Salt-pan bacteria as potential plant growth promoters and their antagonistic activity to fungal pathogens of *Capsicum annuum* L.

M. Pawaskar[1], K. P. Krishnan[2] and S. Kerkar[1,*]

[1]School of Biological Sciences and Biotechnology, Goa University, Goa 403 206, India
[2]Department of Arctic Operations, National Centre for Polar and Ocean Research, Goa 403 804, India

Chilli, an essential condiment worldwide, is generally cultivated in paddy fields and can be infected by fungal pathogens, thus hampering its yield. Due to increasing soil salinization, the efficacy of many biocontrol agents is poor in the fields. In this study, bacteria (about 196) isolated from the Goan salt pans in India were screened for their antifungal activity against *Fusarium oxysporum*, *Fusarium pallidoroseum*, *Rhizoctonia solani* and *Pythium aphanidermatum*. Halotolerant isolates of *Bacillus tequilensis*, *Bacillus subtilis* subsp. *inaquosorum*, *Bacillus cabrialesii*, *Bacillus licheniformis*, *Bacillus paralicheniformis* and *Brevibacterium antiquum* could grow under a wide range of pH, temperature and NaCl concentrations, and also displayed plant growth-promoting attributes.

Keywords: Antagonistic activity, chilli, fungal pathogens, plant growth promotion, salt-pan bacteria.

INDIA is the largest producer of chilli (*Capsicum annuum*; about 1.7 million tonnes) in the world, followed by Thailand and mainland China[1]. Because of its colour, flavour and pungency, chilli, also known as pepper, has become an essential component of Indian cuisine. The phytochemicals of pepper have a remarkable potential in the history of new bioactive compounds and natural ingredients for agro-food, cosmetic and pharma industry[2]. However, every year, a considerable loss in the yield of chilli is observed due to various factors[3].

The major disease is damping off, caused by the genus *Pythium*, affecting up to 90% of the chilli crop[4]. Likewise, the wilt causing *Fusarium* species not only reduces the growth but also affects the fruit quality and causes about 10–80% yield loss in the total global production of chilli[5]. Another root rot-causing saprophytic pathogen, *Rhizoctonia solani* causes up to 33.2% yield loss due to disease incidence at the seedling stage under greenhouse conditions and about 40.2% in the main field[6]. Biocontrol measures are desirable to control these phytopathogens to boost the economy of chilli cultivation.

Biocontrol inoculants based on naturally occurring antagonists are environmental-friendly and effective agents against many soil-borne pathogens[7]. Additional properties like salt tolerance and plant growth promotion can improve their potential application even in saline soils. Among the abiotic factors, soil salinization is the most detrimental and is considered one of the significant limiting factors of agricultural productivity and food security[8]. Worldwide, about 20% of the agricultural land is inundated with salt water, and this is continuously increasing[9]. Halophilic and halotolerant microorganisms from solar salt pans are known to produce several secondary metabolites which can be exploited for various applications[10]. The present study aims to evaluate the potential of halotolerant salt-pan bacteria as plant growth promoters and inhibitors of fungal pathogens affecting chilli crops in saline soils.

Materials and methods

Sample collection and isolation of bacteria from salt pans

Isolation of bacteria was carried out on different strengths of Zobell marine (ZM) agar from water, sediment and biofilm samples from three different salt pans (Ribandar, Batim and Agarwado) in Goa, India. Various physico-chemical properties of these samples, like salinity, temperature, pH, dissolved oxygen, conductivity and total dissolved solids, were assessed[11].

In-vitro antifungal test

Fungal phytopathogens *Fusarium oxysporum* (8302), *Fusarium pallidoroseum* (7890), *Rhizoctonia solani* (5338) and *Pythium aphanidermatum* (4746) were procured from the Indian Type Culture Collection (ITCC), New Delhi. The dual culture assay was carried out to estimate the inhibition percentage (L) of fungal mycelia by the bacterial isolates using the formula $L = [(C - T)/C] \times 100$, where C is the mycelial inhibition in control and T is mycelial inhibition in test[12].

In-vitro screening of salt-pan bacterial isolates for plant growth promoting activity

Isolates with significant antifungal activity (>49%) against all four fungi were screened for plant growth-promoting

*For correspondence. (e-mail: drsavitakerkar@gmail.com)

(PGP) activity. Antagonistic bacteria (7) were tested for the production of ammonia in peptone water, siderophore on ChromauzoralS agar and hydrogen cyanide (HCN) on ZM agar amended with glycine[13]. To test indole acetic acid (IAA) and exopolysaccharide (EPS) production, the ZM medium was supplemented with tryptophan, sucrose and Congo red respectively[14]. Production of cell wall-degrading enzymes chitinase, glucanase, amylase, protease, cellulase and lipase was determined by plate assay using 0.2% colloidal chitin, 0.2% laminarin, 0.2% starch, 1% skimmed milk, 1% carboxymethylcellulose and 1% tributyrin as substrate respectively[15]. Isolates were spot-inoculated on Jensen's agar plates to determine the nitrogen-fixing ability and on Dorwin–Fostwer medium supplemented with 1-aminocyclopropane-1-carboxylate (ACC) for testing the ACC deaminase activity[14,16]. Solubilization of insoluble phosphate, zinc, potassium and silicate by the bacterial isolates was determined by calculating the solubilization index of each bacterial isolate on Pikovskaya's, zinc solubilizing, Aleksandrow and modified Bunt and Rovira medium agar plates respectively[13,17,18].

