×10^9	0.330	2.56×10^9	0.170
BS_6	3.13×10^9	0.216	2.03×10^9	0.198	3.9×10^9	0.324	1.70×10^{10}	0.632	1.29×10^9	0.201	1.90×10^9	0.187
BS_7	3.56×10^9	0.240	4.26×10^9	0.211	1.03×10^9	0.187	5.21×10^9	0.247	4.00×10^9	0.212	3.63×10^9	0.200

BS_8	4.53×10^9	0.265	4.53×10^9	0.220	1.06×10^9	0.192	4.23×10^9	0.217	9.63×10^9	0.376	2.86×10^9	0.194
BS_9	3.26×10^9	0.229	5.83×10^9	0.305	1.20×10^9	0.204	6.26×10^9	0.312	4.53×10^9	0.302	1.66×10^9	0.172
BS_{10}	1.22×10^9	0.129	8.23×10^9	0.390	9.46×10^8	0.169	4.18×10^9	0.203	3.66×10^9	0.205	6.66×10^8	0.152
BS_{11}	2.43×10^9	0.188	8.41×10^9	0.396	8.33×10^8	0.156	9.66×10^9	0.401	1.32×10^{10}	0.428	1.33×10^9	0.164

Table 4: Effect of different pH on colony forming units (CFUs/ml) and optical density (OD 600nm) of *Bacillus subtilis* isolates

Isolates of *Bacillus subtilis*	4pH		6pH		7pH		8pH	
	CFUs/ml	OD	CFUs/ml	OD	CFUs/ml	OD	CFUs/ml	OD
BS_1	1.01×10^8	0.038	1.00×10^{10}	0.352	1.15×10^{10}	0.401	7.56×10^9	0.321
BS_2	1.66×10^8	0.051	9.03×10^8	0.208	4.80×10^9	0.388	8.93×10^8	0.242
BS_3	7.33×10^8	0.201	9.06×10^8	0.212	1.00×10^{10}	0.394	9.00×10^8	0.250
BS_4	1.60×10^8	0.050	1.56×10^9	0.293	1.06×10^{10}	0.396	2.26×10^9	0.273
BS_5	4.33×10^8	0.096	1.06×10^9	0.242	8.90×10^9	0.393	8.66×10^8	0.229
BS_6	2.66×10^8	0.081	8.33×10^8	0.112	2.23×10^9	0.382	4.33×10^8	0.080
BS_7	1.33×10^8	0.042	1.66×10^9	0.302	1.16×10^9	0.380	8.30×10^8	0.207
BS_8	5.66×10^8	0.110	8.66×10^8	0.184	7.66×10^8	0.301	3.90×10^8	0.073
BS_9	2.00×10^8	0.070	1.23×10^9	0.263	1.00×10^9	0.350	8.10×10^8	0.192
BS_{10}	4.00×10^8	0.090	9.00×10^8	0.193	8.66×10^8	0.342	5.00×10^8	0.091
BS_{11}	2.33×10^8	0.073	7.33×10^8	0.110	6.66×10^8	0.290	5.66×10^8	0.109

Table 5: Effect of different concentrations of NaCl on colony forming units (CFUs/ml) and Optical density (OD 600nm) of *Bacillus subtilis* isolates

Isolates of *Bacillus subtilis*	Designation	Control (un-amended NaCl)		0.25M		0.5M		0.75M	
		CFUs/ml	OD	CFUs/ml	OD	CFUs/ml	OD	CFUs/ml	OD
***Bacillus subtilis* BS_1**	**T1**	1.15×10^{10}	0.401	4.56×10^9	0.372	2.76×10^9	0.348	3.63×10^9	0.250
***Bacillus subtilis* BS_2**	**T2**	4.80×10^9	0.388	4.76×10^9	0.375	2.03×10^9	0.303	1.86×10^9	0.201
***Bacillus subtilis* BS_3**	**T3**	1.00×10^{10}	0.394	3.26×10^9	0.368	2.46×10^9	0.341	1.96×10^9	0.211
***Bacillus subtilis* BS_4**	**T4**	1.06×10^{10}	0.396	4.96×10^9	0.388	4.56×10^9	0.374	8.33×10^8	0.121
***Bacillus subtilis* BS_5**	**T5**	8.90×10^9	0.393	2.90×10^9	0.355	2.83×10^9	0.351	5.66×10^8	0.100
***Bacillus subtilis* BS_6**	**T6**	4.23×10^9	0.384	1.56×10^9	0.330	8.00×10^8	0.261	2.00×10^8	0.030
***Bacillus subtilis* BS_7**	**T7**	5.46×10^9	0.392	5.36×10^9	0.390	2.43×10^9	0.322	1.73×10^9	0.190
***Bacillus subtilis* BS_8**	**T8**	7.66×10^8	0.293	6.33×10^8	0.290	4.33×10^8	0.183	3.66×10^8	0.080
***Bacillus subtilis* BS_9**	**T9**	2.30×10^9	0.380	2.16×10^9	0.341	1.26×10^9	0.282	1.03×10^9	0.130
***Bacillus subtilis* BS_{10}**	**T10**	1.06×10^9	0.301	6.66×10^8	0.300	6.33×10^8	0.240	4.66×10^8	0.091
***Bacillus subtilis* BS_{11}**	**T11**	1.66×10^9	0.352	7.33×10^8	0.297	5.33×10^8	0.205	2.33×10^8	0.072

