Research Article
ISSN:2249-1236
CODEN: IJRRBI
V, Haritha *et al.*, *Int*. J. Res. Rev. Pharm. Appl. Sci.*, (2024) 14(1) 008-024
International Journal of Research and Reviews in Pharmacy and Applied Sciences

Isolation of Bacteria from Rhizosphere of Custard Apple and Studies on Bioreduction of Chromium

V Haritha,
Department of Microbiology, University college of Sciences,Osmania University,Amberpet-500007 , Hyderabad, Telangana,India.

Corresponding author

V.Haritha

Abstract:

Plant Growth-Promoting Rhizobacteria (PGPR) live in the rhizosphere - a vast place for microbes to inhabit - but they are the only kind of bacteria that can pass on benefits to host plants like growth promotion and disease suppression. Apart from the bacteria that are known for promoting plant growth which can suppress the pathogens, the PGPR restricts the negative impact of the pathogens in different ways. Besides, it is not always the case that a cumulative or synergistic effect is produced by the application of a bacterial consortium. In this review, the PGPR disease suppressive mechanisms are re-visited and the diversity of function, stability, or both among PGPR taxa regarding these mechanisms are presented. Furthermore, we demonstrate that PGPR mixture applications, rather than single PGPRs, are good for crop protection against many diseases and emphasize the main factors for the successful application of PGPR mixtures.

Keywords: Rhizosphere, PGPR, Pathogen

Research Article
ISSN:2249-1236
CODEN: IJRRBI
V, Haritha *et al.*, *Int*. J. Res. Rev. Pharm. Appl. Sci.*, (2024) 14(1) 008-024
International Journal of Research and Reviews in Pharmacy and Applied Sciences

Introduction:

Plant growth-promoting rhizobacteria (PGPR), due to the diversity of their reps, are distributed in many plant species and function by empowering the plant's stress tolerance and enhancing its growth. Nevertheless, scientists have proven through numerous publications that PGPR is an alternative to chemical fertilizers and fungicides or a biological agent against pathogens. Furthermore, P. Pseudomonas, Bacillus, Azospirillum, and Streptomyces are some of the most well-researched biocontrol bacteria. The "mechanisms of action" that have been suggested as the reason for the development of this defense can be direct competition for nutrients and niches, antibiosis, enzyme lysis, signal interference, and indirect induction of host resistance.

Researchers from across the world are focusing on sustainable agricultural practices such as the use of slow-release fertilizers, microbial inoculants, PGPB (endophytes or epiphytes), and especially rhizobacteria. The microbiome of plants is the immune system of plants and phytopathogen exclusion is the proof of it. The idea is, that microbial community transplantation would serve as the best tool for the use of plant-related microbial community complexity to improve plant health, thus, the transfer of these species would provide unique insights into the skills of species sets (Jousset and Lee 2022). Plant growth-promoting bacteria produce auxins, gibberellins, cytokinins, mineral solubilization (phosphorous, potassium, zinc), iron chelation, antagonistic activities, enzyme synthesis, and ACC deaminase synthesis that lead to plant growth (Dutta S and Podile 2010, Mir et al. 2021).

Biosurfactants are molecules or chemical compounds Prepared by microorganisms. These are amphiphilic molecules consisting of both hydrophilic and hydrophobic moiety (The hydrophilic moiety can be a carbohydrate amino acid, a phosphate group, or some other compounds; The hydrophobic moiety is mostly long carbon chain fatty acid) that partition preferentially at the interface between fluid phases having different degrees of polarities and hydrogen bonding eg. interfaces between oil and water or air and water (Benincasa et al., 2001; Bodour et al., 2003).

Objectives of work:

Screening methods of biosurfactant produced from bacterial cultures
Characterization of biosurfactants (TLC,) and applications (Haemolytic assay, antifungal activity, antioxidant activity, plant growth promotion, Protein estimation, DPCZ method for chromium-reduction).

Material Method:

Separation of bacteria from custard apple soil sample
The bacteria strains are collected from the custard apple soil sample by serial dilution method in the laboratory. These isolates are used for the characterization of PGP and Biosurfactant production and chromium reduction activity by the DPCZ method.

Preliminary Evaluation of Biosurfactant Synthesis:

Biosurfactant production by Bacterial isolates was studied in nutrient broth media with various glucose concentrations (1% w/v) and was studied in nutrient broth amended with 1% glucose as a carbon source. For this, 200 ml. of media was taken in three 500 ml. conical flasks separately and inoculated with 3 ml, of activated bacterial culture, and incubated at 37 °C on an orbital shaker (120

rpm) for 72 h. OD was calculated every 24 hours, and the bacterial growth was evaluated. The culture is centrifuged at 10,000 rpm for 15 minutes, and the cell-free supernatant is collected into a flask The obtained supernatant is stored at 4°C for future screening techniques.Screening of bacterial isolates for biosurfactant production.

Microplate Assay:
CFS (100 µL) of each Bacterial strain was transferred into a separate 96-well microplate and placed on graph paper. The formation of concave surface distortion of grid lines on graph paper was compared with the negative control (water) and positive control (DMSO), where the concave distortion similar to DMSO indicates the presence of active molecule biosurfactant (Vaux and Cottingham, 2007)

Oil Displacement Activity:
Oil displacement of both strains of Stenotrophomonas sp. was carried out based on the method described by Morikawa et al. (1993) with slight modifications. 50 ml. of distilled water was taken in a Petri plate and overlaid with 100µl of crude oil. To the center of the crude oil layer, 50 µL. of cell-free supernatant (CFS) was added and observed for the formation of clear due to displacement of oil. The diameter of the clear zone on the oil layer appears due to the activity of biosurfactant activity.

