Survival of phosphate-solubilizing bacteria against DNA damaging agents

Manoj Shrivastava, Yogendra S. Rajpurohit, Hari S. Misra, and S.F. D'Souza

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Abstract: Phosphate-solubilizing bacteria (PSBs) were isolated from different plant rhizosphere soils of various agroecological regions of India. These isolates showed synthesis of pyrroloquinoline quinone (PQQ), production of gluconic acid, and release of phosphorus from insoluble tricalcium phosphate. The bacterial isolates synthesizing PQQ also showed higher tolerance to ultraviolet C radiation and mitomycin C as compared to *Escherichia coli* but were less tolerant than *Deinococcus radiodurans*. Unlike *E. coli*, PSB isolates showed higher tolerance to DNA damage when grown in the absence of inorganic phosphate. Higher tolerance to ultraviolet C radiation and oxidative stress in these PSBs grown under PQQ synthesis inducible conditions, namely phosphate starvation, might suggest the possible additional role of this redox cofactor in the survival of these isolates under extreme abiotic stress conditions.

Key words: DNA damaging agents, gluconic acid, phosphate-solubilizing bacteria, pyrroloquinoline quinone.

Résumé : Des bactéries solubilisant le phosphate ont été isolées de différents sols de la rhizophère de végétaux présents dans des régions agro-écologiques variées de l'Inde. Ces isolats montraient des activités de synthèse de la pyrroloquinoline quinone (PQQ), de production d'acide gluconique et de libération de phosphore à partir de phosphate tricalcique insoluble. Les isolats bactériens qui synthétisaient de PQQ étaient également plus tolérants aux UVC et à la mitomycine C qu'*Escherichia coli*, mais moins tolérants que *Deinococcus radiodurans*. Contrairement à *E. coli*, les isolats bactériens solubilisant le phosphate étaient plus tolérants aux dommages à l'ADN lorsque cultivés en absence de phosphate inorganique. La tolérance plus élevée aux radiation ultraviolette C et au stress oxydatif de ces bactéries solubilisant le phosphate lorsqu'elles étaient cultivées en condition de synthèse inductible de PQQ, c.-à-d. privées de phosphate, pourrait suggérer que ce cofacteur impliqué dans les processus d'oxydoréduction joue un rôle supplémentaire dans la survie de ces isolats cultivés dans des conditions de stress abiotique extrême.

Mots-clés : agents endommageant l'ADN, acide gluconique, bactérie solubilisant le phosphate, pyrroloquinoline quinone.

[Traduit par la Rédaction]

Introduction

Phosphate-solubilizing microorganisms are able to convert insoluble mineral phosphate into soluble forms and play an important role in the biogeochemical cycle of phosphorus in soil (Goldstein 1994). Mineral phosphate solubilization (MPS) by plants and microbes occurs by 2 primary mechanisms, H^+ excretion and organic acid production. While H^+ excretion based solubilization of phosphate occurs in plants (Hinsinger et al. 2003), fungi (Ahuja et al. 2007), and some species of *Bacillus* (Vora and Shelat 1998), MPS activity by organic acid production has been reported in both plants and microbes. In bacteria, the major excreted acids include gluconic, citric, and oxalic acids (Richardson 2001), while plants produce mainly citric, oxalic, and malic acids among others (Zheng et al. 2005).

The MPS phenotype (MPS^+) in bacteria generally requires the presence of both apo-glucose dehydrogenase and its redox cofactor, pyrroloquinoline quinone (PQQ) (Goldstein 1994). Many bacteria confer an incomplete system for MPS activity. For example, the *Escherichia coli* genome encodes apo-glucose dehydrogenase but not PQQ synthase (Blattner et al. 1997), while some bacteria, including *Deinococcus radiodurans*, encode PQQ synthase and lack apo-glucose dehydrogenase (White et al. 1999), making them MPS^- (Khairnar et al. 2003). Therefore, the MPS^+ phenotype in bacteria is regarded as an indicator of the presence of both PQQ synthase and apo-glucose dehydrogenase. Presence of PQQ in MPS^- bacteria could have a role beyond MPS (Khairnar et al. 2003).

PQQ has been shown to act as an antioxidant and prooxidant in mammalian systems (He et al. 2003). It is also involved in protection of DNA and proteins from oxidative stress and DNA double-strand breakage caused by γ radiation (Misra et al. 2004; Rajpurohit et al. 2008). Hence, the effect of various DNA damaging agents on the survival of PQQ-producing phosphate solubilizing bacteria (PSBs) has been investigated.

In the present paper, we studied the survival of different

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PSBs, isolated from the rhizosphere soils of various plants growing in different agroecological regions of India, against various DNA damaging agents. In this study, we report that PQQ synthesized by PSB isolates was a key factor involved in the tolerance against DNA damaging agents.

Materials and methods

All the chemicals of molecular biology grade were obtained from Sigma Chemical Company (St. Louis, Mo.), Bethesda Research Laboratory (Bethesda, Md.), and Sisco Research Laboratory (Mumbai, India). The restriction enzymes and DNA modifying enzymes were obtained from Roche Molecular Biochemicals (Mannheim, Germany), New England Biolabs (Ipswich, Mass.), and Bangalore Genei (Bengaluru, India), and growth media from Difco Research Laboratories (Sparks, Md.).