Table 6: Effect of two concentrations of PEG on colony forming units (CFUs/ml) and optical density (OD 600nm) of *Bacillus subtilis* isolates

Isolates of *Bacillus subtilis*	Designation	Control (un-amended PEG)		5% PEG		10% PEG	
		CFUs/ml	OD	CFUs/ml	OD	CFUs/ml	OD
BS_1	**T1**	1.15×10^{10}	0.401	1.26×10^9	0.340	5.30×10^8	0.249
BS_2	**T2**	4.80×10^9	0.388	1.63×10^9	0.351	9.00×10^8	0.295
BS_3	**T3**	1.00×10^{10}	0.394	2.16×10^9	0.360	8.30×10^8	0.283
BS_4	**T4**	1.06×10^{10}	0.396	1.83×10^9	0.354	1.66×10^9	0.325
BS_5	**T5**	8.90×10^9	0.391	1.20×10^9	0.320	1.10×10^9	0.301
BS_6	**T6**	3.03×10^9	0.387	4.26×10^9	0.366	4.33×10^8	0.231
BS_7	**T7**	1.36×10^9	0.385	5.30×10^9	0.375	1.36×10^9	0.319
BS_8	**T8**	7.66×10^8	0.290	5.33×10^8	0.251	5.00×10^8	0.243
BS_9	**T9**	1.00×10^9	0.380	1.23×10^9	0.330	2.06×10^9	0.339
BS_{10}	**T10**	8.66×10^8	0.301	9.60×10^8	0.290	2.33×10^8	0.203
BS_{11}	**T11**	6.66×10^8	0.280	1.30×10^9	0.345	5.33×10^8	0.263