Hemolytic Activity:
Two strains of bacterial isolates were screened for hemolysis by spot inoculation of actively grown bacterial strains on blood agar plates containing 5% v/v of blood and incubated at 37 C for 48 h.° Lysis of blood cells around the colonies due to the rupture of the cell membrane indicates surface active molecules (Carrillo et al., 1996).

Phenol-sulfuric test:
This test was carried out by Ellaiah et al, using the Dubois et al. approach. I end CYS was mixed with 1 ml of phenol solution (5%, W/V), and 2-5 ml of conc. H, SO, was added drop by drop until the distinctive orange color appeared the mixture was shaken and left at room temperature for 10 minutes. The presence of glycolipids is shown by the development of orange color.

Protein estimation:
The BCA protein detection pack was used to determine the overall unbound protein quantity (in milligrams) containing subtilisin, utilizing conventional bovine serum albumin (BSA) as a baseline. The examination was executed in threefold followed by outcomes offered in the form of mean concentration (mg/mL) ± variance by the standard deviation (Bagewadi et al., 2017, Shettar et al., 2023a)Characterization of biosurfactant from two potential strains of Bacterial isolates

Extraction of crude sample:
The precipitated biosurfactant was recovered by centrifugation at 8,000 rpm, for 10 min. Detection of biosurfactant by TLC
A qualitative analysis of biosurfactants was carried out by TLC. The stationary phase used in this study was silica gel 60-120 mesh size (2 mm), and the solvent system consisted of chloroform methanol-water (70:10:0.5). Various color-developing reagents, such as ninhydrin 8.2% in ethanol for lipopeptide with red-pinkish spots, for glycolipid with brown spots, and iodine vapors for lipid,

were used to visualize the type of biosurfactant. The crude sample was dissolved with methanol and loaded on the TLC plate at 1 cm above the edge of the plate, and the plate was placed in the solvent, where precautions were taken for not immerse the sample in the solvent. After the solvent reached 3/4th, the plate was marked for the solvent front, and ninhydrin was sprayed and left to dry in a hot air oven for about 15 minutes. Observation was done by spot formation, and the RF value was calculated as

Retention Factor=Distance traveled by solute / Distance traveled by solvent font color in the supernatant.

Screening of Bacterial isolates for PGP traits:

Actively grown Stenotrophomonas strains were used to screen PGP traits. They were prepared by inoculation of the single isolated colony of each Stenotrophomonas strain into the sterilized nutrient broth and incubated for 24h at 37°C for epiphyte and 30°C for endophyte on a shaking incubator.

Indole acetic acid (IAA) production:

The IAA was qualitatively screened by inoculating actively grown Stenotrophomonas strains as a spot-on nutritional agar medium enriched with 5mM L-tryptophan and incubating for 48 hours at 37°C. Following incubation, the colonies were covered with Whatman no. I filter paper discs (5mm) soaked in Salkowski reagent. The formation of pink color indicates the LAA is being produced (Gordon and Weber, 1951).

Ammonia production:

Ammonia production by two Stenotrophomonas strains was screened in peptone water and cultured for 48 hours at 37°C in a shaker incubator. Ammonium accumulation was examined by adding 0.1 ml. Nessler's reagent and looking for the formation of a yellow-browncolor (Kumar et

Solubilization of phosphate:

Bacterial cultures were inoculated on NBRIP (National Botanical Research Institute's Phosphate growth) medium (Nautiyal, 1999) containing tricalcium phosphate as insoluble phosphate. plates were incubated at 37°C for 72 hours. The formation of a transparent halo zone around colonies was regarded as a beneficial result.

Solubilisation of Zinc:

Bacterial cultures were inoculated as spots on the ZSB medium using 0.1 g of zinc oxide as an insoluble zinc source. Plates were incubated at 37°C for 72 hours. The formation of a transparent halo zone around colonies was regarded as a beneficial result.

Antioxidant-ABTS (2,2'-azino-bis-3-ethylbenthiazoline-6-sulphonic acid

Assay Components:

ABTS solution: For 7 mM ABTS dissolve 0.36g of ABTS in 100ml of double distilled water. Potassium persulphate solution: For 140 mM potassium persulphate dissolve 0.066g of salt in 100ml of distilled water.

ABTS reagent preparation and experiment: ABTS radical solution was prepared by gently mixing 5ml of 7mM ABTS solution and 88µl of 140mM potassium persulphate solution. This solution was allowed to stand in the amber flask at room temperature for 12-16 hr. During this, the ABTS radical will be generated and the reagent could be usable for 15 days. This ABTS radical cation stock solution was diluted in water (1:50) to an absorbance of 0.70±0.02 at 720 nm. Different concentrations of methanolic extract along with ABTS radical solution were pipetted into a 96well microplate and the reagents were mixed by shaking the plate and allowed to stand at 37°C for 6min. The absorbance was read at 720nm and subtracted from solvent block absorbance.