Isolation of PSBs

For isolation of bacteria, the soil samples were collected from the rhizosphere of different plants growing in distinct agroecological zones of India. Characteristics of isolation sites are given in Table 1. The serially diluted samples were streaked onto sterile Pikovskaya's agar medium (pH 7.0–7.5), containing tricalcium phosphate as the sole phosphorus source (Pikovskaya 1948), and incubated at 37 °C for 48 h. The PSBs were isolated on the basis of the appearance of a clear halo around the colonies, and the phosphate-solubilizing efficiency of these bacterial isolates was evaluated (Seshadri et al. 2002). Six of the 30 PSB isolates showing very high phosphate-solubilizing efficiency were selected for further study (Table 1).

Molecular studies

Genomic DNA was isolated from overnight-grown cultures of the selected isolates as described earlier (Sambrook and Russell 2001). The gene encoding 16S rRNA was amplified from selected strains by PCR using bacterial universal primers (forward, 5′-AGAGTTTGATCCTGGCTCAG-3′; reverse, 5′-AAGGAGGTGAT CCAGCC-3′) following the standard protocol (Sambrook and Russell 2001). The PCR products were purified using a gel extraction kit (Qiagen, Hilden, Germany) following the manufacturer's protocol and sequenced. Nucleotide sequences were subjected to multiple sequence alignment using the BlastN program (Altschul et al. 1997), and the closest match of known phylogenetic affiliation was used to assign the isolated strains to specific taxonomic groups. The rRNA gene nucleotide sequences of these PSBs were submitted to GenBank (Table 2).

For southern hybridization, approximately 10 μg genomic DNA from different bacterial isolates was digested with *Bam*HI. Fragments were separated in a 1% agarose gel and transferred to Hybond N^+ membrane. Southern hybridization was carried out at 55 °C as described earlier (Misra and Tuli 2000). In brief, the PCR product of the deinococcal *pqqE* gene (Khairnar et al. 2003) was PCR amplified using gene-specific primers, PqqF (5′-ATGGTGGCATTTCTCCGT-GGCCCA-3′) and PqqR (5′-TCATGC GTGACTTACCAA-TGGA-3′), labeled with DIG-dCTP using a multiprime labeling system (Roche Molecular Biochemicals) and used as a *pqqE* gene probe for hybridization with genomic DNA from these isolates. *Deinococcus radiodurans* and *E. coli* genomic DNA digested with *Bam*HI were used as positive and negative controls, respectively. The hybridization signal was detected using a chemiluminescence detection system as described in the manufacturer's protocol (Roche Molecular Biochemicals).

Biochemical studies

PQQ analysis in CFE

For the detection of the presence of PQQ in bacterial cells, the cells were grown at 37 °C, and CFE was prepared from stationary-phase cells of bacterial isolates, as described earlier (Rajpurohit et al. 2008). For the analysis of PQQ, 20 μL of extracted sample was injected into the high-performance liquid chromatography (HPLC) system (Waters 515 HPLC pump, Waters, Milford, Mass.) equipped with a Waters 2487 dual λ absorbance detector. The PQQ separation was carried out on C-18 Bondapak column with 45% methonal in HPLC-grade water as mobile phase with a flow rate 1 $mL \cdot min^{-1}$. The retention time of each signal was recorded at a wavelength of 289 nm.

MPS activity and organic acid production

MPS activity was measured by broth assay. One millilitre of bacterial culture (10^8 $cells \cdot mL^{-1}$) was inoculated in 100 mL of Pikovskaya's liquid medium (pH 6.8–7.0) containing tricalcium phosphate (1000 mg $P \cdot L^{-1}$) as an insoluble phosphorus source. The cells were incubated with Pikovskaya's liquid medium for 3 days at 37 °C with constant shaking. The aliquots were aseptically transferred into 1.5 mL microfuge tubes and centrifuged at 18 000*g* for 5 min. Supernatant was filtered through a 0.22 μm filter (Millex GS, Millipore, Billerica, Mass.) and used for phosphate and gluconic acid determinations. The soluble phosphorus in the filtrate was estimated using the colorimetric Mo-blue color method (Murphy and Riley 1962). The concentration of gluconic acid in the filtrate was determined by HPLC (Waters 515 HPLC pump; detector: Waters 2487 dual λ absorbance; column: Waters T 90541Q 27, C-18 Bondapak column, 4.6 × 250 mm; mobile phase: 0.1% H_3PO_4 and 98 acetonitrile (2:98); flow rate 1 $mL \cdot min^{-1}$; UV detector at 210 nm; duration 20 min; injection volume 20 μL). The identification of gluconic acid in filtrate was based on a comparison of the elution time of the unknown acids in filtrate with the elution times of the gluconic acid standard.

Catalase activity assay

Catalase activity was estimated in CFE prepared from bacterial cells grown under optimal growth conditions. Enzyme activity was measured spectrophotometrically by monitoring the decrease in absorbance at 240 nm due to the decomposition of hydrogen peroxide in 50 $mmol \cdot L^{-1}$ potassium phosphate buffer (pH 7.0) at 25 °C, as described earlier (Beers and Sizer 1951). Protein concentration was determined by Lowry's method (Lowry et al. 1951).

