BRAZILIAN JOURNAL OF MICROBIOLOGY

http://www.bjmicrobiol.com.br/

Environmental Microbiology

Exploring plant growth-promotion actinomycetes from vermicompost and rhizosphere soil for yield enhancement in chickpea

M. Sreevidya[a,b], *S. Gopalakrishnan*[b,*], *H. Kudapa*[b], *R.K. Varshney*[b]

[a] *Centre for Biotechnology, Jawaharlal Nehru Technological University, Kukatpally 500072, Telangana, India*
[b] *International Crops Research Institute for the Semi-Arid Tropics, Patancheru 502324, Telangana, India*

ARTICLE INFO

Article history:
Received 19 April 2015
Accepted 17 August 2015

Associate Editor: Jerri Édson Zilli

Keywords:
Chickpea
Actinomycetes
PGP
Streptomyces
Biocontrol
Gene expression

ABSTRACT

The main objective of the present study was to isolate and characterize actinomycetes for their plant growth-promotion in chickpea. A total of 89 actinomycetes were screened for their antagonism against fungal pathogens of chickpea by dual culture and metabolite production assays. Four most promising actinomycetes were evaluated for their physiological and plant growth-promotion properties under *in vitro* and *in vivo* conditions. All the isolates exhibited good growth at temperatures from 20 °C to 40 °C, pH range of 7–11 and NaCl concentrations up to 8%. These were also found highly tolerant to Bavistin, slightly tolerant to Thiram and Captan (except VAI-7 and VAI-40) but susceptible to Benlate and Ridomil at field application levels and were found to produce siderophore, cellulase, lipase, protease, chitinase (except VAI-40), hydrocyanic acid (except VAI-7 and VAI-40), indole acetic acid and β-1,3-glucanase. When the four actinomycetes were evaluated for their plant growth-promotion properties under field conditions on chickpea, all exhibited increase in nodule number, shoot weight and yield. The actinomycetes treated plots enhanced total N, available P and organic C over the un-inoculated control. The scanning electron microscope studies exhibited extensive colonization by actinomycetes on the root surface of chickpea. The expression profiles for indole acetic acid, siderophore and β-1,3-glucanase genes exhibited up-regulation for all three traits and in all four isolates. The actinomycetes were identified as *Streptomyces* but different species in the 16S rDNA analysis. It was concluded that the selected actinomycetes have good plant growth-promotion and biocontrol potentials on chickpea.

* *Corresponding author.*
E-mail: s.gopalakrishnan@cgiar.org (S. Gopalakrishnan).
http://dx.doi.org/10.1016/j.bjm.2015.11.030

Introduction

Chickpea (*Cicer arietinum* L.) is the second most widely grown food legume crop after common bean, with annual production of 13.8 mt worldwide.[1] Globally, more than 90% of chickpea production occurs in the semi-arid tropics of Asia and Africa. Asia accounts for 88% of global chickpea production whereas India is the largest producer accounting for 75% of Asia's chickpea production.[2] Several biotic and abiotic factors were involved in low production of chickpea. The biotic factors include fungi, bacteria, viruses, nematodes, mycoplasma and insect pests. Fungi are the largest group affecting stems, roots, leaves, flowers and pods. Chickpea crop is mainly affected by Fusarium wilt, dry root rot, collar rot, Ascochyta blight and Botrytis gray mold caused by *Fusarium oxysporum* f. sp. *ciceri* (FOC), *Macrophomina phaseolina*, *Sclerotium rolfsii*, *Ascochyta rabiei* and *Botrytis cinerea*, respectively resulting in reduced crop yield.[3,4]

The microbes in rhizosphere help plants in growth-promotion and yield. Actinomycetes are one of the major components of rhizosphere microbial populations and are useful in soil nutrient cycling[5,6] as well as plant growth-promotion (PGP).[7] Actinomycetes produce secondary metabolites such as lytic enzymes, PGP substances and antibiotics.[8] The actinomycetes, mainly those belonging to *Streptomyces* spp., make up an important group of soil microbes. *Streptomyces* are abundant in soil and help in the degradation of complex molecules to simple molecules for plant growth and development.[9,10] These are also reported to decompose organic matter, promote plant growth and control plant pathogens.[11]

In the present study, actinomycetes isolated from rhizosphere and herbal vermicompost were characterized and evaluated for PGP properties and for biocontrol-related traits. The promising strains were also evaluated for their PGP in chickpea under field conditions.

