Biocatalysis and Agricultural Biotechnology 11 (2017) 312–321

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

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

Isolation, characterization and inter-relationship of phosphate solubilizing bacteria from the rhizosphere of sugarcane and rice

Muhammad Awais[a,b], Muhammad Tariq[a], Arfan Ali[a,b,*], Qurban Ali[a], Anwar Khan[a], Bushra Tabassum[a], Idrees Ahmad Nasir[a], Tayyab Husnain[a]

[a] Centre of Excellence in Molecular Biology, University of the Punjab, Lahore 53700, Pakistan
[b] Institute of Molecular Biology and Biotechnology, University of Lahore, Lahore, Pakistan

ARTICLE INFO

Keywords:
Phosphate solubilizing bacteria
16S rRNA
Antagonistic activity

ABSTRACT

Phosphate solubilizing bacteria (PSB) have the ability to solubilize insoluble phosphorus to make it available for plant roots to be absorbed. In this study, isolation of rhizobacteria and screening of their ability for phosphate solubilization, production of indole acetic acid (IAA), antagonistic activity against fungal pathogen and intrinsic antibiotic resistance was studied. In total, 15 isolates of PSB were found gram negative with rod shaped cells. Different levels of antibiotic resistance was seen against four antibiotics (Ampicillin, Kanamycin, Tetracycline and Streptomycin 25, 30, 30 and 10 μg/ml respectively) by Rhizobacterial isolates (Seven isolates were found to show antifungal activity against *Fusarium oxysporum*). All PSB isolates were found to solubilize insoluble phosphorous $Ca_3(PO_4)$ and produce IAA. Two PSB isolates sequences were found novel and were submitted in NCBI database. Conclusively, combine application of rhizobacterial isolates, Sugarcane (SC-22) in addition with antifungal agent can prove to be an excellent combination to solubilize insoluble phosphorus and to act as antifungal remedy. Further this combination can be eco-friendly and prove to be cost effective strategy to improve crop production.

1. Introduction

Phosphorus is considered to be one of the essential macro-element required for growth and development of plant (Kumar et al., 2010). Plants obtain phosphorus from the soil solution. However, due to low solubility and fixation in soils, only a small fraction of phosphorus exists in soil solution (1 ppm or 0.1%), which is readily available to plants. The roots take up several forms of phosphorus, out of which the greatest part is absorbed in the forms of $H_2PO_4^-$ and HPO_4^{2-} depending upon soil pH (Mahidi et al., 2011). Certain bacteria called PSB have the ability to solubilize insoluble phosphorus and make it available to the plant roots for absorption. PSB are ubiquitous whose numbers vary from soil to soil. PSB constitute 1–50% of the total respective population in soil (Chen et al., 2006). They are predominately concentrated in the rhizosphere, where they are known to be metabolically more active than those isolated from other sources (Vessey, 2003). Evidence of naturally occurring rhizospheric PSB dates back to 1903 (Kiani et al., 2013). Strains from bacterial genera *Pseudomonas*, *Bacillus*, *Rhizobium*and *Enterobacter* are the most powerful phosphate solubilizer (Fu et al., 2016; Wakelin et al., 2004; Zaidi et al., 2009). The major phosphate solubilizing mechanism involves the production of organic acids accompanied by acidification of the medium. The organic acids convert tricalcium phosphate to di- and mono- basic phosphates with the net result of an enhanced availability of the element to the plant. The type of organic acids produced and their amounts differ with different organisms. Among them, Gluconic acid and 2-ketogluconic acid seems to be the most frequent agents of mineral phosphate solubilization. Other organic acids, such as acetic, citric, lactic, propionic, glycolic, oxalic, malonic, succinic acid, fumaric, tartaric acids have also been implicated in phosphate solubilization (Ahmad and Sahab, 2011). Indoleacetic acid (IAA) is a molecule that is synthesized by plants and a few microbes. In plants, IAA plays a key role in both root and shoot development. The hormone moves from one part of the plant to another by a designated importer (AUX1) and efflux pumps (PIN1–7). Plant Growth promoting hormone (PGPR) help plants to synthesize this molecule for better growth and root developmentsSo this also a parameter to check wether PGPR are working or not (Sheikhian and Bina, 2016).

Global crop production is facing a serious threat of food security and stability because of the current yield losses caused by pathogenic microbs. Today's intensified agricultural practices utilize huge amounts of

* Corresponding author at: Centre of Excellence in Molecular Biology, University of the Punjab, Lahore 53700, Pakistan.
E-mail addresses: m.awais_virk@hotmail.com (M. Awais), seetariq2000@yahoo.com (M. Tariq), arfan.ali@cemb.edu.pk (A. Ali), Saim1692@gmail.com (Q. Ali), bushra_cemb@gmail.com (B. Tabassum), dr.idrees@gmail.com (I.A. Nasir), husnaint@yahoo.com (T. Husnain).

http://dx.doi.org/10.1016/j.bcab.2017.07.018
Received 7 September 2015; Received in revised form 27 July 2017; Accepted 28 July 2017
Available online 29 July 2017

Table 1
Soil analysis of rhizospheric samples taken from the rhizosphere of sugarcane and rice.