Safety evaluation

The safety of the bacterial isolates was assessed by determining their hemolytic activity with plates containing 5% sterile human blood in agar base and checking the sensitivity of the isolates towards various antibiotics[19]. The antibiotics tested were amikacin, amoxyclav, ampicillin : sulbactam, cephalexin, cephalothin, cephotaxime, chloramphenicol, ciprofloxacin, clindamycin, co-trimoxazole, doxycycline hydrochloride, erythromycin, gentamycin, kanamycin, levofloxacin, lincomycin, nalidixic acid, neomycin, nitrofurantoin, ofloxacin, streptomycin, tetracyclin, tobramycin and vancomycin. The isolates were categorized as sensitive (S), intermediate (I) or resistant (R), as recommended by the Clinical and Laboratory Standards Institute, Pennsylvania, USA, depending on the zone of inhibition[20].

Phenotypic and biochemical characterization

For preliminary phenotypic identification, test strains were Gram-stained and checked for sulphur and nitrate reduction, indole and enzyme (oxidase, catalase, gelatinase and urease) production, citrate and *O*-nitrophenylbeta-D-galactopyranoside (ONPG) utilization and methyl red–Voges Proskauer (MR–VP) test. The utilization of various carbohydrates by bacterial isolates was checked using KB009 HiCarbo™ kits and the results were interpreted according to the manufacturer's instructions. The ability of isolates to grow and replicate in the soil was validated by streaking them on soil extract agar; the oxygen requirement was assessed by growing the isolates in an anaerobic agar medium. Tolerance of the isolates to a broad range of temperature (0–65°C), pH (2.0–14.0) and NaCl concentration (0–20% (w/v)) was also assessed.

SEM analysis and molecular characterization

Cells of 24-h-old bacterial isolates were fixed overnight in 1.5% glutaraldehyde onto glass slides, followed by washing in phosphate buffer and dehydration using ethanol (10–100% v/v concentration). Samples were then mounted on SEM stubs, Sparta-coated and photomicrographs were captured at 15K magnification. Further, the genomic DNA was isolated and the 16S rRNA gene of each isolate was amplified using universal bacterial primers 27F: AGAGTTTGATCCTGGCTCCAG and 1492R: TACGGTTACCTTGTTACGACTT. The PCR product was then sequenced and matched with the GenBank database using NCBI-BLAST.

Statistical analysis

Experimental data were analysed using SPSS software (version 2020). Standard errors were calculated for all mean values. Post-hoc analysis was carried out using Duncan's multiple range test (DMRT) and means were considered significant at the $P \leq 0.05$ level.

Results

Isolation of bacteria from salt pans

A total of 196 bacterial isolates were obtained from the three salt pans of Goa. The physico-chemical properties and number of isolates obtained from each sampling site were compiled (Supplementary Table 1 and Figure 1). The bacterial isolates obtained from water samples (55), sediment samples (48) and biofilm samples (93) were stored on ZM agar for further analysis.

In-vitro antifungal assay

Antifungal dual culture assay revealed that 21 out of 196 bacterial isolates had an inhibitory effect on the mycelia of the fungal pathogens tested (Table 1). The isolates MPSK14, MPSK22, MPSK23, MPSK28, MPSK186, MPSK109 and SK473 showed >49% mycelial inhibition, while MPSK109 showed the highest inhibition against *R. solani* (65.9%), *P. aphanidermatum* (56%), *F. oxysporum* (60.5%) and *F. pallidoroseum* (55%) (Figure 1).

In-vitro screening of bacterial isolates for plant growth promoting activity

Table 2 shows the results of the PGP traits of the selected bacterial isolates. All seven strains tested positive for the

Table 1. Antagonistic activity of salt-pan bacteria against chilli plant pathogens

Isolate	Inhibition (%)			
	Rhizoctonia solani	*Pythium aphanidermatum*	*Fusarium oxysporum*	*Fusarium pallidoroseum*
MPSK109	65.9 ± 1.2^{k}	56 ± 1.5^{j}	60.5 ± 1.5^{j}	55 ± 1.6^{fg}
ABSK11	37.4 ± 1.8^{b}	45 ± 1.3^{b}	47.4 ± 1.2^{ef}	40 ± 1.5^{c}
ABSK171	37.6 ± 0.5^{b}	48 ± 1.4^{cde}	47.4 ± 1.2^{ef}	34.5 ± 0.5^{ab}
MPSK186	56.7 ± 1.3^{h}	51 ± 1.8^{fgh}	55.3 ± 2.1^{i}	56 ± 0.6^{g}
ABSK9	50 ± 1.7^{ef}	49 ± 0.7^{def}	47.4 ± 1.8^{ef}	40 ± 0.5^{c}
BGUM136	47.2 ± 2.2^{cd}	46.9 ± 2.3^{bcd}	42.1 ± 0.8^{d}	32 ± 1.6^{a}
MPSK14	55.5 ± 1.9^{h}	53 ± 1.5^{hi}	52.6 ± 1.6^{ghi}	51 ± 2.1^{de}
BGUM14B	54.3 ± 1.6^{gh}	52.2 ± 1.3^{ghi}	47.4 ± 1.8^{ef}	49 ± 0.3^{d}
BGUM256	47.6 ± 1.3^{de}	44.4 ± 2.5^{b}	47.4 ± 2^{ef}	32 ± 2.2^{a}
BGUM359	44.5 ± 0.5^{c}	45 ± 0.5^{b}	42.1 ± 1.3^{d}	32 ± 1.3^{a}
BGUM370	36 ± 0.5^{b}	45.5 ± 1.5^{bc}	47.4 ± 0.9^{ef}	40 ± 0.7^{c}
BGUM440	45 ± 2.2^{cd}	44.4 ± 2^{b}	34.2 ± 1.7^{b}	36 ± 0.6^{b}
SK473	61.6 ± 2.3^{ij}	54 ± 1.5^{ij}	54.2 ± 1.8^{hi}	60 ± 0.5^{h}
BGUM93	47 ± 1.3^{cd}	44 ± 1.9^{b}	42.1 ± 1.3^{d}	40 ± 1.5^{c}
MPSK20	51 ± 1.1^{f}	50 ± 1.7^{efg}	45 ± 1.5^{e}	50 ± 1.3^{de}
MPSK22	51.4 ± 1.8^{ef}	56 ± 1.2^{j}	52 ± 2.1^{gh}	54.2 ± 1.9^{fg}
MPSK23	63.2 ± 0.9^{j}	61 ± 1.1^{k}	54.2 ± 1.7^{hi}	56.3 ± 1.9^{g}
MPSK51	52.5 ± 0.6^{fg}	51 ± 0.5^{fgh}	39 ± 0.5^{c}	34.2 ± 1.1^{ab}
MPSK28	52.4 ± 1.2^{fg}	49.7 ± 1.8^{efg}	50 ± 0.7^{fg}	49.6 ± 2.2^{de}
MPSK6	37 ± 1.1^{b}	40 ± 0.5^{a}	32 ± 1^{b}	34.2 ± 0.9^{ab}
MPSK8	29.5 ± 1.8^{a}	40 ± 1.5^{a}	29 ± 2.2^{a}	35.4 ± 1.6^{b}