Materials and methods

Actinomycetes isolation

Actinomycetes were isolated from herbal vermicompost (*Jatropha curcas*, *Annona squamosa*, *Parthenium hysterophorus*, *Gliricidia sepium* and *Azadirachta indica*) and chickpea rhizosphere soils. Ten grams of each vermicompost and rhizosphere soils were suspended in 90 mL of sterile physiological saline (0.85% NaCl in distilled water) in a bottle and kept for shaking on an orbital shaker (at 100 rpm) at 28 ± 2 °C for 1 h. At the end of shaking, the samples were serially diluted up to 10^5 dilutions and samples from 10^4 and 10^5 dilutions were spread plated (0.1 mL) on actinomycetes isolation (AIA) agar (HiMedia Laboratories, Mumbai, India) and incubated at 28 ± 2 °C for 7 days. Prominent colonies were isolated and stored on AI agar slants.

Selection of antagonistic actinomycetes against fungal pathogens of chickpea

A total of 89 actinomycetes were screened for their antagonistic activity against *S. rolfsii*, *M. phaseolina* (three strains *viz.* MP-6, MP-24 and MP-115) and FOC (acquired from legumes pathology, ICRISAT, Patancheru) by dual culture assay as per the protocols of Gopalakrishnan et al.[12] on glucose casaminoacid yeast extract agar plates. The culture filtrates of the promising isolates, based on the dual culture assay, were extracted by partitioning against ethyl acetate (EtOAc) and the resultant organic (EtOAc) and aqueous fractions were evaporated on a rotary evaporator and collected in a minimal volume of methanol. Both the fractions were evaluated for their antagonistic potential against the three fungal pathogens of chickpea (*M. phaseolina*, FOC and *S. rolfsii*). For this, a fungal disc of 6 mm diameter was bored and kept at center of the potato dextrose agar plate amended with either organic or aqueous fractions (at a concentration of 0.5%). Control plates contained only methanol. The plates were incubated at 28 ± 2 °C for 5 days and inhibition of the pathogen was recorded for both dual culture and metabolite production assays on a scale of 0–4 as follows: 0 = no inhibition; 1 = slight inhibition; 2 = moderate inhibition; 3 = good inhibition and 4 = excellent inhibition.

Colony morphology of selected actinomycetes

Selected isolates of actinomycetes were streaked on AI agar by quadrant streaking technique and incubated for 5 days at 28 ± 2 °C. At the end of incubation, the isolated colonies were observed for their morphology. Gram staining was also performed[13] and observed under light microscope.

Molecular identification of the selected actinomycetes

The selected actinomycetes were sent to Macrogen Inc., Seoul, Korea for identification by 16S rDNA analysis. The sequences obtained from Macrogen were compared with similar sequences from GenBank, compared using the BLAST program,[14] aligned using the Clustal W software[15] and the dendrogram inferred by neighbor-joining method.[16] Bootstrap analysis was performed using the MEGA version 4 program to estimate the statistical stability of the branches in cluster with 1000 replications. The sequences (1460 bp for SAI-13, 1474 bp for SAI-291,475 bp for VAI-7 and 1472 bp for VAI-40) were submitted to NCBI and accession numbers obtained.

In vitro evaluation of actinomycetes for their physiological traits and fungicide tolerance

Physiological properties such as tolerance to pH, temperature, salinity and fungicides were studied for the selected actinomycetes. The actinomycetes were streaked on Bennett's agar (HiMedia Laboratories, Mumbai, India), adjusted to different pH (5, 7, 9 and 11) and saline concentrations (0–12% NaCl at the interval of 2%) and incubated at 28 ± 2 °C for 5 days. For temperature, the Bennett's agar plates were streaked with the actinomycetes and incubated at different temperatures (20 °C, 30 °C, and 40 °C) for 5 days. For test at 50 °C, the isolates were inoculated in Bennett's broth and incubated at 50 °C. The fungicide tolerance was evaluated as per the protocols of Gopalakrishnan et al.[17] The actinomycetes were streaked on AI agar plates amended with fungicides Bavistin, Thiram, Benlate, Captan and Ridomil at field application levels (2500, 3000, 4000, 3000 and 3000 ppm) and incubated at 28 ± 2 °C for