S. no.	Location	Depth (in.)	EC (mS cm^{-1})	pH	Organic matter (%)	Available phosphorus (mg kg-1)	Available potassium (mg kg-1)	Saturation (%)	Texture
1	Gujranwala	0–12	3.50	8.3	0.63	2.80	93	36	Loam
2	Gujranwala	0–12	2.73	8.4	0.70	3.33	100	49.33	Loam
3	Shaikhupura	0–12	1.83	7.9	0.86	4.53	87.33	52	Clay
4	Shaikhupura	0–12	1.47	7.7	0.96	5.57	119.33	52	Clay
5	Gujranwala	0–12	3.23	7.6	0.84	9.30	105	52	Clay
6	Lahore	0–12	1.80	7.7	1.05	5.30	129	52	Clay
7	Lahore	0–12	3.20	7.6	0.90	12.04	111	52	Clay
8	Lahore	0–12	1.60	7.8	0.80	5.70	90	52	Clay

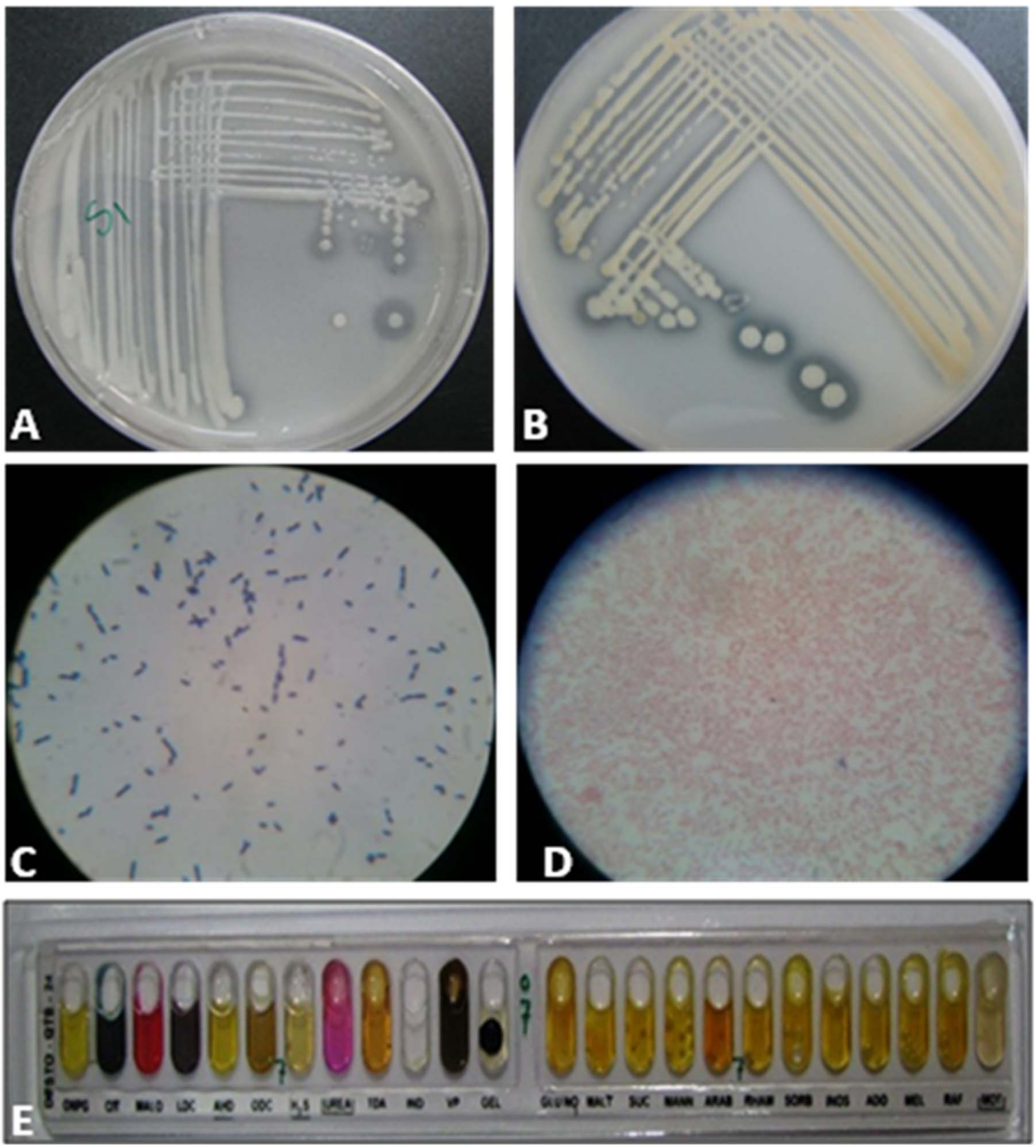

Fig. 1. Biochemical Identification of Rhizobacterial isolates. Purified PSB isolates identified on the basis of their (A, B): capability of forming clear zones of phosphate solubilization around colonies on PVK/NBRIP agar media, (C,D): Gram staining differentiating Rod shaped cells and round shaped cells, (E): QTS-24 for identification of phosphate solubilizing rhizobacterial isolates.