The Pharma Innovation Journal 2023; 12(4): 33-38

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(4): 33-38

www.thepharmajournal.com
Received: 11-02-2023
Accepted: 19-03-2023

Kshama G Deshpande
Assistant Professor, MGM College of Agricultural Biotechnology, Aurangabad, Maharashtra, India

Nilesh S Chavan
Assistant Professor, MGM College of Agricultural Biotechnology, Aurangabad, Maharashtra, India

Shubhum S Pandit
MGM College of Agricultural Biotechnology, Aurangabad, Maharashtra India

Corresponding Author:
Kshama G Deshpande
Assistant Professor, MGM College of Agricultural Biotechnology, Aurangabad, Maharashtra, India

Characterization of fig rhizospheric bacteria

Kshama G Deshpande, Nilesh S Chavan and Shubhum S Pandit

Abstract
The experiment was conducted for the isolation and characterization of fig rhizospheric bacteria and its potential as plant growth promoting rhizobacteria was assessed. The fig was supposed to have attacked with bacterial diseases. The purpose of this experiment is to control the bacterial diseases of fig by using the antagonistic actions of the rhizospheric bacteria. The fig rhizospheric bacteria were isolated from the soil. Four isolates from soil were selected. The selective media such as Kings B, Ashbys media, Pikoviaskos media and Zinc solubalizing media were used for this isolation to get the desired bacteria. They were characterized by morphological, biochemical and genetic characters. Three gram negative motile rods were found which were able to produce catalase, nitrate reductase, cellulase and urease enzyme and Indole acetic acid. This was confirmed by using various tests. Only one isolate was found nonmotile and lacking the catalase enzyme. The bacterial isolates were further subjected for the genomic DNA analysis and its purity was checked. The PCR and gel electrophoresis results and banding pattern showed that the isolate C3 was found diversity over rest of the other isolates. Among the four isolates isolate C2 and C3 were found to have better purity (1.93). From the obtained results It can be indicated that three isolates C1, C3 and C4 were found to have much resemblance with the *Pseudomonas* species from fig rhizosphere.

Keywords: Fig rhizosphere, soil isolates, characterization identification, bio control

Introduction
Most soils are simply a graveyard for dead bacteria cells. Bacteria are so simple in structure that they have often been called a bag of enzymes and/or soluble bags of fertilizer (Dick, R., 2009) [2]. Since bacteria live under starvation conditions or soil water stress, they reproduce quickly when optimal water, food, and environmental conditions occur. Bacteria population may easily double in 15-30 minutes. Flourishing microbial populations increase soil productivity and crop yields over time. Bacteria perform many important ecosystem services in the soil including improved soil structure and soil aggregation, recycling of soil nutrients, and water recycling. Soil bacteria form micro-aggregates in the soil by binding soil particles together with their secretions. These micro-aggregates are like the building blocks for improving soil structure. Improved soil structure increases water infiltration and increases water holding capacity of the soil (Ingham, 2009) [3].

Bacteria perform important functions in the soil, decomposing organic residues from enzymes released into the soil. Ingham (2009) [3] describes the four major soil bacteria functional groups as decomposers, mutualists, pathogens and lithotrophs. Each functional bacteria group plays a role in recycling soil nutrients.

The decomposers consume the easy-to-digest carbon compounds and simple sugars and tie up soluble nutrients like nitrogen in their cell membranes. Bacteria dominate in tilled soils but they are only 20-30 percent efficient at recycling carbon (C). Bacteria are higher in nitrogen (N) content (10-30 percent nitrogen, 3 to 10 C:N ratio) than most microbes (Islam, 2008) [4] of the mutualistic bacteria, there are four bacteria types that convert atmospheric nitrogen (N2) into nitrogen for plants. There are three types of soil bacteria that fix nitrogen without a plant host and live freely in the soil and these include Azotobacter, Azospirillum and Clostridium.