Research Article
ISSN:2249-1236
CODEN: IJRRBI
V, Haritha *et al.*, *Int*. J. Res. Rev. Pharm. Appl. Sci.*, (2024) 14(1) 008-024
International Journal of Research and Reviews in Pharmacy and Applied Sciences

Quantification was performed using a Trolox calibration curve. Assessment of plant growth by tube method using bacterial treatments

Plant growth of green gram was analyzed by paper towel method. Seeds were washed thoroughly with sterile distilled water and surface sterilized as described in the previous step. Surface sterilized seeds were treated 24hr actively growing bacterial cultures for 2 h. Untreated seeds were taken as control. All the seeds with respective treatments and control were sowed in the soft agar (0.8% agar) in duplicates incubated for 10 days in room temperature. Plant growth studies were performed in aseptic conditions, at 25°C for 10 days, and growth parameters (shoot and root lengths), are compared with the control.

Chromium Reduction:

The sample was diluted appropriately and inoculated on Nutrient broth (3 peptone, 5 Beef extract, 5 g peptone in 1 l, pH 7.2) tubes having different concentrations of Cr (0.1to0.7 gr/100ml) as K2Cr2o7 In agar plates 0.1 to 0.8 gm/100ml chromium is added asK2Cr2O7 as Cr(VI). The colonies that could tolerate 2 mM Cr were selected randomly and assessed for their Cr(VI) removal ability. The isolate that showed significant Cr (VI) removal was selected for further experiment in this study and the isolate was designated as KUCr3. The isolate was then purified to have a pure culture by cycles of single colony isolation and maintained on solid agar plates supplemented with 2 mM Cr (VI). Two bacteria were eventually chosen for a further experiment based on their Cr(VI) removal ability and were being maintained. The minimum inhibitory concentrations (MIC) of Cr (VI) to this strain were measured both in nutrient agar media supplemented with higher concentrations of Cr. Chromium (VI) analysisChromate removal was measured as the decrease of chromate with the time using Cr (VI) specific colorimetric reagent Diphenyl carbazide (DPCZ). About 1 ml of 0.05% DPCZ (w/v in acetone) was added to 1 ml of culture supernatant and additionally, 3 ml of 0.16 M sulfuric acid was poured to minimize the deterioration (Urone1955). A purple complex was formed due to the reaction of DPCZ with chromate after incubation colour changed into pinkish violet. The absorbance was taken immediately at 540 nm in a spectrophotometer (Cecil CE7200, England). The quantity of Cr
(VI) was measured by obtaining the calibration curve using K2CrO4 solution as standard. Growth and chromate removal in nutrient broth. The effect of Cr (VI) on cell growth under aerobic conditions. Effects of initial inoculum concentration on chromate removal To assess the effect of initial inoculum load on chromate removal young cell suspension of KUCr3 (finally to have *4.2, 5.1, and 6.0 log CFU ml-1 separately) was inoculated to nutrient broth supplemented with 2 mM Cr (VI) as K2CrO4 and incubated on a rotary shaker at 37˚C. The chromate removal was measured at an interval of 24 h, and 48 by measuring the residual Cr (VI) in the cell-free supernatant following centrifugation.

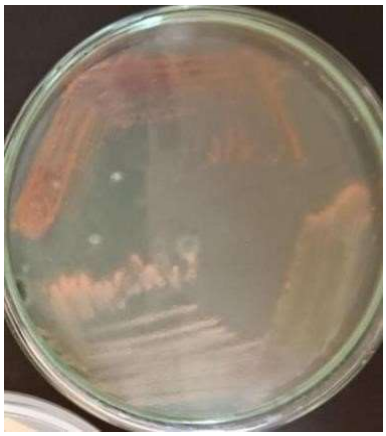
Results:

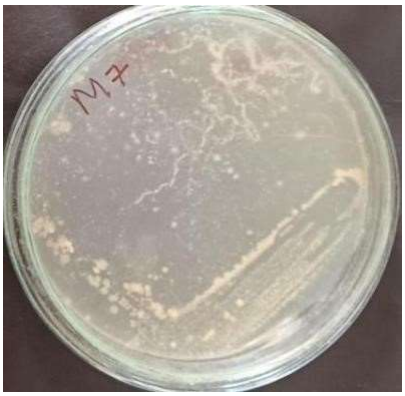
Cultural characteristics

Colony characteristics for the bacterial isolates

Size	Moderate
Shape	Round
Color	Creamy white
Texture	Smooth
Elevation	Convex
Margine	Even