Cell survival studies under ultraviolet (UV) radiation, mitomycin C (MMC), and hydrogen peroxide treatment

For UV and hydrogen peroxide treatments, the bacterial

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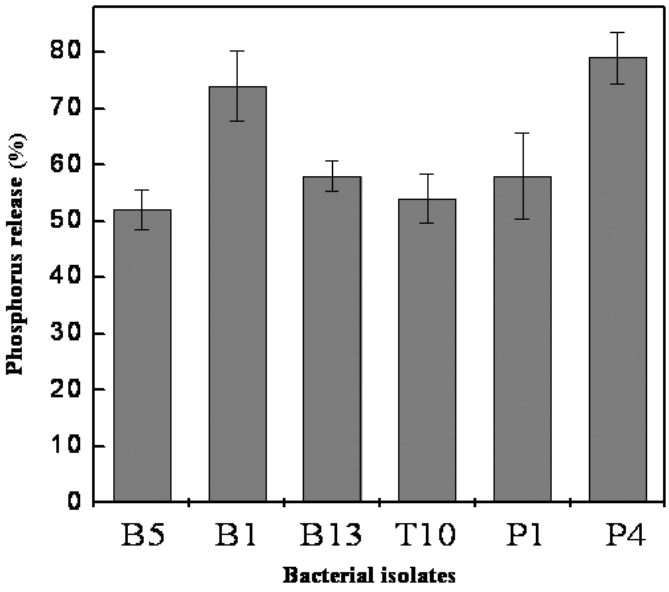
Table 1. Phosphate-solubilizing bacterial isolates from different agroecological regions of India.

Isolates (plant rhizosphere)	Agroecological region of India	pH	Olsen P content (mg P·kg soil^{-1})
TMPSB2 (B1) (groundnut)	Indian agroecological region No. 6, hot semiarid ecoregion, with shallow and medium black soil, Dharwar (Karnataka)	8.5	3.8
TMPSB5 (B5) (groundnut)	Indian agroecological region No. 6, hot semiarid ecoregion, with shallow and medium black soil, Dharwar (Karnataka)	8.5	3.8
TMPSB13 (B13) (red gram)	Indian agroecological region No. 6, hot semiarid ecoregion, with shallow and medium black soil, Dharwar (Karnataka)	8.5	3.8
TMPSB-P1 (P1) (wheat)	Indian agroecological region No. 9, northern plain, hot subhumid ecoregion, with alluvium-derived soils, Pantnagar (Uttarakhand)	7.7	4.3
TMPSB-P4 (P4) (wheat)	Indian agroecological region No. 9, northern plain, hot subhumid ecoregion, with alluvium-derived soils, Pantnagar (Uttarakhand)	7.7	4.3
TMPSB-T10 (T10) (mangrove)	Indian agroecological region No. 19, west coastal plain, hot humid–perhumid ecoregion, with alluvium-derived soils, Mumbai (Maharashtra)	6.6	8.2

Fig. 1. Mineral phosphate-solubilizing activity of phosphate-solubilizing bacteria. Bacterial isolates were tested for solubilization of tricalcium phosphate (1000 mg P·L^{-1}) in Pikovskaya's medium.



cells were grown either in Luria–Bertani (LB) broth at 37 °C or in minimal medium with and without inorganic phosphates, as described earlier (Pausz and Herndl 2002). One millilitre of bacterial culture (2×10^8 cells·mL^{-1}) was centrifuged to pellet cells; these cells were resuspended in sterile phosphate-buffered saline and treated with different doses of UV, as described earlier (Kota and Misra 2006). The resuspended cells (0.2 mL samples) were applied in a thin layer in sterile Petri dishes (4.5 cm diameter) and exposed to different doses (0–200 J·m^{-2}) of UV radiation at 254 nm, at a dose rate of 0.295 J·s^{-1}·m^{-2} (PSV-3303 laser power meter, Gentec, Quebec, Que.) at room temperature. All UV irradiation procedures were performed under red light to prevent possible photoreactivation activity. After UV irradiation, the appropriate dilutions were plated in triplicate on LB plates. Plates were wrapped with aluminum foil and incubated in the dark for 24 h at 37 °C for measurement of colony-forming units (CFU). Hydrogen peroxide treatment of different bacterial isolates was given using a modified protocol, as described earlier (Arrage et al. 1993). In brief, bacterial cells were treated with different concentrations of hydrogen peroxide (0–5 mmol·L^{-1}) for 30 min. Cells were appropriately diluted with normal saline and plated on LB agar plates and CFU were scored after 24 h incubation at 37 °C.

For MMC (0–5 μg·mL^{-1}) effects, the cells were treated with different concentrations of MMC for 30 min, as described earlier (Keller et al. 2001). Appropriate dilutions of treated cells were plated on LB agar plates, and CFU were determined after incubation of plates at 37 °C for 24 h. The cell survival against different DNA damaging agents of treated cultures was determined as the percent CFU of treated cultures relative to CFU of an untreated control culture.

Statistical analysis

Statistical analysis of data was carried out to measure the significance difference in treatments. The results are presented as means ± SE. Data presented are the mean of 3 replicates that had coefficients of variation <10%. All data are taken from 2 independent, reproducible experiments.

Results and discussion

Identification of bacteria isolated from different agroecological regions

Bacteria present in soil samples were screened for MPS$^+$ phenotype in minimal medium supplemented with tricalcium phosphate as the only source of inorganic phosphorus. The 30 bacterial isolates exhibiting differential levels of MPS activity were isolated (data not shown). Of these, 6 isolates showing a significantly higher MPS$^+$ activity were selected for this study. These MPS$^+$ bacteria were identified on the

Table 2. Identification of phosphate-solubilizing bacterial isolates by 16S rDNA sequencing.