Values are mean ± standard deviation of three independent experiments. Means followed by the same letter within columns are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

Table 2. *In-vitro* characterization of salt-pan bacterial isolates for plant growth promotion attributes and hydrolytic enzymes

Isolate	MPSK22	MPSK23	MPSK186	MPSK109	MPSK14	SK473	MPSK28
IAA							
Siderophore							
HCN							
Ammonia							
Amylase							
Protease							
Lipase							
Chitinase							
β-Glucanase							
Cellulase							
N fixation							
Zinc							
Silicon							
Potassium							
Phosphate							
EPS							
ACC deaminase							

Scale: No activity | Low activity | Moderate activity | High activity

production of IAA, ammonia and ACC deaminase, and were able to grow on nitrogen-free media. Except for MPSK28, all the isolates produced EPS and amylase, lipase, protease and cellulase enzymes. These cultures were also able to solubilize zinc in the media. None of the isolates exhibited the production of HCN or solubilization of silicon, potassium and phosphate. Only two isolates, viz. MPSK186 and MPSK109 produced siderophores and three isolates, viz. MPSK22, MPSK23 and MPSK186 utilized colloidal chitin and laminarin.

Safety evaluation

All seven isolates exhibited *γ*-hemolysis on human blood agar, indicating negative hemolysin production against human blood cells (Figure 2). The sensitivity of the seven isolates to 24 different antibiotics and the interpretive criteria are given in Supplementary Table 2. The results indicate the susceptibility of the isolates to >50% of the antibiotics tested.

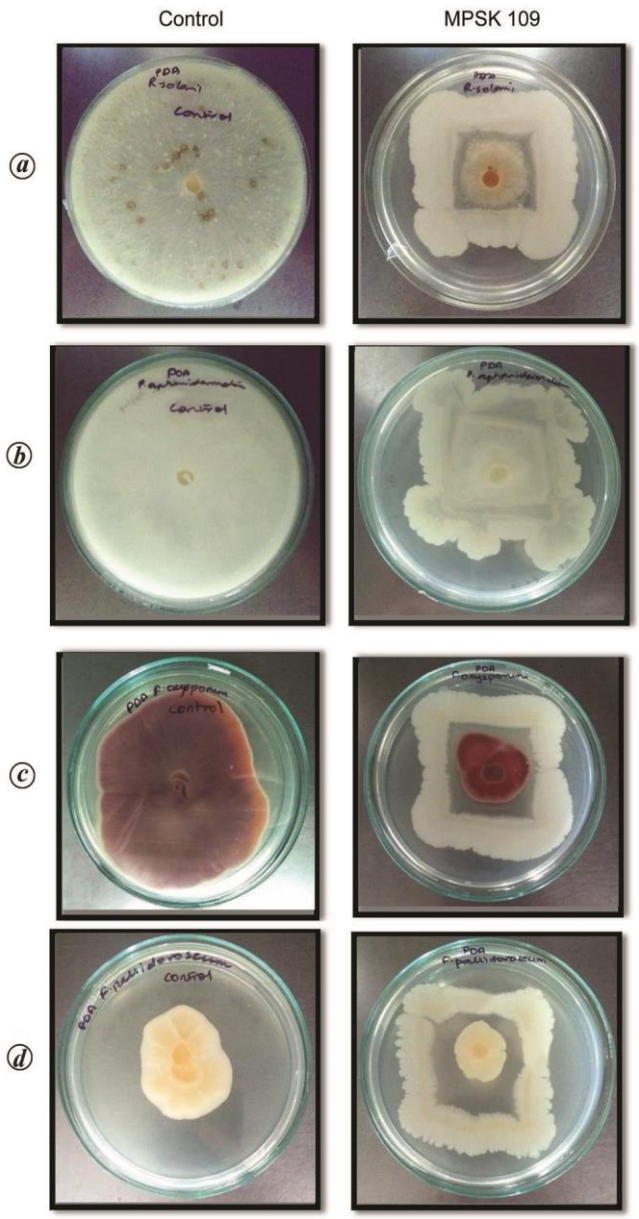

Figure 1. Antagonism exhibited by *Bacillus cabrialesii* strain MPSK 109 against fungal phytopathogens. ***a***, *Rhizoctonia solani*; ***b***, *Pythium aphanidermatum*; ***c***, *Fusarium oxysporum*; ***d***, *Fusarium pallidoroseum*.