Opacity	Opaque

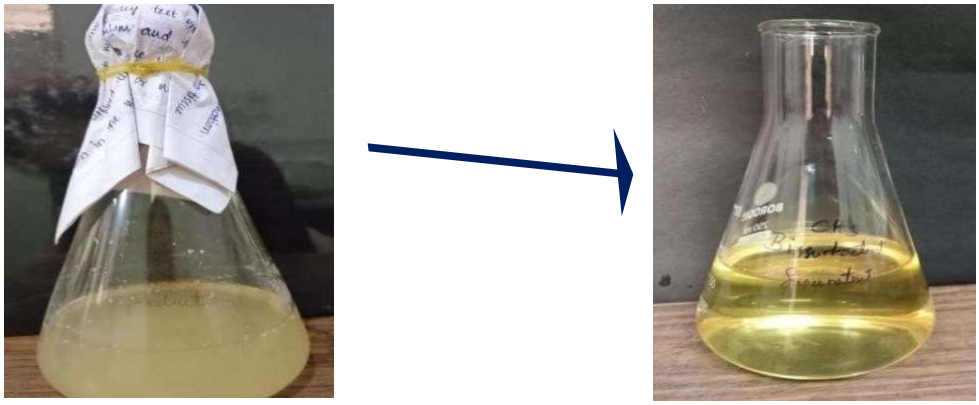




Research Article
ISSN:2249-1236
CODEN: IJRRBI
V, Haritha *et al., Int*. J. Res. Rev. Pharm. Appl. Sci., *(2024) 14(1) 008-024*
International Journal of Research and Reviews in Pharmacy and Applied Sciences

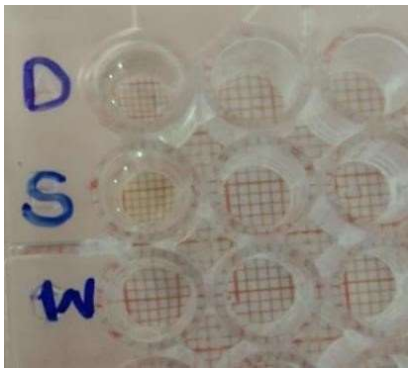
Biosurfactant production by bacterial isolates was studied in Nutrient Broth media with glucose concentration (1%, w/v). Growth for the Bacteria is seen better in Nutrient Broth containing 1% w/v Glucose, growth in 1% glucose is found more predominant.

Production Of Biosurfactant From Two Potential:
Bacterial Isolates:



Biosurfactant production flask **Supernatant**

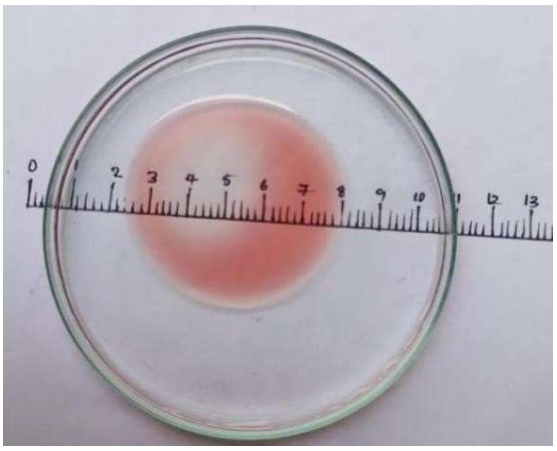
Microplate Assay:
The presence of Biosurfactant in the supernatant of bacterial strains was indicated by a change in the surface to concave resulting in a diverging lens, which is compared with the Negative control (Water), and positive control (Dimethyl sulphoxide). Both the strains showed a concave surface indicating the presence of biosurfactant.



Microplate Assay (W=Water, S=Sample, D=DMSO

Research Article
ISSN:2249-1236
CODEN: IJRRBI
V, Haritha *et al.*, *Int*. J. Res. Rev. Pharm. Appl. Sci.*, (2024) 14(1) 008-024
International Journal of Research and Reviews in Pharmacy and Applied Sciences

The bacterial isolates show oil displacement activity. Where csb1 shown 2 cm of oil displacement activity.

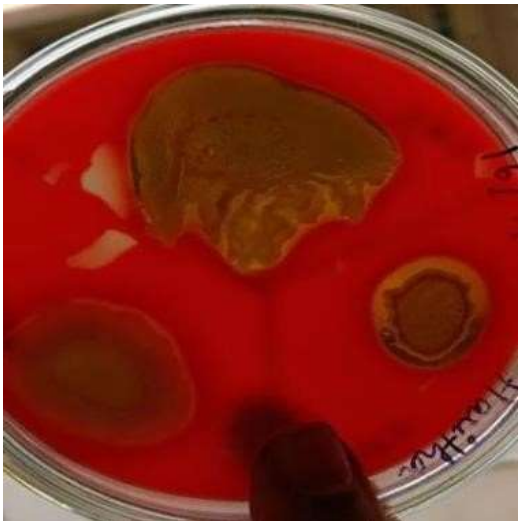


Csb1 showing 2cm of oil displacement activity

Phenol-sulfuric test:
All the bacterial isolates showed an orange colour which indicates, the biosurfactant sugars.

Research Article
ISSN:2249-1236
CODEN: IJRRBI
V, Haritha *et al.*, *Int*. J. Res. Rev. Pharm. Appl. Sci.*, (2024) 14(1) 008-024
International Journal of Research and Reviews in Pharmacy and Applied Sciences

Hemolysis Activity:
Bacterial isolates showing gamma and alpha hemolysis on blood agar after 48hrs