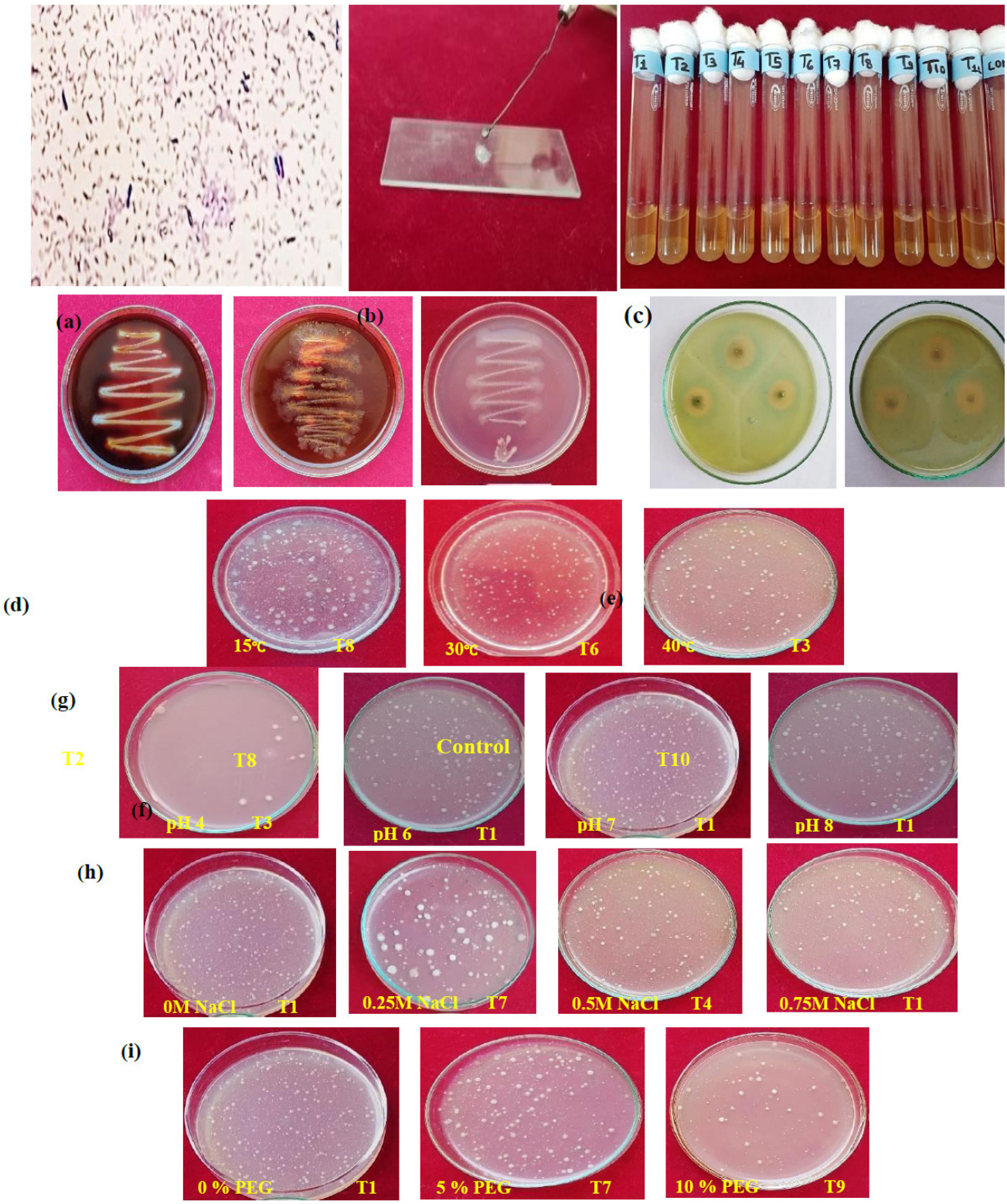

Fig. 1 : Biochemical and Physiological characterization of *Bacillus subtilis* isolates (a) Gram stain reaction, (b) KOH test, (c) Gelatin hydrolysis, (d) Starch hydrolysis (e) Siderophore production (f) Colony forming units (CFUs) of *B. subtilis* isolates at different temperature (g) CFUs at different pH (h) CFUs at different concentration of NaCl (i) CFUs at different concentration of PEG

Acknowledgements

The authors extend their appreciation to the State Bio Control Laboratory (SBCL), Chorbhatti, Bilaspur and Section of Plant Pathology, BTC college of Agriculture and Research Station Bilaspur (IGKV), Chhattisgarh for their support offered during the course of research work.

Declaration

Conflict of interest. Authors declare no conflict of interest.

References

Blake, C., Christensen, M. N. and Covacs, A.T. (2020). Molecular aspects of plant growth promotion and protection by *Bacillus subtilis*. *Molecular Plant-Microbe Interactions*, **34**(1), 15-25.

Buchanan, R.E. and Gibbonsn N.E. (1974). Bergey's Mannual of determinative bacteriology.8th Edition, Williams and Wilkins, Baltimore,1268.

Cao, Y., Zhang, Z.H., Ling, N., Yuan, Y.J., Zheng, X.Y., Shen, B.A., Shen, Q.R. (2011). *Bacillus subtilis* SQR9 can control Fusarium wilt in cucumber by colonizing plant roots. *Biol. Fert. Soils*., **47**, 495–506.

Chen, H., Wang, L., Su, C. X., Gong, G. H., Wang, P. and Yu, Z. L. (2008). Isolation and characterization of lipopeptide antibiotics produced by *Bacillus subtilis*. *Letters in App. Microbiol*., **47**, 180-186.

Compant, S., Duffy, B., Nowak, J., Clement, C. and Barka, E.A. (2005). Use of plant growth-promoting bacteria for biocontrol of plant diseases, principles, mechanisms of action, and future prospects. *App. Environ. Microbiol*.,**71**, 4951–4959.

Cowan, S.T. (1974). Cowan & Steel's manual for the identification of medical bacteria, (2nd ed), vol **12**, 148-162. Cambridge University Press, Cambridge, England.

Delgadillo, R. J., Rodriguez, S. E. V., Portugal, V. O., Jaurez, R. A. and Hernandez, J. L.G. (2018). Effect of pH and temperature on the growth and antagonistic activity of *Bacillus subtilis* on *Rhizoctonia solani*. *Mexican J. Phytopathol*., **36**(2), 256-275.