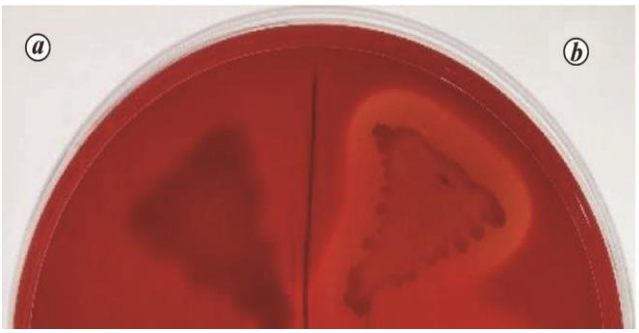

Figure 2. ***a***, γ-Hemolysis of human blood cells by MPSK 28. ***b***, β-Hemolysis of human blood cells by *Vibrio parahaemolyticus*.

Phenotypic and biochemical characterization

In the sulphur, indole, motility (SIM) medium, all the isolates except MPSK28 exhibited diffused growth suggesting motility; however, all isolates tested negative for sulphur reduction. The biochemical tests revealed that all the bacterial isolates were positive for oxidase and catalase production and negative for gelatinase and indole production. Only two isolates, viz. MPSK22 and MPSK23 showed the production of urease enzyme; nitrate reduction was observed in all the isolates except MPSK28. MPSK14, MPSK22, MPSK23 and SK473 utilized ONPG, whereas MPSK14, MPSK22, MPSK23, MPSK109 and MPSK186 utilized citrate. It was also observed that except for MPSK28, all the isolates showed a positive VP reaction and MPSK186 solely showed a positive MR reaction. Based on the Gram-staining and the above results, isolates MPSK14, MPSK22, MPSK23, MPSK109, MPSK186 and SK473 were inferred to belong to the genus *Bacillus*, whereas MPSK28 to the genus *Brevibacterium*. The results showed that all the isolates were halotolerant and could tolerate pH values up to 12. MPSK22, MPSK23 and MPSK28 grew in the media with up to 17% NaCl and MPSK14, MPSK109, MPSK186 and SK473 could tolerate up to 15% of NaCl. The minimum temperature which supported the growth of all the isolates was 15°C; however, the maximum temperature was 30°C for MPSK28, 50°C for MPSK22 and MPSK23, and 60°C for the remaining isolates. Further, MPSK22 and MPSK23 were determined to be facultative aerobes, whereas the remaining isolates were obligate aerobes. The ability of all these isolates to replicate in the soil was confirmed by the growth observed on the soil extract agar. The utilization pattern of 31 different carbohydrates by the seven halotolerant isolates is given in Supplementary Table 3.

SEM analysis and molecular characterization

Scanning electron microscope images confirmed that all the isolates except MPSK28 were long rods, with dimensions ranging from 2.056 μm × 715.3 nm to 2.563 μm × 889.5 nm (Figure 3). Cells of MPSK28 appeared as joint short rods with two cells orienting to give a V-shape, which is typical of the genus *Brevibacterium*. Molecular sequencing of MPSK22 and MPSK23 showed similarity to *Bacillus licheniformis* and *Bacillus paralicheniformis* respectively. Similarly, MPSK186 showed similarity to *Bacillus subtilis* subsp. *inaquosorum* and MPSK109 showed similarity to *Bacillus cabrialessi*. Both MPSK14 and SK473 showed high similarity to *Bacillus tequilensis*, and MPSK28 showed high similarity to *Brevibacterium antiquum*. The partial 16S rRNA gene sequences of the antagonistic strains were deposited at NCBI GenBank and the accession numbers were obtained (Figure 4).

Discussion

Several *Bacillus* spp. have been reported as potential biocontrol agents (BCAs) against phytopathogens affecting