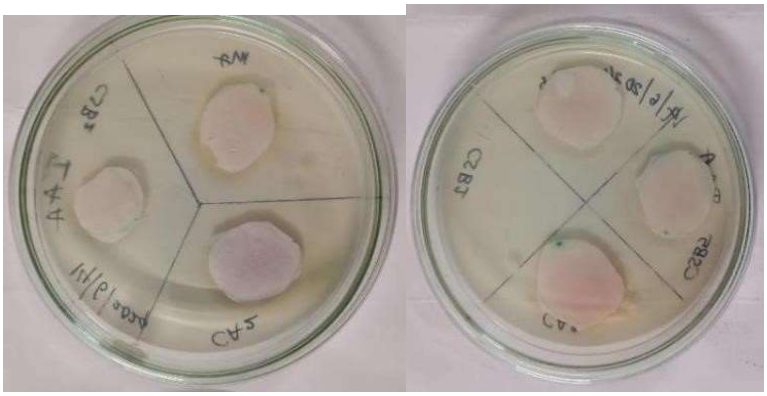


Detection of biosurfactant by TLC:
Qualitative analysis of partially purified biosurfactant produced by CA3Bacterial isolate was performed by TLC. On spraying the silica plates with ninhydrin reagent, purple colored spot was developed which is a characteristic feature of the protein unit. Rf value was calculated and found to be

CA3 bacterial isolate

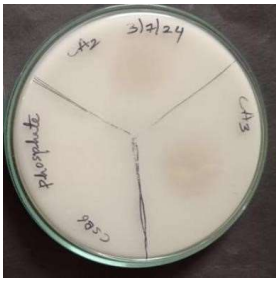
Research Article
ISSN:2249-1236
CODEN: IJRRBI
V, Haritha *et al.*, *Int*. J. Res. Rev. Pharm. Appl. Sci.*, (2024) 14(1) 008-024
International Journal of Research and Reviews in Pharmacy and Applied Sciences

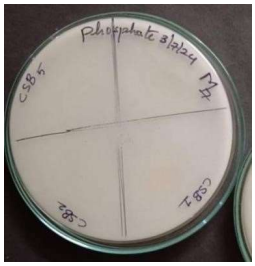
IAA Production
All the bacterial isolates showed positive for IAA production on plates which was indicated by the formation of a pink colour on the paper disc which was pre-soaked with the Salkowski reagent.



Ammonium production:
All the bacterial strains showed positive results for ammonium production by forming a yellow-orange colour upon Nessler's reagent.

Solubilization of phosphate:
No bacterial isolates showed phosphate solubilization on the NBRIP medium by the formation of the hallow zone around the colon





Research Article
ISSN:2249-1236
CODEN: IJRRBI
V, Haritha *et al., Int*. J. Res. Rev. Pharm. Appl. Sci.*, (2024) 14(1) 008-024*
International Journal of Research and Reviews in Pharmacy and Applied Sciences

Antifungal Activity

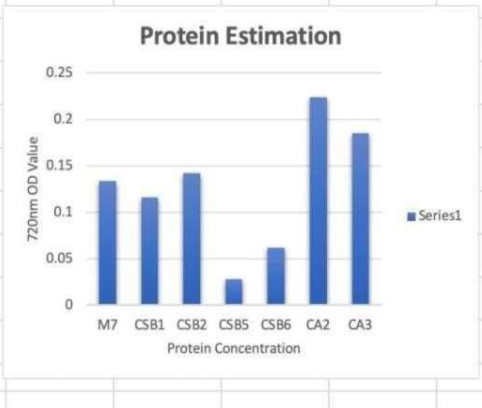
All the bacterial cultures showed no antifungal activity, csb 6 showed maximum antifungal activity against *Fusarium.*

Fusarium *A. flavus* *Macrophomina*

Rhizoctonia *Rhizoctonia*

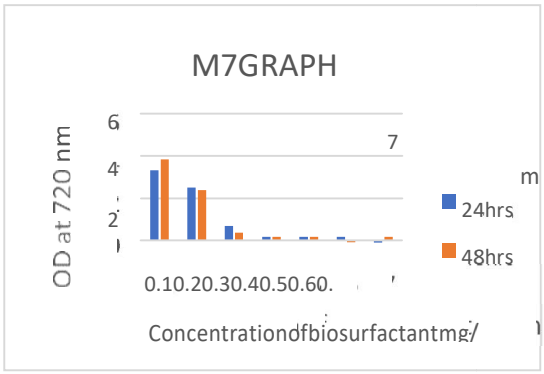
Antioxidant ABTS (2,2-azino-bis-3-ethylbenthiazoline-6-sulphonic acid) Assay Protein Estimation

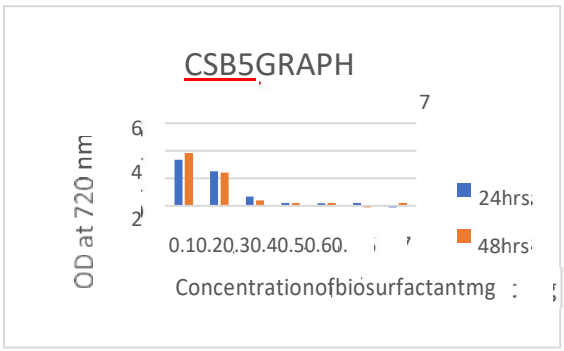
Protein Concentration	720nm OD Value
M7	0.134
CSB1	0.116
CSB2	0.142
CSB5	0.028
CSB6	0.062
CA2	0.224
CA3	0.185