Isolate	Length of gene sequence (bp)	16s rDNA identification	Gene identity (%)	GenBank acc. No.
TMPSB2 (B1)	1495	*Proteus mirabilis*	99	EF626945
TMPSB5 (B5)	1500	*Pseudomonas oleovorans*	99	EU047553
TMPSB13 (B13)	1476	*Burkholderia cepacia*	99	EU047554
TMPSB-P1 (P1)	1505	*Pantoea agglomerans*	99	EU047555
TMPSB-P4 (P4)	1491	*Enterobacter* sp.	97	EU047557
TMPSB-T10 (T10)	1499	*Enterobacter hormaechei*	99	EU047556

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basis of their 16S rDNA gene sequence homology with sequences available in NCBI GenBank databases (Table 2). The selected isolates were identified as *Proteus mirabilis* TMPSB1 (B1), *Pseudomonas oleovorans* TMPSB5 (B5), *Burkholderia cepacia* TMPSB13 (B13), *Pantoea agglomerans* TMPSBP-1 (P1), *Enterobacter* sp. TMPSB-P4 (P4), and *Enterobacter hormaechei* TMPSB-T10 (T10). Thus, preliminary phylogenetic placements based on the results of the BLAST analysis revealed that the majority of the isolates clustered together into members of well-characterized bacterial species. Diverse PSBs have been reported earlier from various geographical regions (Chen et al. 2006; Pérez et al. 2007).

Mineral phosphate solubilization

The MPS potential of these 6 isolates was quantified under liquid culture conditions. Result showed very high levels of phosphorus (50%–80%) release from tricalcium phosphates (Fig. 1) by these PSBs in 72 h. It has been well established that microorganisms produce and exude organic acids and (or) chelating agents to release phosphorus from insoluble mineral phosphate (Rodríguez and Fraga 1999). Most PSBs produce gluconic acid from the enzymatic oxidation of glucose. This process requires the enzyme glucose dehydrogenase and its redox cofactor, PQQ (Goldstein 1994). The bacteria lacking either the apoenzyme or PQQ cofactor showed MPS$^-$ phenotype, and *E. coli* and *D. radiodurans* are examples of the few bacteria having an incomplete system for mineralization of insoluble phosphates (Babu-Khan et al. 1995; White et al. 1999; Khairnar et al. 2003). Expression of the PQQ synthase gene, *pqqE*, makes transgenic *E. coli* cells positive for MPS activity (Babu-Khan et al. 1995). Therefore, PSBs used in this study would most likely have both apo-glucose dehydrogenase and the PQQ cofactor. The synthesis of gluconic acid for solubilization of insoluble tricalcium phosphate could be hypothesized.

Production of gluconic acid and PQQ synthesis by MPS bacterial isolates

These isolates were analyzed for the production of gluconic acid and the presence of the deinococcal-type *pqqE* gene. HPLC analysis showed that all isolates released gluconic acid into the medium (Table 3). The levels of gluconic acid produced by MPS$^+$ isolates varied from 8.6 to 56.3 mmol·L^{-1}, which can be related to the levels of MPS activity (Fig. 1). This strongly suggested the presence of an MPS mechanism dependent on gluconic acid production by direct oxidation of glucose and the presence of an active glucose dehydrogenase.

Fig. 2. Detection of *pqqE*-related sequences in genomic DNA of phosphate-solubilizing bacteria. Genomic DNA from the bacteria were isolated, digested with *Bam*HI, and hybridized with DIG-dCTP labeled heterologous gene *pqqE* probe from *Deinococcus radiodurans*.

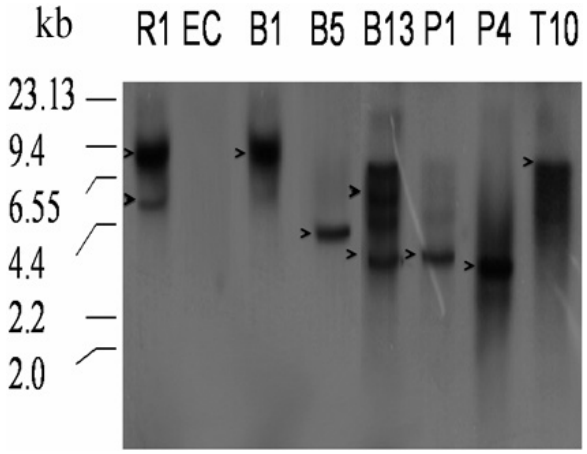

Table 3. Gluconic acid production and pyrroloquinoline quinone (PQQ) synthesis by phosphate-solubilizing bacterial isolates.

Isolate	PQQ synthesis	Gluconic acid (mmol·L^{-1})
TMPSB2 (B1)	Yes	8.6
TMPSB5 (B2)	Yes	12*
TMPSB13 (B13)	Yes	18.4
TMPSB-P1 (P1)	Yes	12.5
TMPSB-P4 (P4)	Yes	45.4
TMPSB-T10 (T10)	Yes	56.3
Escherichia coli	No	nd

Note: nd, not detected. Gluconic acid released into Pikovskaya's medium by PSBs and PQQ in cell-free extract of PSBs were analyzed by HPLC. The elution profile of bacterial gluconic acid and PQQ were compared with commercially available gluconic acid and PQQ.

*Ketogluconic acid.