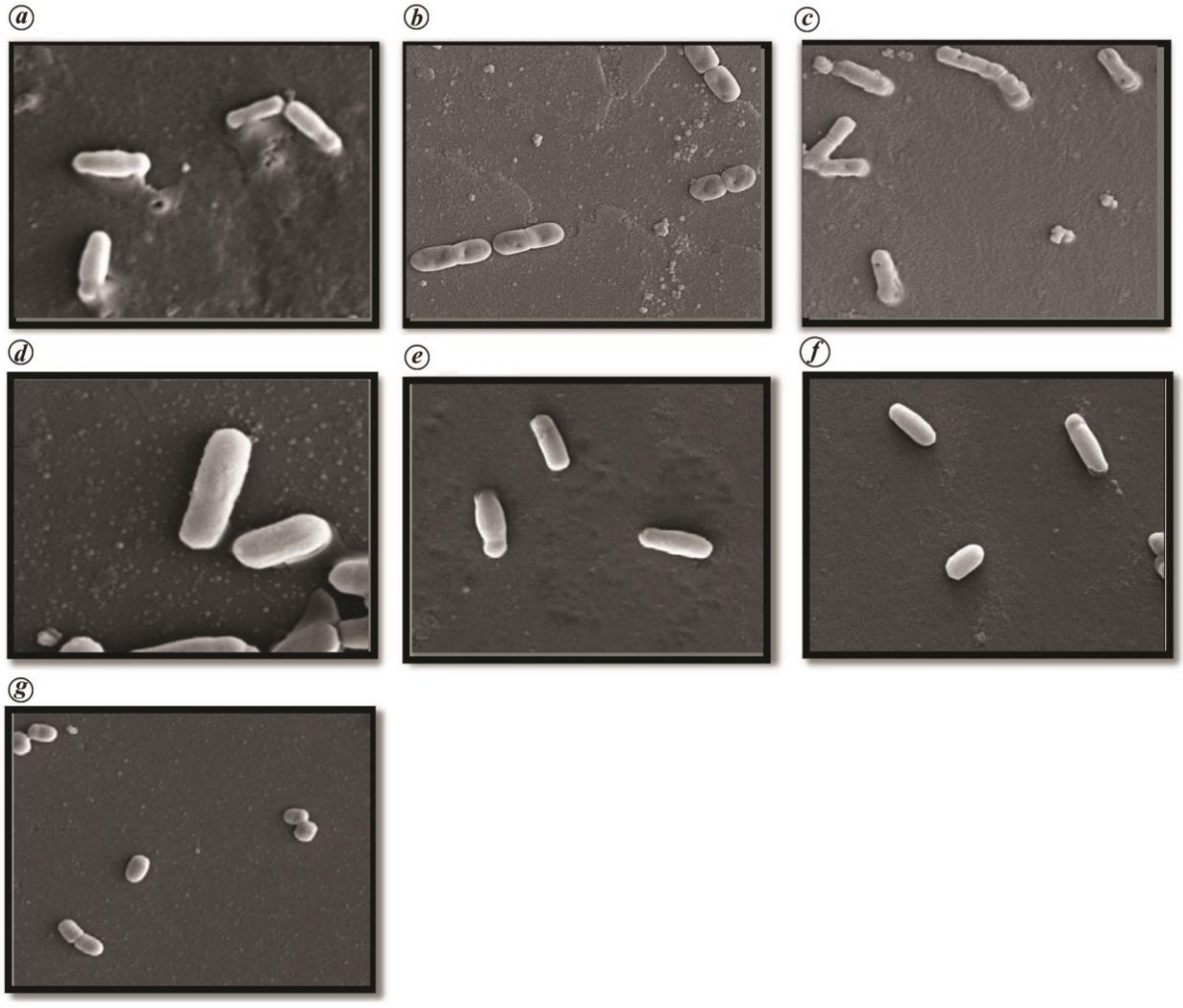

Figure 3. Scanning electron photomicrographs showing individual cells of (***a***) MPSK 22, (***b***) MPSK 23, (***c***) MPSK 186, (***d***) MPSK 109, (***e***) MPSK 14, (***f***) SK 473, (***g***) MPSK 28.

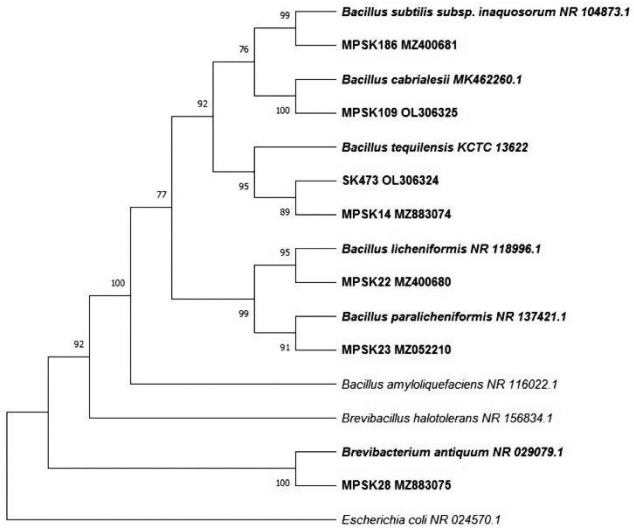

Figure 4. Phylogenetic tree of partial 16S rDNA sequences of halotolerant strains with those of maximum similar entries of type strains from NCBI nucleotide database. The tree was made maximum likelihood method with 1000 bootstrap resamplings.

chilli plants. However, no studies have reported the application of salt-pan bacteria as BCAs against fungal pathogens *R. solani*, *P. aphanidermatum*, *F. oxysporum* and *F. pallidoroseum*[3]. In this study, we explored halotolerant *Brevibacterium* and *Bacillus* strains from different salt pans of Goa (Batim, Ribandar and Agarwada) for their antagonistic activity against fungal pathogens. The isolates of *B. tequilensis* (MPSK14 and SK473), *B. lichenifromis* (MPSK22), *B. paralichenifromis* (MPSK23), *B. antiquum* (MPSK28), *B. subtilis* subsp. *inaquosorum* (MPSK186) and *B. cabrialessi* (MPSK109) were all able to grow on soil extract agar and exhibited tolerance towards a wide range of temperature (15–60°C), pH (5–12) and NaCl concentration (0–17%), thus demonstrating their ability to survive and proliferate in the varying dynamics of the soil. These isolates did not haemolyse human erythrocytes *in-vitro*, proving safe for *in-vivo* applications[20]. Further, the results of the antibiotic susceptibility test showed their strong susceptibility to tetracycline, a broad-spectrum antibiotic which could be used in case of an emergency.