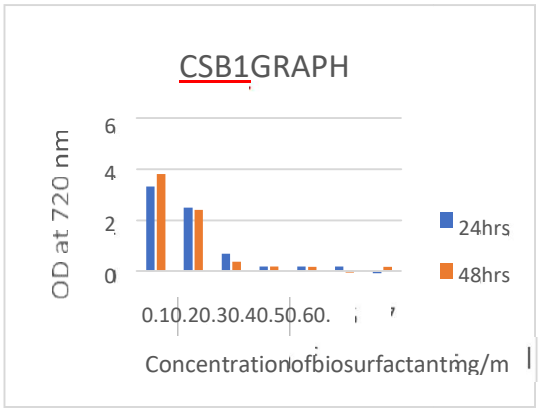


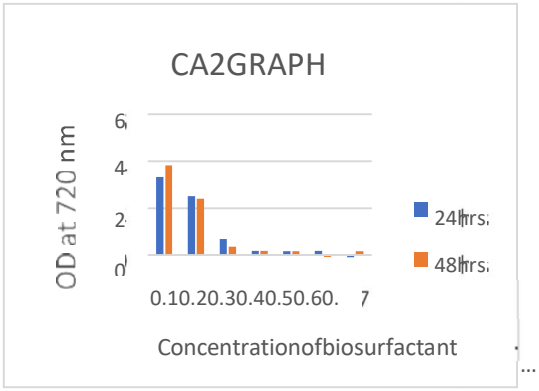
Bio reduction of 100 ppm Cr iv by selected isolates.

Research Article
ISSN:2249-1236
CODEN: IJRRBI
V, Haritha *et al.*, *Int*. J. Res. Rev. Pharm. Appl. Sci., *(2024) 14(1) 008-024*
International Journal of Research and Reviews in Pharmacy and Applied Sciences



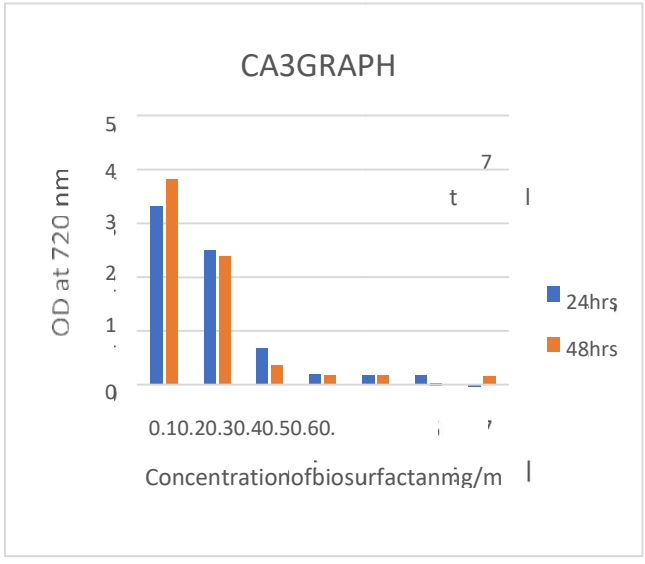






Assessment of plant growth by Tube method using bacterial treatment.

The seed which is with bacterial isolates of M7 and CA3 showed better growth when compared with the control.




M7, control CA3, control

Seeds that are treated with bacterial isolates of M7 and CA3 are grown on soft agar embedded with 0.4,0.5gm/100ml of chromium (VI) K2Cr207.

Bio reduction of 100 ppm Cr iv by selected isolates


M7, control CA3, contr

Discussion:

All the bacterial isolates demonstrated notable PGP traits except bacterial isolates M7, CSB1, CSB2, CSB5, CSB6, CA2, and CA3 exhibited better antagonistic activity and lytic enzyme syntheses; cumulative effects crafted seven of ten bacterial isolates as remarkable and potential PGPB. Previous researchers demonstrated that PGPBs improve plant growth and yield, as they aid plants by providing available nutrients from the environment and protecting them from various biotic and abiotic stresses (Tan et al. 2017, Li et al. 2017, Mir et al. 2021). The PGPB resides in the roots and rhizosphere and metabolizes tryptophan, which is exuded excessively from plants and these bacteria synthesize secondary metabolites, phytohormones like auxin, and IAA.

These bacteria significantly help in the availability of phosphorus, an essential macronutrient required by the plant. The plant may benefit from retaining a certain PGPB population in a particular niche as it aids in competing for nutrient availability and pathogen stress elimination (Banik et al. 2016). Bacteria are able to synthesize HCN i.e., 30% of isolated bacteria compete on plants' behalf with phytopathogens and iron deficiency eventually aids in plant growth.

All Bacterial isolates could synthesize HCN, which is a byproduct of ethylene biosynthesis. Ethylene, which is vigorously produced during stress, is one of the significant phytohormones that regulate growth and senescence. Higher amounts of ethylene would affect plant growth, which may be reduced by ACC deaminase activity and cope-up with the stress. ACC usage as a nitrogen source and ammonia synthesis was observed by all bacterial isolates and hence regulates the growth of plants (Rasool et al. 2021, Mir et al. 2021).

In anti-fungal activity studies of the isolated bacteria, have shown the maximum inhibition shown by Csb1 against the Fusarium. This might be due to the production of antifungal metabolites by isolated bacteria, such as HCN, extracellular enzymes, and/or siderophores. Various lytic enzymes synthesized by PGP bacteria are responsible for damaging pathogenic fungal components. Our results are similar to those of earlier researchers who worked on fungal inhibition by different species of Stenotrophomonas, Pseudomonas, and Bacillus (Ziedan and El-Mohamedy 2008, Hameeda et al. 2010). In various studies, phytopathogens were shown to be managed by the fungi-static effect of PGPB instead of unsafe chemical fungicides.

