C. R. Biologies xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Comptes Rendus Biologies

www.sciencedirect.com

Microbiology: bacteriology, mycology, parasitology, virology/Microbiologie : bactériologie, mycologie, parasitologie, virologie

Characterization of culturable bacteria isolated from hot springs for plant growth promoting traits and effect on tomato (*Lycopersicon esculentum*) seedling

Kinjal Samir Patel, Jinal Hardik Naik, Sejal Chaudhari, Natarajan Amaresan *

C.G. Bhakta Institute of Biotechnology, Uka Tarsadia University, Maliba Campus, Bardoli, Surat 394350, Gujarat, India

ARTICLE INFO

Article history:
Received 16 December 2016
Accepted after revision 26 February 2017
Available online xxx

Keywords:
Bacillus spp.
Biocontrol
Hot springs
PGP properties
Salinity

ABSTRACT

To elucidate the functional diversity of hot spring bacteria, 123 bacteria were isolated and screened for evaluating their multifunctional plant growth promoting (PGP) properties. The antagonistic activity against different phytopathogens showed the presence of a high amount of biocontrol bacteria in the hot springs. During screening for PGP properties, 61.0% isolates showed production of indole acetic acid and 23.6% showed inorganic phosphate solubilization qualitatively. For production of extracellular enzymes, it was found that 61.0% isolates produced lipase, 56.9% produced protease, and 43.9% produced cellulase. In extreme properties, half of the isolates showed tolerance to 5% NaCl (w/v) and 48.8% isolates survived heat shock at 70 °C. The identification of 12 multipotential bacteria based on 16S rRNA gene sequencing revealed that the bacteria belonged to *Aneurinibacillus aneurinilyticus* and *Bacillus* spp. Bacterization of tomato seeds showed that the hot spring bacteria promoted shoot height, fresh shoot weight, root length, and fresh root weight of tomato seedlings, with values ranging from 3.12% to 74.37%, 33.33% to 350.0%, 16.06% to 130.41%, and 36.36% to 318.18%, respectively, over the control. This research shows that multifunctional bacteria could be isolated from the hot springs. The outcome of this research may have a potential effect on crop production methodologies used in saline and arid environments.

1. Introduction

Bacteria are ubiquitous and highly diverse. Bacterial population gains different significant properties according to their habitat and surrounding environment. In Gujarat, some areas are well known for natural hot water resources. Well-known hot springs are located in Tuwa, and Unnai. Hot water springs are manifestations of geological activity and represent extreme environment. Extremophiles isolated from extreme environments have a tremendous role in the production of thermopile enzymes that have wide applications in pharmaceutical and other industries [1]. Rhizobacteria growing in the site of hot spring can produce different compounds and enzymes that may have a beneficial role in plant growth promotion and can be used as an alternative chemical for plant growth promotion [2].

In this study, the bacteria isolated from different hot springs located in the province of Gujarat, India, were screened for their (i) plant growth promoting (PGP) properties, (ii) biocontrol properties against different plant pathogens, (iii) extracellular enzymes production, (iv)

* Corresponding author.
E-mail address: na.amaresan@gmail.com (N. Amaresan).

http://dx.doi.org/10.1016/j.crvi.2017.02.005
1631-0691/

extreme properties such as salt tolerant and thermotolerant, and (v) identification of multipotential strains by 16S rDNA sequencing.

2. Materials and methods

2.1. Collection of samples and isolation of bacteria

Rhizosphere and nonrhizosphere soil samples from natural hot water springs were collected aseptically from Unnai (20°85′33″N; 73°33′42″E), and Tuwa (22°47′58″N; 73°27′37″E). The soil samples were collected in sterile polythene bags and transferred to a laboratory in cool boxes. The samples were kept in a cold room until use. One gram of the soil sample was suspended in 100 ml distilled water and then incubated in an orbital shaker with shaking at 200 rpm for 30 min. The mixtures were allowed to settle and serial dilutions up to 10^{-5} were prepared using sterile distilled water. Isolation of bacteria from this mixture was carried out with serial dilution technique in nutrient agar (HiMedia, Mumbai, India). Purification of bacteria was carried out by repeated streaking and checked for purity. The mixture was maintained at 4 °C until use.