Bacillus tequilensis as a BCA has been reported previously[21–23]. In the present study, we show the antagonistic activity of salt-pan *B. tequilensis* (MPSK14 and SK473) against *F. pallidoroseum*. Previous studies have also proved the antifungal activity of *B. paralicheniformis* against *F. oxysporum* and *R. solani*[24,25]. However, to our knowledge, no previous studies on salt-pan *B. paralicheniformis* (MPSK23) were found to be antagonistic to *F. pallidoroseum* and *P. aphanidermatum*. The present study shows the potential of a halotolerant *B. subtilis* subsp. *inaquosorum* (MPSK186) as a BCA against *F. pallidoroseum, P. aphanidermatum* and *R. solani*. Another interesting finding of this study is the antifungal activity exhibited by the salt-pan *B. cabrialesii* (MPSK109) against *P. aphanidermatum, F. oxysporum* and *F. pallidoroseum.* Although previous studies have reported antifungal activity of *B. cabrialesii* against *Bipolaris sorokiniana* affecting wheat and *Botrytis cinerea*, *Rhizoctonia solani, Verticillium dahlia* and *Phytophthora infestans* affecting tomato[26,27], no report is available on its activity against the pathogens tested in the present study. In the case of *B. antiquum*, there is a report on its antagonistic activity against *Macrophomina phaseolina*, the causal agent of charcoal rot[28]. The present study demonstrates the biocontrol ability of *B. antiquum* (MPSK 28) against other disease-causing phytopathogens.

These salt-pan isolates exhibited several PGP activities which have not been reported previously. *B. licheniformis* strain MPSK22, *B. paralicheniformis* strain MPSK23 and *B. cabrialesii* strain MPSK109 showed solubilization of insoluble zinc from the medium. Micronutrients such as Zn, Fe and Mn are deficient in most soils[29]. Zn solubilization by microorganisms in this context proves to be beneficial and economical in agricultural applications. *B. paralicheniformis* strain MPSK23 also exhibited ACC deaminase activity, and production of cell wall-degrading chitinase and glucanase. Various bacterial isolates have been shown to excrete chitinases and glucanases to digest chitin and β-glucans from the fungal hyphae, and the products of digestion are used as energy sources[30]. This enzymatic digestion of the fungal pathogens could present an effective method for biological control by bacteria[31]. ACC deaminase acts by degrading ACC, the precursor of ethylene, resulting in the production of α-ketobutyrate and ammonia, which prevents excessive increase in the synthesis of ethylene under various stress conditions. It is one of the most efficient mechanisms to induce plant tolerance to salt stress[32]. *B. subtilis* subsp. *inaquosorum* strain MPSK186, used in the present study, has shown siderophore production, dinitrogen fixation and ACC deaminase activity. It is known that microbial siderophores provide plants with iron nutrition to enhance their growth when the bioavailability of Fe is low. Siderophores also compete with phytopathogens for iron, thus negatively affecting the growth of several fungal pathogens[33]. Furthermore, due to the ability of the bacteria to fix atmospheric nitrogen, the dinitrogen is converted to ammonia which is assimilated by the plants[34]. The present study has also demonstrated nitrogen fixation, ammonia production and ACC deaminase activity in the salt-pan isolates of *B. cabrialesii* strain MPSK109 and *B. antiquum* strain MPSK28. Ammonia production is known to not only satisfy the nitrogen demand of the host plant but, in excess, can reduce the colonization of plants by pathogens, thus protecting the plant from various diseases. *B. cabrialesii* strain MPSK109 also produced cellulase, lipase and amylase enzymes. Cell wall-degrading enzymes like cellulases help in the penetration of plant tissue by PGP bacteria, and enzymes like lipases, amylases and proteases are known to degrade the cell wall of pathogens to a considerable extent[35,36].

Conclusion

The results of this study indicated the potential of halotolerant bacteria isolated from the salt pans of Goa as promising antifungal agents, with additional PGP activities against pathogens of chilli. Further field trials will confirm their synergistic effect on the environment, ascertaining their use as sustainable BCAs. Overall, the present study showed the potential application of such bacteria as candidates for yield improvement and biological control for integrated use in disease and nutrient management strategies in saline soils.

Conflict of interest: The authors declare that there is no conflict of interest.

1. FAO, FAOSTAT 2020. Food and Agriculture Organization, Rome, Italy, retrieved from https://www.fao.org/faostat/en/#data/QCL/visualize
2. Baenas, N., Belović, M., Ilic, N., Moreno, D. A. and García-Viguera, C., Industrial use of pepper (*Capsicum annum* L.) derived products: technological benefits and biological advantages. *Food Chem.*, 2019, **274**, 872–885; https://doi.org/10.1016/j.foodchem.2018.09.047.
3. Pawaskar, M. and Kerkar, S., Microbial biocontrol agents against chilli plant pathogens over synthetic pesticides: a review. *Proc. Indian Natl. Sci. Acad.*, 2021, **87**, 578–594; https://doi.org/10.1007/s43538-021-00053-2.
4. Arora, H., Sharma, A., Sharma, S., Haron, F. F., Gafur, A., Sayyed, R. Z. and Datta, R., *Pythium* damping-off and root rot of *Capsicum annuum* L.: impacts, diagnosis, and management. *Microorganisms*, 2021, **9**, 823; https://doi.org/10.3390/microorganisms9040823.
5. Bashir, M. R., Atiq, M., Sajid, M., Mohsan, M., Abbas, W., Alam, M. W. and Bashair, M., Antifungal exploitation of fungicides against *Fusarium oxysporum* f. sp. *capsici* causing Fusarium wilt of chilli pepper in Pakistan. *Environ. Sci. Pollut. Res.*, 2018, **25**, 6797–6801; https://doi.org/10.1007/s11356-017-1032-9.
6. Rini, C. R. and Sulochana, K. K., Management of seedling rot of chilli (*Capsicum annuum* L.) using *Trichoderma* spp. and fluorescent pseudomonads (*Pseudomonas fluorescens*). *J. Trop. Agric.*, 2007, **44**, 79–82; http://jtropag.kau.in/index.php/ojs2/article/view/159/159.
7. Lugtenberg, B. and Kamilova, F., Plant-growth-promoting rhizobacteria. *Annu. Rev. Microbiol.*, 2009, **63**, 541–556; https://doi.org/10.1146/annurev.micro.62.081307.162918.
8. Daliakopoulos, I. N., Tsanis, I. K., Koutroulis, A., Kourgialas, N. N., Varouchakis, A. E., Karatzas, G. P. and Ritsema, C. J., The threat of soil salinity: a European scale review. *Sci. Total Environ.*, 2016, **573**, 727–739; https://doi.org/10.1016/j.scitotenv.2016.08.177.