Crop disease remains a major problem to global food production. Excess use of pesticides through chemical disease control measures is a serious problem for sustainable agriculture as we struggle for higher crop productivity. The use of plant growth promoting rhizobacteria (PGPR) is a proven environment friendly way of controlling plant disease and increasing crop yield. PGPR suppress diseases by directly synthesizing pathogen-antagonizing compounds, as well as by triggering plant immune responses. It is possible to identify and develop PGPR that both suppress plant disease and more directly stimulate plant growth, bringing dual benefit.

A number of PGPR have been registered for commercial use under greenhouse and field conditions and a large number of strains have been identified and proved as effective biocontrol agents (BCAs) under environmentally controlled conditions. However, there are still a number of challenges before registration, large-scale application, and adoption of PGPR for the pest and disease management. Successful BCAs provide strong theoretical and practical support for application of PGPR in greenhouse production, which ensures the feasibility and efficacy of PGPR for commercial horticulture production. This could be pave the way for widespread use of BCAs in agriculture, including under field conditions, to assist with both disease management and climate change conditions.

Carica L. is an important member of the genus *Ficus.* It is ordinarily deciduous and commonly referred to as "fig". The common fig is a tree native to southwest Asia and the eastern Mediterranean, and it is one of the first plants that were cultivated by humans. The fig is an important harvest worldwide for its dry and fresh consumption. Its common edible part is the fruit which is fleshy, hollow, and receptacle. The dried fruits of *F. carica* have been reported as an important source of vitamins, minerals, carbohydrates, sugars, organic acids, and phenolic compounds. The fresh and dried figs also contain high amounts of fiber and polyphenols. Figs are an excellent source of phenolic compounds, such as proanthocyanidins, whereas red wine and tea, which are two good sources of phenolic compounds, contain phenols lower than those in fig. Its fruit, root, and leaves are used in traditional medicine to treat various ailments such as gastrointestinal (colic, indigestion, loss of appetite, and diarrhea), respiratory (sore throats, coughs, and bronchial problems), and cardiovascular disorders and as anti-inflammatory and antispasmodic remedy.

A fig tree can suffer from several diseases

Anthracnose: This is a group of fungal diseases that cause black/brown spots on the leaves, which gradually turn yellow and wilt Treat with a fungicide

Fig rust: The leaves develop small orange spots that increase in size as the season progresses. The leaves themselves may droop. Fig rust can be controlled with copper-based fungicides

Fig mosaic: This is caused by a virus that produces blotches on the leaves the virus is spread by mites and the only way to treat it is to kill the mites with miticide or horticultural oil

Endosepsis: It spread by the pollinating wasp that enters the green fig to lay eggs when the wasp dies inside the fig, the fungus develops on its body. The tree should be destroyed and the surrounding soil treated with a fungicide.

Materials and Methodology

Sample collection

Samples were taken from the rizosphere of fig plants on the MGM Gandheli Hills campus. By vigorously shaking the root, soil that was involved with it was collected and placed right into a sterile zip-lock polythene bag. Before analysis, the samples were labelled and kept in the dark.

Serial dilution of soil sample

1 gm of soil sample from fig rhizosphere was weighed, diluted in 99 ml of water, and stirred on magnetic stirrer for one hour. Label the five test tubes 1, 2, 3, 4, and 5. Each test tube should contain 9 ml of water. After waiting for 10 minutes, remove the supernatant and place 1 ml in test tube 1 that has 9 ml of distil water. Take 1 ml from test tube 1,and add to test tube 2, and so on up to test tube 5.

Inoculation and incubation

These serially diluted samples were inoculated into the various isolation media as shown in the table 1 four isolation media such as ashbys medium, Kings B medium, pikoviasko's medium and Zinc soluibalizing medium plates were prepared and inoculated with fifth number tube dilution by streak plate technique. These bacterial cultures were incubated at room temperature for next 24 -48 hrs until the isolated colonies were grown.