Gauvry, E., Mathot, A. G., Couvert, O., Leguerinel, I. and Coroller, L. (2021). Effects of temperature, pH and water activity on the growth and the sporulation abilities of *Bacillus subtilis* BSB1. *Int. J. Food Microbiol*., **337**, 108915.

Harrigen, W. F. and Maegeret, E. M. (1966). Laboratory Methods in Microbiology. London and NewYork, Academic Press. London and NewYork, 51.

Huang, Y., Wu, Z., He, Y., Ye, B.C. and Li, C. (2017). Rhizospheric *Bacillus subtilis* exhibits biocontrol effect against *Rhizoctonia solani* in pepper (*Capsicum annuum*). *Biomed Res. Int*. 9

Jabborova, D. P., Narimanov, A. A., Enakiev, Y. I. and Davranov. K. D. (2020). Effect of *Bacillus subtilis*1 strain on the growth and development of wheat (*Triticum aestivum* L.) under saline condition. *Bulg. J. Agric. Sci.*, **26**(4), 744–747.

Jadhav, G. G., Salunkhe, D. S., Nerkar, D. P. and Bhandekar, R. K. (2010). Isolation and characterization of salt tolerant nitrogen-fixing microorganism from food. *Eur. Asia. J. Biosci*.,**4**, 33-40.

Jamali, H., Sharma, A., and Shrivastava, A. K. (2019). Biocontrol potential of *Bacillus subtilis* RH5 against sheath blight of rice caused by *Rhizoctonia solani. J. Basic Mircobiol.*, **60**(3), 268-280.

Janisiewicz, W. J. (1988). Biocontrol of postharvest diseases of apples with antagonistic mixtures. *Phytopathol*., **78**, 379-84.

Karimi, K., Amini, J., Harighi, B. and Bahramnejad, B. (2012). Evaluation of biocontrol potential of *Pseudomonas* and *Bacillus* spp. against *Fusarium* wilt of chickpea. *Aus. J. Crop Sci*., **6**(4), 695-703.

Khan, Haleel A., Naseem, Lavarti, R. and B, Pratibha. (2011). Screening and Potency Evaluation of Antifungal from Soil Isolates of *Bacillus subtilis* selected fungi. *35I Advanced Biotech.*, **10**(07), 101-105.

Khan, N., Bano, A., Rahman, M. A., Guo, J., Kang, Z. and Babar, M. A. (2019). Comparative physiological and metabolic analysis reveals a complex mechanism involved in drought tolerance in Chickpea (*Cicer arietinum* L.) Induced by PGPR and PGRs. Scientific Reports, **9**, 2097.

Khan, M. Y., Zahir, Z. A., Asghar, H. N. and Waraich, E. A. (2017). Preliminary investigations on selection of synergistic halo tolerant plant growth promoting rhizobacteria for inducing salinity tolerance in wheat. *Pak. J. Bot*., **49**, 1541–1551

Killani, A. S., Abaidoo, R. C., Akintokun, A. K. and Abiala, M. A. (2011). Antagonistic effect of indigenous *Bacillus subtilis* on root-/soil-borne fungal pathogens of cowpea. *Researcher*. **3**(3), 11-18.

Kim, P.I., Bai, H., Bai, D., Chae, H., Chung, S., Kim, Y., Park, R. and Chi, Y.T. (2004). Purification and characterization of a lipopeptide produced by *Bacillus thuringiensis* CMB26. *J. App.Microbiol*., **97**, 942–949.

Kumar, G. P., Ahmed, S. K. M. H., Desai, S., Amalraj, E. L. D. and Rasul, A. (2014). *In vitro* screening for abiotic stress tolerance in potent biocontrol and plant growth promoting strains of *Pseudomonas* and *Bacillus* spp. *Int. J. Bacteriol*., 1-6.

Kumar, S., Tiwari, R. K. S., Nirmalkar, V. K., Dayasagar and Singh, Y. (2024). Ecological Niches of *Bacillus* Species and their Multifaceted Mechanisms in Combatting Plant Pathogens. *J. of Scientific Res. and Reports*., **30**(2),514-525

Kumar, S., Tiwari, R. K. S., Nirmalkar, V. K., Keshri, P. K. and Sahu, J. (2023). Evaluation of novel bacterial isolates of Chhattisgarh for salinity tolerance. *The Pharma Innovation J.* **12** (7), 51-54

Kumar, S., Tiwari, R. K. S., and Nirmalkar, V. K. (2023). Evaluation of effective bacterial isolates for their plant growth promoting (PGP) activities in chickpea. *J. of Curr. Opinion in Crop Sci.,* **4**(2), 47-49

Li, S., Zhang, N., Zhang, Z., Luo, J., Shen, B., Zhang, R. and Shen, Q. (2013). Antagonist *Bacillus subtilis* HJ5 controls *Verticillium* wilt of cotton by root colonization and biolm formation. *Biol. Fertil. Soils*, **49**, 295–303.