9. Gupta, B. and Huang, B., Mechanism of salinity tolerance in plants: physiological, biochemical, and molecular characterization. *Int. J. Genomics*, 2014, **2014**, 1–18; https://doi.org/10.1155/2014/701596.
10. Waditee-Sirisattha, R., Kageyama, H. and Takabe, T., Halophilic microorganism resources and their applications in industrial and environmental biotechnology. *AIMS Microbiol.*, 2016, **2**, 42–54.
11. Kamat, T. and Kerkar, S., Bacteria from salt pans: a potential resource of antibacterial metabolites. *Rec. Res. Sci. Technol.*, 2011, **3**, 46–52; http://irgu.unigoa.ac.in/drs/bitstream/handle/unigoa/2521/RecentRes_Sci_Technol_3_46.pdf?sequence=1&isAllowed=y
12. Karimi, E., Safaie, N., Shams-Baksh, M. and Mahmoudi, B., *Bacillus amyloliquefaciens* SB14 from rhizosphere alleviates *Rhizoctonia* damping-off disease on sugar beet. *Microbiol. Res.*, 2016, **192**, 221–230; https://doi.org/10.1016/j.micres.2016.06.011.
13. Sahu, P. K. *et al.*, Endophytic bacilli from medicinal-aromatic perennial Holy basil (*Ocimum tenuiflorum* L.) modulate plant growth promotion and induced systemic resistance against *Rhizoctonia solani* in rice (*Oryza sativa* L.). *Biol. Control*, 2020, **150**, 104353; https://doi.org/10.1016/j.biocontrol.2020.104353.
14. Shet, S. A. and Garg, S., Plant growth promotion of *Vigna unguiculata* in arid sandy soil using bacterial species from coastal sand dune. *Agric. Res.*, 2022, **11**, 1–12; https://doi.org/10.1007/s40003-022-00613-y.
15. Karimi, E., Sadeghi, A., Abbaszadeh Dahaji, P., Dalvand, Y., Omidvari, M. and Kakuei Nezhad, M., Biocontrol activity of salt tolerant *Streptomyces* isolates against phytopathogens causing root rot of sugar beet. *Biocontrol. Sci. Technol.*, 2012, **22**, 333–349; https://doi.org/10.1080/09583157.2012.658552.
16. Kayasth, M., Gera, R., Dudeja, S. S., Sharma, P. K. and Kumar, V., Studies on salinization in Haryana soils on free-living nitrogen-fixing bacterial populations and their activity. *J. Basic Microbiol.*, 2014, **54**, 170–179; https://doi.org/10.1002/jobm.201200158.
17. Pirhadi, M., Enayatizamir, N., Motamedi, H. and Sorkheh, K., Screening of salt tolerant sugarcane endophytic bacteria with potassium and zinc for their solubilizing and antifungal activity. *Biosci. Biotechnol. Res. Commun.*, 2016, **9**, 530–538; https://www.researchgate.net/profile/Naeimeh-Enayatizamir/publication/333712846_Screening_of_salt_tolerant_sugarcane_endophytic_bacteria_with_potassium_and_zinc_for_their_solubilizing_and_antifungal_activity/links/5d0b9f6ba6fdcc11782233a2/Screening-of-salt-tolerant-sugarcane-endophytic-bacteria-with-potassium-and-zinc-for-their-solubilizing-and-antifungal-activity.pdf
18. Chandrakala, C., Voleti, S. R., Bandeppa, S., Kumar, N. S. and Latha, P. C., Silicate solubilization and plant growth promoting potential of *Rhizobium* sp. isolated from rice rhizosphere. *Silicon*, 2019, **11**, 2895–2906; https://doi.org/10.1007/s12633-019-0079-2.
19. Fernandes, S., Kerkar, S., Leitao, J. and Mishra, A., Probiotic role of salt pan bacteria in enhancing the growth of whiteleg shrimp, *Litopenaeus vannamei*. *Probiot. Antimicrob. Protein.*, 2019, **11**, 1309–1323; https://doi.org/10.1007/s12602-018-9503-y.
20. Performance standards for antimicrobial susceptibility testing. CLSI supplement M100. Clinical and Laboratory Standards Institute, 2017.
21. Kim, M. J. *et al.*, Safety evaluations of *Bifidobacterium bifidum* BGN4 and *Bifidobacterium longum* BORI. *Int. J. Mol. Sci.*, 2018, **19**, 1422; https://doi.org/10.3390/ijms19051422.
22. Bhattacharya, A. *et al.*, Intervention of bio-protective endophyte *Bacillus tequilensis* enhance physiological strength of tomato during *Fusarium* wilt infection. *Biol. Control*, 2019, **139**, 104074; https://doi.org/10.1016/j.biocontrol.2019.104074.
23. Zhou, H., Zhu, H., Ren, Z., Li, X., Zhong, J. and Liu, E., Efficacy of *Bacillus tequilensis* strain JN-369 to biocontrol of rice blast and enhance rice growth. *Biol. Control*, 2021, **160**, 104652; https://doi.org/10.1016/j.biocontrol.2021.104652.