Southern hybridization of total genomic DNA of these MPS$^+$ isolates and *D. radiodurans* R1 showed a positive signal with the heterologous gene probe (Fig. 2). *Escherichia coli* DNA did not hybridize with the *pqqE* gene probe, indicating that the specific hybridization of this probe with genomic DNA from MPS$^+$ bacteria was sequence specific and confirming the presence of a *pqqE*-like sequence in the genome of all MPS$^+$ isolates used in this study. The absence of *pqqE* hybridization with *E. coli* genome was in agreement

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with earlier reports where *E. coli* had been used as a host for cloning of *pqqE* by functional complementation (Babu-Khan et al. 1995) and by genome sequence analysis (Blattner et al. 1997). The binding of DIG-dCTP labeled probe at more than one place in some of the isolates (B13, T10, and R1) showed the presence of more than one *pqqE*-like sequence in their genome. The presence of the PQQ synthase gene was further confirmed by detecting the presence of PQQ in vivo by HPLC analysis. The HPLC analysis of CFE of these MPS$^+$ bacteria showed the presence of a compound with retention time identical to the retention time (2.02 min) of standard PQQ on C-18 Bondapak column (Table 3). These results thus confirmed the presence of an active PQQ synthesis system in metabolically distinct bacteria isolated from the rhizosphere of different plants.

Survival of MPS bacterial isolates against UVC and MMC

The responses of MPS$^+$ bacteria possessing a PQQ synthesis system for MPS activity to DNA damaging agents were investigated. Results indicated that the tolerance of all PQQ-producing bacteria against UVC and MMC were higher than that of *E. coli* and lower than that of *D. radiodurans* (Fig. 3A, 3B). These isolates showed a nearly 2 $\log_{10}$ cycle higher tolerance to UVC and MMC as compared with *E. coli*, which does not synthesize PQQ. Zhang and Sundin (2004) reported higher tolerance of *Pseudomonas* spp. and *Burkholderia cepacia* cepacia to UVB radiation. The higher tolerance of *Enterobacter cloacae* to UVA was attributed to the presence of less sensitive UVA targets and (or) the existence of a more effective defense systems against oxidative stress (Oppezzo and Pizarro 2001). Both UVB and UVC exert different effects on living cells. UVC produces a relatively low level (<1%) of oxidative stress compared with that produced by UVB (Cadet et al. 2005), while DNA strand breakage produced under UVC exposure is much higher than that caused by UVB radiation by way of unrepaired cyclobutadipyrimidine lesion accumulation (Garinis et al. 2005) and replication fork arrest (Cox et al. 2000). UV causes primarily intrastrand cross-links in DNA, whereas MMC inhibits DNA synthesis by reacting with guanines of complementary DNA, causing the production of interstrand cross-links (Keller et al. 2001; Vidal et al. 2006). Such changes in DNA can lead to the arrest of the replication fork and production of DNA strand break (Niedernhofer et al. 2004). Thus, the possibility of PQQ contributing to repair of DNA strand breaks produced during UVC and MMC exposure is suggested. The molecular mechanism by which PQQ could enhance the DNA repair capability of the bacterial isolates used in this study remains to be understood. However, it has already been shown that the expression of deinococcal PQQ synthase in *E. coli* makes these cells more tolerant to higher doses of UV radiation (Khairnar et al. 2007). *Deinococcus radiodurans* lacking PQQ becomes highly sensitive to γ radiation and other DNA damaging agents and shows an impairment of double-strand break repair (Rajpurohit et al. 2008). The results from this study support the role of PQQ in the protection of host cells from oxidative stress and DNA damage caused by abiotic stresses like drought and UV radiation in the environment. Battista et al. (2001) reported that in *D. radiodurans* DNA strand breaks caused by desiccation (drought) is similar to that caused by γ radiation and X-rays.

Survival of MPS bacterial isolates against peroxide and catalase activity

The results from the experiment conducted to evaluate the tolerance of bacterial isolates to hydrogen peroxide treatment showed that 4 of the 6 strains tested showed higher tolerance to hydrogen peroxide, while isolates B1 and P4 showed a hydrogen peroxide sensitivity similar to that of *E. coli* (Fig. 4A). It is known that both antioxidant enzymes and antioxidant metabolites contribute to the oxidative stress tolerance of any organism (Finkel and Holbrook 2000; Shashidhar et al. 2010). Chang et al. (2005) reported that DNA repair proteins and catalases are the main defense systems in *Pseudomonas aeruginosa* for preventing the lethal effect of hydrogen peroxide. PSBs producing PQQ but showing differential response to oxidative stress might suggest the possibility of an inefficient antioxidant enzyme system. Catalase, an important enzyme involved in hydrogen peroxide dismutation, was studied in these isolates. The results showed the different levels of catalase activity in these isolates (Fig. 4B). The B1 and P4 isolates showed relatively less catalase activity compared with that of other isolates, but at par to that of *E. coli*. The level of catalase activity in other isolates, which showed higher tolerance to hydrogen peroxide, was significantly higher. These results suggested that the higher tolerance to oxidative stress is a cumulative effect of both antioxidant enzymes and antioxidant metabolites like PQQ in these MPS$^+$ bacterial isolates. The antioxidant role of PQQ has been explicitly demonstrated in vivo (He et al. 2003; Khairnar et al. 2003) and in vitro (Misra et al. 2004).

Survival of MPS bacterial isolates against UVC and hydrogen peroxide under phosphate starvation conditions

The effect of phosphate on UVC and hydrogen peroxide tolerance was investigated in these PSB isolates and compared with that of *E. coli*. *Escherichia coli* showed improved tolerance in the presence of inorganic phosphate, whereas the PSBs were found to be more resistant to oxidative stress under phosphate starvation conditions (Fig. 5). The survival of PSBs against UVC stress under phosphate-rich conditions was found to be at par with their survival under phosphate starvation conditions (Fig. 6). Bacteria solubilizing insoluble mineral phosphates through production of gluconic acid show inducible synthesis of PQQ in the absence of inorganic phosphates (Goldstein and Liu 1987). The improved tolerance to DNA damage and oxidative stress under phosphate starvation therefore argued in favor of PQQ having a role in oxidative stress and DNA damage tolerance in these bacteria. Earlier reports suggested that bacteria that do not produce PQQ show an enhanced tolerance to UVB when grown in the presence of inorganic phosphate compared with those grown in the absence of phosphate (Pausz and Herndl 2002). This indicates that the mechanisms of UV tolerance in PQQ-producing PSBs and those bacteria that do not make PQQ might be different. The molecular basis of the differential effect of phosphates on tolerance to these abiotic stresses in bacteria lacking PQQ versus those synthe-

Fig. 3. Colony-forming units (CFU) of phosphate-solubilizing bacteria and *Escherichia coli* following MMC (A) and UVC (B) treatments in comparison with untreated control cultures. Each point on the graphs represents the mean of at least 3 experiments, and the error bars represent SE.