Different media used for bacterial culture

Sr No	(Treatments)	Media for isolation
1)	T_1	Ashby`s Media
2)	T_2	Kings B agar and broth
3)	T_3	Pikovskaya's Media
4)	T_4	Zinc solubalizing media

Bacterial colonies were selected based on their morphological characteristics.

Biochemical Characterization of the isolates

1. Gram's staining

Homogenize a loop-full of bacterial culture in 1 ml distilled water. Create a smear of bacterial colonies on a microscope slide and heat fix it. Apply a primary stain of crystal violet. Wash thoroughly with the distilled water. Add a mordant, ram's iodine (1min). Wash with 70% ethanol followed by distilled water. Counterstain with safranin for 4min. Wash and air-dry the smear and observe the slide under microscope.

2. Motility

The motility test is used to determine whether an organism is motile or non-motile. Motile organisms are generally bacilli although a few motile cocci do exist. It is also used to aid in differentiation between genera and species. Make suspension culture of isolated colonies. Take a cover slip apply grease on each corner of cover slip. Take inoculation needle collect a droplet from the suspension place in the middle of the cover slip. Place a cavity slide on the cover slip. Observe it under microscope at 40X.

3. Nitrate reductase test

This test demonstrates the presence of catalase, an enzyme that catalyses the release of oxygen from hydrogen peroxide (H2O2). It is used to differentiate those bacteria that produces an enzyme catalase, such as staphylococci, from non-catalase producing bacteria. Inoculate the nitrate broths with bacterial culture. The tubes should be incubated for 24 hours at the optimum temperature of 30 or 37 °C. Add 5 drops of -Naphthylamine and 5 drops of sulfanilic acid, and watch for red colouring. Add zinc powder if the red coloration is not visible.

4. Catalase test

This test demonstrates the presence of catalase, an enzyme that catalyses the release of oxygen from hydrogen peroxide (H2O2). It is used to differentiate those bacteria that produces an enzyme catalase, such as staphylococci, from non-catalase producing bacteria. Prepare suspension culture of the bacterial colonies. Add a few drops of hydrogen peroxide. The test is positive if the bubbles are visible and vice versa.

5. IAA test

This test demonstrates the ability of certain bacteria to decompose the amino acid tryptophane to indole, which accumulates in the medium. Indole production test is important in the identification of Enterobacteria. The production of indole-3acetic acid is an essential tool for rhixobacteria to stimulate and facilitate plant growth. The property of sythesizing IAA is considered as effective tool for screening beneficial microoraganism suggesting that IAA producing bacteria have profound effect on plant growth. inoculation with IAA producing bacteria induces the proliferation of lateral roots and root hairs.

Bacterial culture was grown for 7Hrs in kings B broth at 36 °C. Centrifugation was carried out at 3000 rpm for 30 min. The supernatant was collected and homogenize with two drops of orthophosphoric acid and 4ml of Salkowaski reagent. Pink colour emergence indicates IAA production.

6. Cellulose test

Cellulase degradation test is used to determine whether the bacteria have the ability to degrade cellulose with the help of filter paper. black spots appearing on paper states the sign of cellulose degrading bacteria. In a sterile test tube, make cellulase mineral salt medium. Pour media in test tube, autoclave. Incubate a loop-full of sample (C1 - C4 plate) in the medium. Insert blotting paper strip in the medium and plug with cotton, wrap it with aluminium foil. Keep it at room temperature for seven days. Black spot will be visible on the blotting paper.

7. Urease test

The urease test is used to determine the ability of an organism to split urea, through the production of the enzyme urease. Urea Agar was developed by Christensen in 1946 for the differentiation of enteric bacilli. The Urease test is used to determine the ability of an organism to split urea, through the production of the enzyme Urease. Inoculate slant with 1 to 2 drops from an overnight infusion broth culture Incubate the tube for 7 days at 35 to 37°C in ambient air by leaving the cap on loosely. Examine for the development of a pink color after 7 days.