Morin, A., Poirier, N., Vallee, S. and Porter, A. (2000). Methods for confirming the gram reaction of gram variable *Bacillus* species isolated from tobacco. *Beitrage*

Zur Tabak Forschung Int. Contributions to Tobacco Res., **19**(2),97,101

Nagendran, S., Kulanthaivelu, S. and Sundararajan, T. (2019). Assessment on antagonistic potential of bacterial bio agents *Psuedomonas fluorescens* and *Bacillus subtilis* against *Rhizoctonia solani* Kuhn. An incitant of Sheath blight of rice. *J. Entomol. And Zool. Studies*, **7**(3), 128-142.

Nirmalkar, K., Tiwari, R. K. S. and Singh, S. (2018). Efficacy of bio-agents against damping off in solanaceous crops under nursery conditions. *Int. J. Pl. Protec*. **11**(1) 1-9

Patel, S., Jinal, H. N. and Amaresan, N. (2017). Isolation and characterization of drought resistance bacteria for plant growth promoting properties and their effect on chilli (*Capsicum annuum*) seedling under salt stress. *Biocatalysis and Agricultural Biotechnol*., **12**, 85-89.

Pramer, D., Schmidt E. L., (1965). Experimental Soil Microbiology. Buffer Publ. Co., Minneapolis, 107.

Rangaswami, G. and Mahadevan, A. (2008). Diseases of crop plants in India. 4th edn. Prentice Hall of India (Pvt). Ltd, New Delhi, 177-179.

Satapute, P. P., Olekar, H. S., Shetti, A. A., Kulkarni, A. G., Hiremath, G. B., Patagundi, B. I., Shivssharan, C. T. and Kaliwal, B. B. (2012). Isolation and characterization of nitrogen fixing *Bacillus subtilis* strains AS-4 form agricultural soil. *Int. J. Recent Scientific Res*., **3**(9), 762-765.

Schwyn, B., and J. B. Neilands. (1987). Universal chemical assay for the detection and determination of siderophores. *Analytical Biochem*., **160**, 47-56.

Spadaro, D., Rossana, V., Serenella, P. and Maria, L.G. (2002). Mechanisms of action and efficacy of four isolates of the yeast Metschnikowia pulcherrima active against postharvest pathogens on apples. *Postharvest Biol. Technol*., **24**, 123–134

Suslow, T. V., Schroth, M. N. and Isaka, M. (1982). Application of a rapid method for Gram- differentiation of plant pathogenic and saprophytic bacteria without staining. *Phytopathol*.,**72**, 917-918.

Thakur, P., Tiwari, R. K. S., Nirmalkar, V. K., Verma, S. K. and Chaure, N. K. (2022). Antagonistic activity and bio-efficacy of *Bacillus subtilis* against sheath blight of Rice (*Oryza sativa* L) caused by *Rhizoctonia solani*. *Biological forum- An int. j.* **14**(3), 1305-1311

Thakur, L., Tiwari, R.K.S. and Nirmalkar, V. K. (2023). Efficacy of bio-control agents against soil borne pathogens under *in-vitro* condition. *J. of soils and crops,* **33**(2), 415-419

Wafula, E. N., Kinyua, J., Karuiki, D., Muigai, A. and Mwirichia, R. (2014). Isolation and characterization of *Bacillus* species from soil in Ngeretea catchment area of Muranga's county, Kenya. *Int. J. LifeSci. Res*., **2**(3), 27-35.

Woo, O. G., Kim, H., Kim, J. S. Keum, H. L., Lee, K. C., Sul, W. O. and Lee, J. H. (2020). *Bacillus subtilis* strain GOT9 confers enhanced tolerance to drought and salt stresses in *Arabidopsis thaliana* and *Brassica campestris*. *Plant Physiol. and Biochem*., **148**, 359-367.

Yu, X., Ai, C., Xin, L. and Zhou, G. (2011). The siderophore-producing bacterium, *Bacillus subtilis* CAS15, has a biocontrol effect on Fusarium wilt and promotes the growth of pepper. *Eur. J. Soil Biol*., **47**(2), 138–145.