The nitrogen-fixing ability of bacterial isolates was quantified by the most sensitive acetylene reduction assay (ARA), while the qualitative evaluation was by growth on NFB media and nif H gene amplification. Molecular studies earlier 34 researchers affirm that the nitrogenase enzyme is an essential requirement in the nitrogen fixation process. Employing them would eventually improve the nitrogen nutrition of the crop applied. All the bacterial isolates in the study met the characteristics and features of diazotrophs.

The results obtained were similar to earlier reports that included many PGPBs isolated from different crops like rice, wheat, maize, and saffron (Suyal et al. 2016, Naher et al. 2017, Singh and Jha 2017, Mir et al. 2021). We should recognize crop-specific competent bacteria and inoculate them into plants to provide a specific positive stimulus to the autochthonous population of plant and

soil microbes so that they perform multiple tasks such as improving crop yield/quality and reducing the effect of phytopathogens along with ecological services.
The selected bacterial strain was found to be Gram-positive, non-motile, and pleomorphic, which developed creamy-white, circular, and convex colonies on a nutrient agar medium. The strain showed a positive result for catalase, amylase, IAA production, Ammonium production, HCN production, and protein reduction... The differences in MIC values in Nutrient agar might be explained by the fact that bioavailable metal content was reduced due to complexation with undefined components in the nutrient broth medium. Nevertheless, this high tolerance to different toxic metals could be attributed to the fact that the strain was isolated from an industrial waste-fed canal where a selection pressure was posed.

Media Composition Nutrient Broth:
Peptone -5 gm Beef extract-3gm NaCl-5gm

Indole-3-acetic acid (IAA) synthesis- Qualitative and quantitative estimation of IAA was carried out in nutrient agar and nutrient broth (supplemented with 5 mM tryptophan) respectively. The active culture of each bacterial isolate was inoculated and incubated at 30°C for 72 h, after incubation in sterile filter paper disks presoaked in Salkowski reagent (1 mL of 0.5 M FeCl3, 49 mL HClO4) were overlaid on the inoculated spots and observed for pink coloration. The level of positive bacterial isolates in plate culture conditions was determined quantitatively by inoculating 50 µL of active culture in 5 mL nutrient broth supplemented with 5 mM tryptophan and incubating on an orbital shaker at 30 °C, 200 rpm for 48 h. Post-incubation, cultures were centrifuged at 10,000 rpm for 10 min. Apart from this, 1 mL supernatant was mixed with 4 mL of Salkowski reagent, and then incubated for 30 min at 30 °C to allow the development of a pink color. The absorbance was then measured at 530 nm against standard IAA by UV- -spectrophotometer (Shimadzu UV- VIS)(GordonSAandWeber1951).

Phosphate solubilization- The phosphate solubilizing efficiency of the bacterial isolates was tested using the spot technique, ie by inoculating an actively grown culture on National Botanical Research Institute's Phosphate Growth (NBRIP) medium in plate culture conditions (Annexure I). A 0.1 mL of active culture prepared as mentioned above was inoculated separately in NBRIP broth, incubated on an orbital shaker at 200 rpm, 30 °C for 5 days along with uninoculated control. The broth was centrifuged after incubation at 10,000 rpm for 10 min and ANSA reagent (0.5 mL sol A + 0.2 mL sol B) was added to 0.1 mL of supernatant, The Final volume was made to 5 mL by adding double-distilled water and incubated at room temperature for 10 min. The OD was measured at 660 nm by using a UV spectrophotometer (Shimadzu UV-VIS). (Murphy J and Riley 1962).

(HCN) synthesis of Hydrocyanic: HCN synthesis by bacterial isolates was determined using the method devised by Bakker and Schippers (1987). Briefly, active bacterial isolates were plated on King's B agar (Annexure I), and incubated at 30 °C for 72 h.. After incubation, sterile filter paper presoaked in 2% sodium carbonate (Na2CO3) and 0.5% picric acid ($(O_2N)_3C_6H_2OH$) solution was placed in the inoculated plate lid. Plates were sealed with parafilm, then incubated upright for 3-5 days at 28 ± 2 °C and observed for the color change of filter paper from yellow to brown.

Synthesis of ammonia- Ammonia synthesis by the bacterial isolates was tested in peptone water medium (Annexure I). Ten microliters (µL) of actively grown bacterial culture were taken as an inoculant in 10 mL peptone broth, and incubated for 72 h at 30 °C. Nessler's reagent was added to the incubated tubes and a color change from yellow-brown-deep brown was observed for ammonia synthesis (Cappucino and Sherman 1992).

Conclusion:

The study focused on bacterial diversity among culturable fractions, their PGP effects, and Nfixing ability to meet the plant nutrient requirements in green gram soils. In summary, pronounced results of all bacterial isolates, would be significant due to the cumulative effect of various PGP and host defensive activities, which would eventually boost overall seedling growth and vigor. PGP traits such as the synthesis of ammonia, IAA, and ACCD were exhibited by 100% bacterial isolates, 90 and 80% of isolated bacteria were amylolytic and non-ureolytic respectively; No phosphate, Zinc solubilization shown bacterial isolates, and 50% bacterial isolates in study were zinc solubilizers. I while had protease and HCN- synthesizing ability and Ammonium production activity.