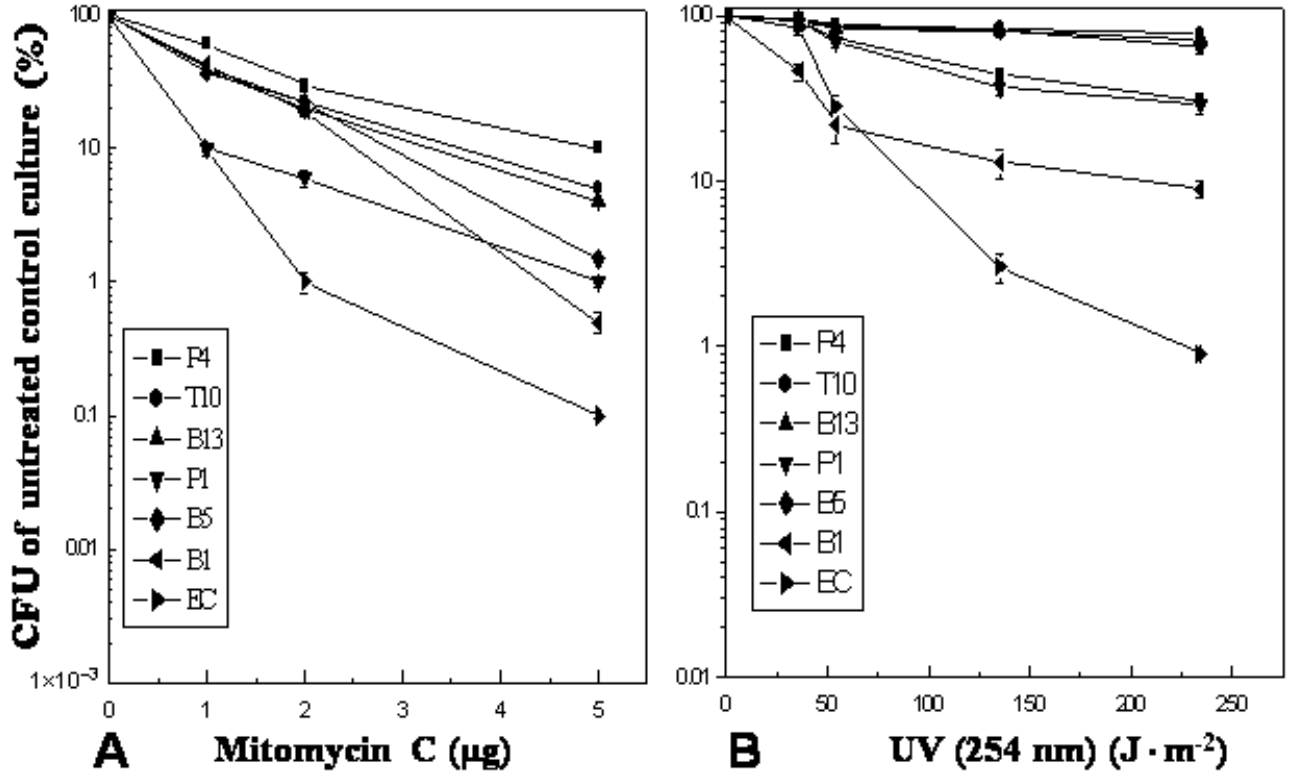
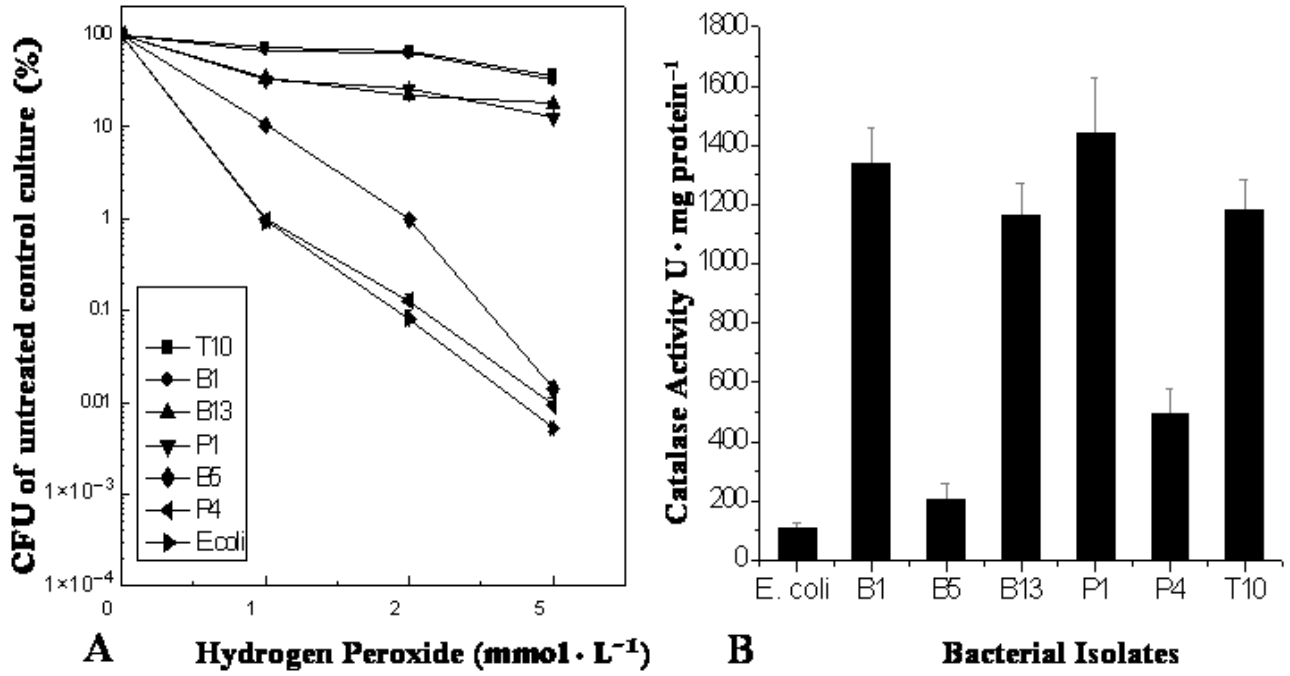