2.2. Indole acetic acid production

Indole acetic acid (IAA) production by the isolates was detected qualitatively by following Sawar and Kremer [3]. Fresh cultures were streaked onto LB medium amended with L-tryptophan (5 μg/ml) and the plates were overlaid with sterile Whatman No. 1 filter paper, incubated at 28 ± 2 °C for 72 h. After incubation, the filter paper was soaked with 2–3 drops of *O*-phosphoric acid and Salkowski's reagent (1 ml of 0.5 M $FeCl_3$ in 50 ml of 35% $HClO_4$) was added. Production of IAA was immediately identified by formation of red colour on the filter paper.

2.3. Phosphate solubilization

All bacterial isolates were screened for inorganic phosphate solubilization [4]. A loopful of fresh bacterial culture was streaked onto Pikovaskaya's medium amended with inorganic phosphate and the plates were incubated at 28 ± 2 °C for 3–4 days. The formation of a clear halo around the bacterial colony indicated solubilization of mineral phosphate.

2.4. Antagonistic properties

The antagonistic ability was determined by the dual-culture technique, as described by Dennis and Webster [5]. Antagonistic activity of the bacterial isolates against tomato pathogens such as *Fusarium fuski*, *Sclerotium rolfsii*, *Sclerotinia sclerotiorum*, *Rhizoctonia batticaloa*, and *Rhizoctonia solani* was tested by dual culturing on PDA plates (HiMedia); 6-mm agar disks containing grown mycelia of any of the five phytopathogenic fungi were placed approximately 3.5 cm (distance) apart on a potato dextrose agar (PDA) plate. Four bacterial isolates were streaked between the agar disks. Inhibition of fungal mycelium around the bacterial colony was scored for 3–4 days by measuring the radial growth of the pathogen. The PDA plate inoculated only with pathogen was taken as control. Percent inhibition of pathogen growth was calculated by the following formula: $I = C - T/C \times 100$, where, I is the percent inhibition of growth, C the mycelial growth in the control plate, and T the mycelial growth in the test plate. All strains were tested in triplicates and tests were carried out twice for each isolate.

2.5. Production of extracellular enzymes

Isolates were analyzed for three enzymes (*i.e.*, protease, cellulase and lipase) by the plate method. Proteolytic activities of the cultures were qualitatively screened in a medium containing skimmed milk (HiMedia). Zones of precipitation of paracasein around the colonies appearing over the next 48 h were taken as evidence of proteolytic activity [6]. For cellulase activity, a mineral–salt agar plate containing 0.4% $(NH_4)_2SO_4$, 0.6% NaCl, 0.1% K_2HPO_4, 0.01% $MgSO_4$, 0.01% $CaCl_2$ with 0.5% carboxymethyl cellulose, and 2% agar (HiMedia) were surface-inoculated. An iodine solution was used to detect cellulase activity [7]. The clear zone formation around the growing colony was considered as positive. The lipase activity of the bacterial isolates was determined according to the diffusion agar methods, *i.e.* the nutrient agar medium was supplemented with $CaCl_2 \cdot H_2O$ 0.01%. Tween 80 sterilized for 20 min at 120 °C was added to the molten agar medium at 45 °C to give a final concentration of 1% [8]. The medium was shaken until Tween 80 dissolved completely and was then poured into Petri plates. The test is considered as positive if an opaque halo around the colonies occurs.

2.6. Screening of salt resistance

The resistance of the bacterial isolates to salinity was determined by observing the growth on the nutrient agar medium amended with 5% NaCl (w/v). The plates were incubated at room temperature for 48 h and the growth on the NaCl-amended media was recorded.

2.7. Screening of thermotolerance

All isolates were examined for their ability to tolerate heat stress. Bacteria were grown overnight in 100-ml nutrient broth on a shaker at 28 ± 2 °C. Subsamples (10 ml) of bacterial suspensions were transferred to test tubes. The bacterial cultures in each tube were then placed at different temperatures of 37, 45, 55, 60, 65, 70, 80, 90, and 100 °C for 1 h. After incubation, a small volume of the broth was spread on the nutrient agar and observed for growth in time intervals of 2–4 days.

2.8. Plant growth promotion ability of tomato plants under pot culture

The tomato seeds were sterilized with 70% ethanol for 2 min and in 2% sodium hypochlorite for 2 min, followed by washing 10 times in sterile water. Pure cultures of isolates were grown in the nutrient broth at room temperature.

Please cite this article in press as: K.S. Patel, et al., Characterization of culturable bacteria isolated from hot springs for plant growth promoting traits and effect on tomato (*Lycopersicon esculentum*) seedling, C. R. Biologies (2017), http://dx.doi.org/10.1016/j.crvi.2017.02.005