Biocatalysis and Agricultural Biotechnology 12 (2017) 85–89

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: www.elsevier.com/locate/bab

Isolation and characterization of drought resistance bacteria for plant growth promoting properties and their effect on chilli (*Capsicum annuum*) seedling under salt stress

Sanjay Patel, H.N. Jinal, N. Amaresan*

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

ARTICLE INFO

Keywords:
Salinity
Kutch
Bacillus spp
PGP properties
Desert

ABSTRACT

The search for efficient drought resistant bacteria from unexplored environments is worldwide to alleviate the negative effects in plant growth. Thus, 67 bacteria were isolated from *Commiphora wightii* rhizosphere soil collected in the desert region. Initial screening for drought tolerance revealed that 29 (43.0%) isolates were able to grow in the presence of 15 g NaCl (w/v), 40 (58.20%) isolates were shown thermo tolerance capacity up to 70 °C and 32 (47.8%) isolates were shown maximum tolerance for polyethylene glycol concentration (13 g/100 ml). A subset of 10 strains was identified based on 16 S rRNA gene sequencing and belonged to four genera (*Bacillus* spp, *Alcaligenes* spp, *Proteus* sp. and *Aneurinibacillus aneurinilyticus*). All strains could produce siderophore and showed indole-3-acetic acid production ranged from 120 to 520 µg/ml. The drought resistant bacterized seeds of chilli were evaluated in the pot soil supplemented with 50 mM NaCl, the isolates showed 23.3–114.6% and 44.2–125.9% higher root and shoot lengths, respectively, compared with control. Our results demonstrated that drought resistant bacteria isolated from the rhizosphere of *C. wightii* grown on desert lands could be used for alleviating salinity stress in crop plants.

1. Introduction

Dessert environment is characterized by high temperature, soil salinity, lack of rainfall and less nutrients. These factors are consider as a major limiting factors of dessert environment and give positive role in adaptation of dessert microorganisms (Martirosyan and Steinberger, 2014). Province of Gujarat, India covers an area of 4953.7 sq km desert named Rann of Kutch. It is located in 22°55" to 24°35" North latitudes and 70°30" to 71°45" East longitudes near the Great Rann of Kutch, Gujarat (Goswamia et al., 2014). In spite of its uniqueness, the diversity of rhizobacteria and its role in adaptation of plants under desert ecosystem is less studied. In arid condition plant growth promotion facilitated by plant growth promoting bacteria (PGPB). Drought resistance PGPB helps direct or indirect way in dessert farming. Many researchers have identified PGPB producing phytohormones such as auxins, cytokinins, gibberellins, and abscisic acid confer the drought resistance to host plant (Wang et al., 2014; Verma et al., 2016; Wani et al., 2016; Fan et al., 2016). For that reasons, the search for new PGPB with multiple traits becomes interesting and, they can be used as inoculants for biofertilization, phytostimulation and biocontrol purposes in desert farming (Kavamura et al., 2013).

Saline soils are known to suppress the growth of plants (Paul, 2012). Plants grown in saline soil are naturally colonized by microorganisms for their adaptation (Pooja and Kumar, 2015). In India, chilli (*Capsicum annuum*) is the major cultivated plant and its fruits are mainly consumed either fresh or dry. However, chilli is exposed to many biotic (virus, fungi) and abiotic stress, especially salinity, which has a negative effect on chilli growth and yield (Zhani et al., 2012). The aim of present study was development of dessert farming using drought resistance rhizobacteria. Rhizospheric microorganisms were isolated from rhizospheric soil of Kutch dessert area of Gujarat. Isolated samples were screened for their drought resistance and PGP properties. Positive isolates were characterized by molecular biological methods. In this way, the aim of this work was to identify and characterize novel bacteria from Kutch desert area of Gujarat exhibiting drought resistance and PGP properties. The plant growth promoting and drought tolerance enhancing ability of those isolates were evaluated on chilli seedlings.

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

http://dx.doi.org/10.1016/j.bcab.2017.09.002
Received 31 March 2017; Received in revised form 2 September 2017; Accepted 6 September 2017
Available online 08 September 2017
1878-8181/ © 2017 Elsevier Ltd. All rights reserved.

2. Materials and methods

2.1. Sampling and isolation of bacteria

Rhizospheric (*Commiphora wightii*) soil was collected in desert zones of the Kutch (22°55" to 24°35" N and 70°30" to 71°45" E), Gujarat. Physicochemical parameters of the soils were determined according to Sparks et al. (1996). For the isolation of bacteria, serially diluted samples (10^4) were spread on full strength nutrient agar and incubated at room temperature for 48 h. Based on colony morphology, the bacterial isolates were purified and maintained on 20% glycerol at −20 °C. All the subsequent experiments were conducted using fresh cultures.

2.2. In vitro assay for stress tolerance properties

2.2.1. Screening of salt tolerance

All bacterial isolates were screened for salt tolerance ability according to Amaresan et al. (2014). A loopful of fresh bacterial culture was streaked onto nutrient agar medium amended with various concentration of NaCl ((5–15 g/100 ml) and plates were incubated at 28 ± 2 °C for 3–4 days. A clear bacterial colony on the NaCl amended plates considered as positive to salt tolerance ability.

2.2.2. Screening of thermo tolerance

For this experiment, freshly grown bacterial cultures were placed at different temperatures (30–80 °C) for 1 h. After heat shock, a small volume of broth was spread on nutrient agar and observed for the growth in time intervals of 2–4 days of incubation.