The bacterial isolates showed antioxidant activity, and antifungal activity csb6 showed max value of antifungal activity and protein reduction activity.

The biosurfactant industry is a highly profitable and competitive industry that uses the biodegradation and development benefits of the medicinal, cosmetic, petroleum, and food industries for renewable energy substrates. There has been a dramatic increase in the need for surfactants in the world, however, most of the surfactants currently available are chemically dependent. This study clarifies the potential benefits of biosurfactants for adapting their actions in several applications.

This study demonstrated that the bacterial isolates when applied to the rhizosphere soil of plants, served two major purposes. First, bio inoculation improved the growth parameters of plants perhaps due to its IAA production and ammonia production HCN production, at least in the tube experiment, secondly and more importantly, it decreased the Cr uptake in plants most likely due to Cr (VI) reduction by the strain in the rhizosphere. Earlier Yu et al. (2006) also reported plant growth-promoting activity of CA3, M7 bacterial isolates on Green gram have shown chromium reduction activity could be applied in the Cr- contaminated rhizosphere soil. It should be a good bioremediation agent although how farit promotes the enhancement of plant growth and reduction in Cr uptake under actual metal- polluted field conditions and its formulation needs further study.

References:

1. Adeleke BS, Ayangbenro AS, Babalola OO (2022) Effect of endophytic bacteriumJVB5 on sunflowers. Plant Protect Sci 58:185–198. https://doi.org/10.17221/171/2021-PPS
2. Ahn JH, Choi MY, Lee HW, et al (2013) Analysis of community structure of metabolically active bacteria in a rice field subjected to long-term fertilization practices. Korean J Soil Sci Fert 46:585–592. https://doi.org/10.7745/KJSSF.2013.46.6.585

3. Ahn JH, Hong IP, Bok JI, et al (2012) Pyrosequencing analysis of the bacterial communities in the guts of honey bees Microbiol 50:735–745.and Apis mellifera in Korea.
4. Ahn JH, Shin AL, Jeong MK et al (2016) Dynamics of bacterial communities in rice field soils as affected by different long-term fertilization practices. J Microbiol 11:724–731. doi:10.1007/s12275-016-6463-3
5. AICRIP, All India Coordinate Rice Improvement Programme (ICAR)- Indian Institute of Rice Research. Report (2022) Crop Production (Agronomy, Soil Science and Plant Physiology). 3:1-148. https://www.icar.org.in/sites/default/files/ICAR-AR- 21-22–E.pdf
6. Ana PS, Luciana NM, Anna S, et al (2021) Extremophilic bacteria restrict the growth by combined secretion of polyamines and lytic enzymes. Biotechnol Rep 15;32:e00674. doi: 10.1016/j.btre.2021.e00674
7. Bakker AW, Schippers B (1987) Microbial cyanide production in the rhizosphere in relation to potato yield reduction and pseudomonas SPP-mediated plant growth- stimulation. Soil Biol Biochem 19:451–7. doi: 10.1016/0038-0717(87)90037-X
8. Banik A, Mukhopadhaya SK, Dangar TK (2016) Characterization of N2-fixing plant growth promoting the endophytic and epiphytic bacterial community of Indian cultivated and wild rice (Oryza spp.) genotypes. Planta 243(3):799-812. doi: 10.1007/s00425- 015- 2444-8.
9. Cappucino JC, Sherman N (1992) Microbiolgy: A laboratory manual. 3rd ed. Benjamin/Cummings Pub Co, New York, pp 125-179.
10. Chandini RK, Kumar R, Om P (2019) The impact of chemical fertilizers on our environment and ecosystem. In: Sharma P (ed) Research Trends in Environmental Sciences, 2nd ed. AkiNik Publications, New Delhi, pp 71–85.
11. Chaudhry V, Rehman A, Mishra A et al (2012) Changes in bacterial community structure of agricultural land due to long-term organic and chemical amendments. Microb Ecol 64(2):450–460.
12. Ding Y, Wang J, Liu Y, Chen S (2005) Isolation and identification of nitrogen-fixing bacilli from plant rhizospheres in Beijing region. J Appl Microbiol 99:1271-1281. https://doi.org/10.1111/j.1365-2672.2005.02738.x
13. Döbereiner J (1988) Isolation and identification of root-associated diazotrophs. Plant Soil 110:207–12. doi: 10.1007/BF02226800
14. Duque LO, Villordon A (2019) Root branching and nutrient efficiency: status and way forward in root and tuber crops. Front Plant Sci 4:10:237. doi: 10.3389/fpls.2019.00237.
15. Dutta S, Podile AR (2010) Plant growth promoting rhizobacteria (PGPR): the bugs to debug the root zone. Crit Rev Microbiol 36:232–44. doi: 10.3109/10408411003766806
16. Engelhard M, Hurek T, Reinhold-Hurek B (2000) Preferential occurrence of diazotrophic endophytes, spp., in wild rice species and landraces ofin comparison with modern races. Environ Microbiol 2(2):131-41. doi: 10.1046/j.1462- 2920.2000.00078.x.