24. Paul, N. C., Ji, S. H., Deng, J. X. and Yu, S. H., Assemblages of endophytic bacteria in chili4 pepper (*Capsicum annuum* L.) and their antifungal activity against phytopathogens *in vitro*. *Plant Omics*, 2013, **6**, 441–448; https://search.informit.org/doi/abs/10.3316/INFORMIT.844523344855871
25. Ramírez-Cariño, H. F. *et al*., Biocontrol of *Alternaria alternata* and *Fusarium oxysporum* by *Trichoderma asperelloides* and *Bacillus paralicheniformis* in tomato plants. *Antonie van Leeuwenhoek*, 2020, **113**, 1247–1261; https://doi.org/10.1007/s10482-020-01433-2.
26. Kannan, C., Mishra, D., Rekha, G., Maruthi, P., Shaik, H. and Sundaram, R. M., Diversity analysis of antagonistic microbes against bacterial leaf and fungal sheath blight diseases of rice. *Egypt. J. Biol. Pest Control*, 2021, **31**, 1–16; https://doi.org/10.1186/s41938-021-00462-x.
27. de los Santos Villalobos, S., Robles, R. I., Cota, F. I. P., Larsen, J., Lozano, P. and Tiedje, J. M., *Bacillus cabrialesii* sp. nov., an endophytic plant growth promoting bacterium isolated from wheat (*Triticum turgidum* subsp. *durum*) in the Yaqui Valley, Mexico. *Int. J. Syst. Evol. Microbiol.*, 2019, **69**, 3939–3945; https://doi.org/10.1099/ijsem.0.003711.
28. Zhou, L., Song, C., Li, Z. and Kuipers, O. P., Antimicrobial activity screening of rhizosphere soil bacteria from tomato and genome-based analysis of their antimicrobial biosynthetic potential. *BMC Genomics*, 2021, **22**, 1–14; https://doi.org/10.1186/s12864-020-07346-8.
29. Gopalakrishnan, S., Humayun, P., Kiran, B. K., Kannan, I. G. K., Vidya, M. S., Deepthi, K. and Rupela, O., Evaluation of bacteria isolated from rice rhizosphere for biological control of charcoal rot of sorghum caused by *Macrophomina phaseolina* (Tassi) Goid. *World J. Microbiol. Biotechnol.*, 2011, **27**, 1313–1321; https://doi.org/10.1007/s11274-010-0579-0.
30. Alloway, B. J., *Zinc in Soils and Crop Nutrition*, International Zinc Association Communications, IZA Publications, Brussels, Belgium, 2004.
31. Garbeva, P., Van Veen, J. A. and Van Elsas, J. D., Assessment of the diversity, and antagonism towards *Rhizoctonia solani* AG3, of *Pseudomonas* species in soil from different agricultural regimes. *FEMS Microbiol. Ecol.*, 2004, **47**, 51–64; https://doi.org/10.1016/S0168-6496(03)00234-4.
32. Arora, N. K., Kim, M. J., Kang, S. C. and Maheshwari, D. K., Role of chitinase and β-1,3-glucanase activities produced by a fluorescent pseudomonad and *in vitro* inhibition of *Phytophthora capsici* and *Rhizoctonia solani*. *Can. J. Microbiol.*, 2007, **53**, 207–212; https://doi.org/10.1139/w06-119.
33. del Carmen Orozco-Mosqueda, M., Glick, B. R. and Santoyo, G., ACC deaminase in plant growth-promoting bacteria (PGPB): an efficient mechanism to counter salt stress in crops. *Microbiol. Res.*, 2020, **235**, 126439; https://doi.org/10.1016/j.micres.2020.126439.
34. Ahmed, E. and Holmström, S. J., Siderophores in environmental research: roles and applications. *Microb. Biotechnol.*, 2014, **7**, 196–208; https://doi.org/10.1111/1751-7915.12117.
35. Olanrewaju, O. S., Glick, B. R. and Babalola, O. O., Mechanisms of action of plant growth promoting bacteria. *World J. Microbiol. Biotechnol.*, 2017, **33**, 1–16; https://doi.org/10.1007/s11274-017-2364-9.
36. Rodrigues, A. A., Forzani, M. V., Soares, R. D. S., Sibov, S. T. and Vieira, J. D. G., Isolation and selection of plant growth-promoting bacteria associated with sugarcane. *Pesqui. Agropecu. Trop.*, 2016, **46**, 149–158; https://doi.org/10.1590/1983-40632016v4639526.
37. Friedrich, N., Hagedorn, M., Soldati-Favre, D. and Soldati, T., Prison break: pathogens' strategies to egress from host cells. *Microbiol. Mol. Biol. Rev.*, 2012, **76**, 707–720; https://doi.org/10.1128/MMBR.00024-12.

ACKNOWLEDGEMENT. We thank Goa University for providing the necessary facilities for this study and National Centre for Polar and Ocean Research, Goa, for assistance.

Received 27 April 2022; revised accepted 11 August 2022

doi: 10.18520/cs/v123/i9/1129-1135