2.2.3. Screening of polyethylene glycol (PEG) tolerance

The osmotic resistance of the bacterial isolates was performed by observing the growth on nutrient agar medium amended with various concentration of PEG 3000 (3–13 g/100 ml). The plates were incubated for 48 h at 28 ± 2 °C, and the growth on PEG amended media was recorded.

2.3. Characterization of bacteria for PGP traits

2.3.1. Indole 3-acetic acid production

The bacterial cultures were inoculated in nutrient broth amended with tryptophan (5 µg/ml), and incubated at 28 ± 2 °C for 5 days. After incubation, the cultures were centrifuged at 3000 rpm for 30 min. Two ml of the supernatant was mixed with two drops of orthophosphoric acid and 4 ml Salkowski's reagent (50 ml, 35% perchloric acid; 1 ml 0.5 $FeCl_3$). Development of pink color considered indole-3-acetic acid (IAA) production; the optical density (OD) was read at 530 nm using a spectrophotometer. The level of IAA produced was estimated from a standard IAA graph and expressed as µg/ml (Patten and Glick, 1996).

2.3.2. Phosphate solubilization

All bacterial isolates were screened for inorganic phosphate solubilization according to Verma et al. (2001). Pikovskaya's medium amended with inorganic phosphate was prepared and a loopful of fresh bacterial culture was streaked on to the plates. Plates were incubated at 28 ± 2 °C for 3–4 days. Solubilization of mineral phosphate was observed by a clear halo around the bacterial colony

2.3.3. Siderophore production

Bacterial culture (48 h old) was streaked on King's B amended with an indicator dye. The tertiary complex chrome azural S (CAS)/Fe3/ hexadecyl trimethyl ammonium bromide served as an indicator. Change of blue color of the medium surrounding the bacterial growth to fluorescent yellow indicated the production of siderophore. The reaction of each bacterial strain was scored either positive or negative to the assay (Schwyn and Neilands, 1987).

2.4. Identification of plant growth-promoting bacteria

Isolates were identified at species level, for this the pure cultures of potential isolates were sent to Gujarat State Biotechnological Mission (GSBTM), Gujarat for identification of bacteria at molecular level by sequencing of 16S rRNA gene using 704 F (5′ -GTA GCG GTG AAA TGC GTA GA- 3′) and 907 R (5′-CCGT CAA TTC MTT TRA GTT T- 3′). After obtaining the sequences that were subjected to the BLASTn search program to look for nucleotide sequence homology (Altschul et al., 1997) with NCBI, GenBank (http://www.ncbi.nlm.nih.gov). The gene sequences were submitted to GenBank and accession number was assigned (KT355722-26; KX168043- 44, KU745405, KX168045 and KX168029).

2.5. Plant growth promotion ability of stress resistant bacteria on chilli

The chilli seeds were sterilized with 70% ethanol for 2 min and in 2% sodium hypochlorite for 2 min, followed by washing ten times in sterile water. Seed bacterization was achieved by immersing surface sterilized chilli seeds in appropriate PGP bacteria suspension (2.1×10^8 CFU ml^{-1}) for 1 h, air-dried and sown immediately. Control treatment was achieved by mixing the seeds with sterilized saline solution (0.85%). The treatments with three replicates were investigated with two independent experiments. For pot experiments, pots were sterilized with 20% sodium hypochlorite solution and filled with sterile loam soil. The 15 chilli seeds were sown in plastic pots filled with 1 kg sterile loam soil. The pots were arranged in a completely randomized factorial design. The seedlings were grown in a glasshouse at a temperature of 30–32 °C and 58% relative humidity in a day–night cycle of 13–14 h natural light. Water-stressed seedlings (irrigated every three days with 50 mM NaCl and alternative days with normal water) and control seedlings were harvested one month after the emergence of seedlings, washed and morphological characteristics viz., root length, shoot length, dry and wet weight of stem and root of each plant was recorded.

2.6. Statistical analysis

IAA production data were statistically analyzed using analysis of variance (ANOVA) with subsequent Duncan's multiple range test (DMRT). Greenhouse experiments were conducted in a completely randomized design. Data were subjected to two-way ANOVA followed by a classification of means with DMRT in order to compare the treatments with the control (uninoculated plants). All statistical analysis was performed with SPSS 14.0. The differences at the 95% confidence level were considered to be significant.

3. Results and discussion

3.1. Isolation and characterization bacteria

A total of 67 bacterial isolates were isolated from rhizospheric (*Commiphora wightii*) soils collected in desert zones of Kutch, Gujarat. Chemical analysis of soil pH was found to be 7.03, electrical conductivity; 0.97 mS cm^{-1} and organic carbon; 59% (Table 1). Initially, all the isolates were subjected for their salt tolerant, thermo tolerant and osmotic stress tolerance properties. The results revealed that 29 (43.0%) isolates were able to grow in the presence of 15 g NaCl (w/v), 40 (58.20%) isolates were shown thermo tolerance capacity up to 70 °C and 32 (47.8%) isolates were shown maximum tolerance for PEG concentration (13 g/100 ml). Plants are constantly exposed to abiotic stresses such as salinity, drought, temperature, etc. leading to poor plant growth and yield loss (Sandhya et al., 2010). Drought being a major abiotic stress may cause huge productivity losses in arid and semiarid regions where the agriculture totally depends on rains (Minakshi et al., 2013). Management of abiotic stress gaining importance through rhizobacteria (Kavamura et al., 2013; Goswamia

Download English Version:

https://daneshyari.com/en/article/5520423

Download Persian Version:

https://daneshyari.com/article/5520423

Daneshyari.com