Fig. 4. Colony-forming units (CFU) of phosphate-solubilizing bacteria and *Escherichia coli* following the hydrogen peroxide (A) treatment in comparison with an untreated control and their catalase activity (B). Each point on the graphs represents the mean of 3 experiments, and the error bars represent SE.



sizing PQQ is not understood and needs to be evaluated independently.

In the present study, we demonstrated that the rhizospheres of plants growing in different agroecological regions of India support a diverse group of potential PSBs. This study showed that the synthesis of PQQ and production of

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Fig. 5. Colony-forming units (CFU) of phosphate-solubilizing bacteria and *Escherichia coli* under phosphate-rich (P) and -free conditions following hydrogen peroxide treatment. The % CFU was determined relative to an untreated control culture. Each point on the graphs represents the mean of 3 experiments, and the error bars represent SE.

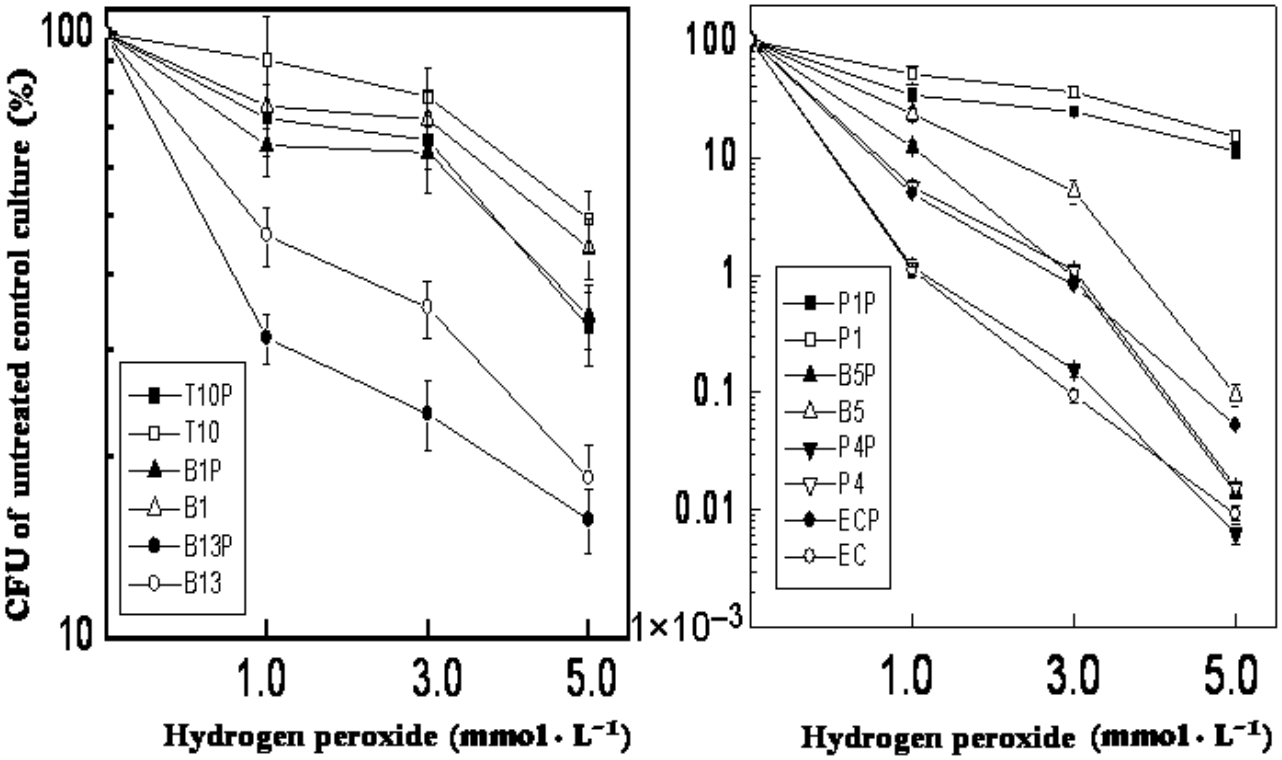
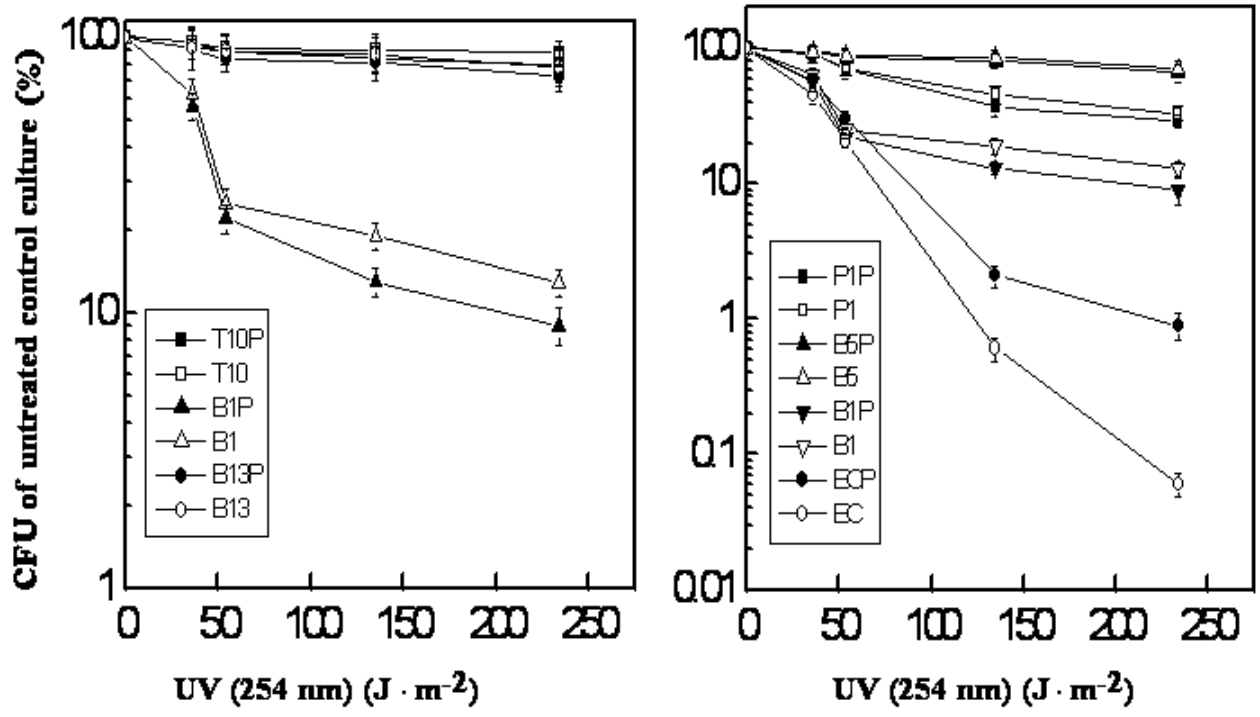


Fig. 6. Colony-forming units (CFU) of phosphate-solubilizing bacteria and *Escherichia coli* under phosphate-rich (P) and -free conditions following UVC treatment. The % CFU was determined relative to an untreated control culture. Each point on the graphs represents the mean of 3 experiments, and the error bars represent SE.



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gluconic acid were strongly associated with MPS activity in these MPS bacterial isolates. Oxidative stress tolerance required the presence of both PQQ and higher catalase activity. Indeed, a clear relationship could be established between higher tolerance to DNA damaging agents and PQQ synthesis, organic acid production, and solubilization of insoluble mineral phosphate. The results thus suggest a possible role of PQQ in response to abiotic stresses in these PSBs.

It has been shown that MPS$^+$ bacterial isolates mobilize insoluble phosphates very efficiently as a consequence of the production of gluconic acid, which results from the extracellular oxidation of glucose via the action of both apoglucose dehydrogenase and its redox cofactor, PQQ (Goldstein 1994). Interestingly, analysis of the culture supernatants and CFE allowed us to detect the presence of gluconic acid and PQQ in all 6 bacterial cultures used in this study. Furthermore, the gene encoding PQQ, the coenzyme responsible for gluconic acid production, was also detected by Southern hybridization.

Although abiotic stress induced signal transduction has been studied in prokaryotes (Parkinson 1993), the DNA damage response elements have not been identified in any bacterial system. PQQ, which can react with artificially produced reactive oxygen species in solution, might act as a signaling molecule in response to abiotic stress and help such bacteria to survive better under adverse conditions. The role of PQQ as an inducer for a periplasmic protein kinase in *E. coli*, in the activation of the Ras signaling pathway of mouse fibroblasts, and in double-strand break repair and γ radiation tolerance of *D. radiodurans* have been demonstrated (Khairnar et al. 2007; Kumazawa et al. 2007; Rajpurohit et al. 2008).

Conclusions

This study thus interestingly demonstrates that bacterial isolates having MPS$^+$ activity supported by a PQQ-dependent mechanism can also withstand various abiotic stresses that exert oxidative stress and DNA damage. These findings suggested a strong possibility of PQQ functioning in improving the survival of host bacteria against DNA damaging agents, along with its principal function in MPS and protection from oxidative stress. Further studies are required to understand the molecular mechanisms of PQQ in DNA damage tolerance and oxidative stresses in PSBs and how this compound helps such organisms to withstand extreme climatic conditions during their growth in nature. Further, the study warrants the use of these isolates as an attractive phosphate-solubilizing biofertilizer for field application under various abiotic stress conditions.

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