8. DNA isolation from bacteria

2ml bacterial culture was taken in a centrifuge tube. Centrifugation was carried out at 10000rpm for 10 min. Discard the supernatant and add TE Buffer 500µl to the cell pellet. Add SDS (100µl); lysozyme (100µl) and incubate for 60 min at room temperature. Add 100 µl NaCl mix well and add CTAB buffer 80µl (10% CTAB, 5M NaCl [1:1]) mix by inverting the tube and incubate at 65˚C for 10 min. Add equal amount of chloroform: Isoamyl alcohol [24:1] mix to emulsify and spin for 5min at 8000 rpm. Collect the supernatant into fresh tube and repeat step (vi). Add equal amount of chilled isopropanol. Centrifuge it at 12000 rpm for 20 min, Discard the supernatant. Wash with 70% ethanol followed by centrifugation at 12000rpm for 20 min; air-dry the pellet. Dissolve the pellet in TE Buffer and store it at -20 ˚C.

Qualitative and quantitative analysis by Nanodrop

Nanodrop Spectrophotometer was used for analysis of purified DNA. Clean the pedestal with tissue paper. Load the solute- TE buffer or distil water for zeroing of the instrument. Clean the pedestal and now load the DNA sample & close the pedestal. Observe the concentration (ng/ml) and the absorbance at 260/280nm.Note the readings for the sample or save the result file.

PCR using RAPD markers

Master Mix was prepared for each primer with the below reagents divided into different PCR tubes. 2µL of different bacterial DNA samples were added in the PCR tubes and final volume was made 25 µL. One RAPD OPA 04 (AATCGGGCTG) primer was used for amplification,

PCR using RAPD markers

SN.	Components	Quantity 1
1	Nuclease free water	15.5 µl
2	10X buffer	2.5 µl
3	DNTPs	2.0 µl
4	Taq polymerase	1.0 µl
5	Primer	2.0 µl
6	DNA sample	2 µl

The amplification cycle of PCR contains Initial denaturation 94 °C for 5 min followed by 40 cycles of Denaturation at 94 °C for 1 min, Annealing at 37 °C for 1 min, Extension at 72 °C for 2 min. The final extension was completed at 72°C for 7 min. The PCR products (25 µL) were mixed with 6X gel loading buffer (4 µL) and 100bp DNA ladder (5 µL) were loaded onto an agarose (2% w/v) gel containing 8µL of EtBr. 1X TBE buffer (Tris-Borate-EDTA) was added and electrophoresis was carried out at 100V until the dye migrates 1/3rd of the gel. The gel was later visualized in UV-transilluminator and the observations were recorded.

Results

The results of isolation of colonies and selection of four different media was shown in fig. 1 only isolate 4 (C4) showed growth on Zinc soluibalising media. C3 does not show growth on Pikovaiskos medium.

Table 1: Growth of colonies on different medias

Colonies/ media	Kings B media	Pikovskaya's media	Ashby's media	Zinc solubilizing media
C1	+	+	+	-
C2	+	+	+	-
C3	+	-	+	-
C4	+	+	+	+

The colony morphology was studied of all four isolates as shown in the table 2

Table 2: Morphological characteristics

Characteristics	C1	C2	C3	C4
Size	6 mm	3 mm	14 mm	4 mm
Shape	Irregular	Circular	Circular	Irregular
Colour	Milky white	Off white	White	yellow
Margine	Cirrate	Entire	Entire	Entire
Elevation	Flat	Flat	flat	Raised
Surface	Rough	Smooth	Smooth	Smooth
Opacity	Opaque	Opaque	Opaque	Opaque
Consistency	Vitrous	Buterous	Vitrous	Buterous
Motility	Motile	nonmotile	motile	motile

All the four isolates showed following biochemical Characterization All four isolates showed catalase production, nitrate reductase production, cellulase production, urease production and IAA production positive. It was shown in table 3.Also it is shown in fig no 3, 4, 5, 6.

Table 3: Biochemical Test Result

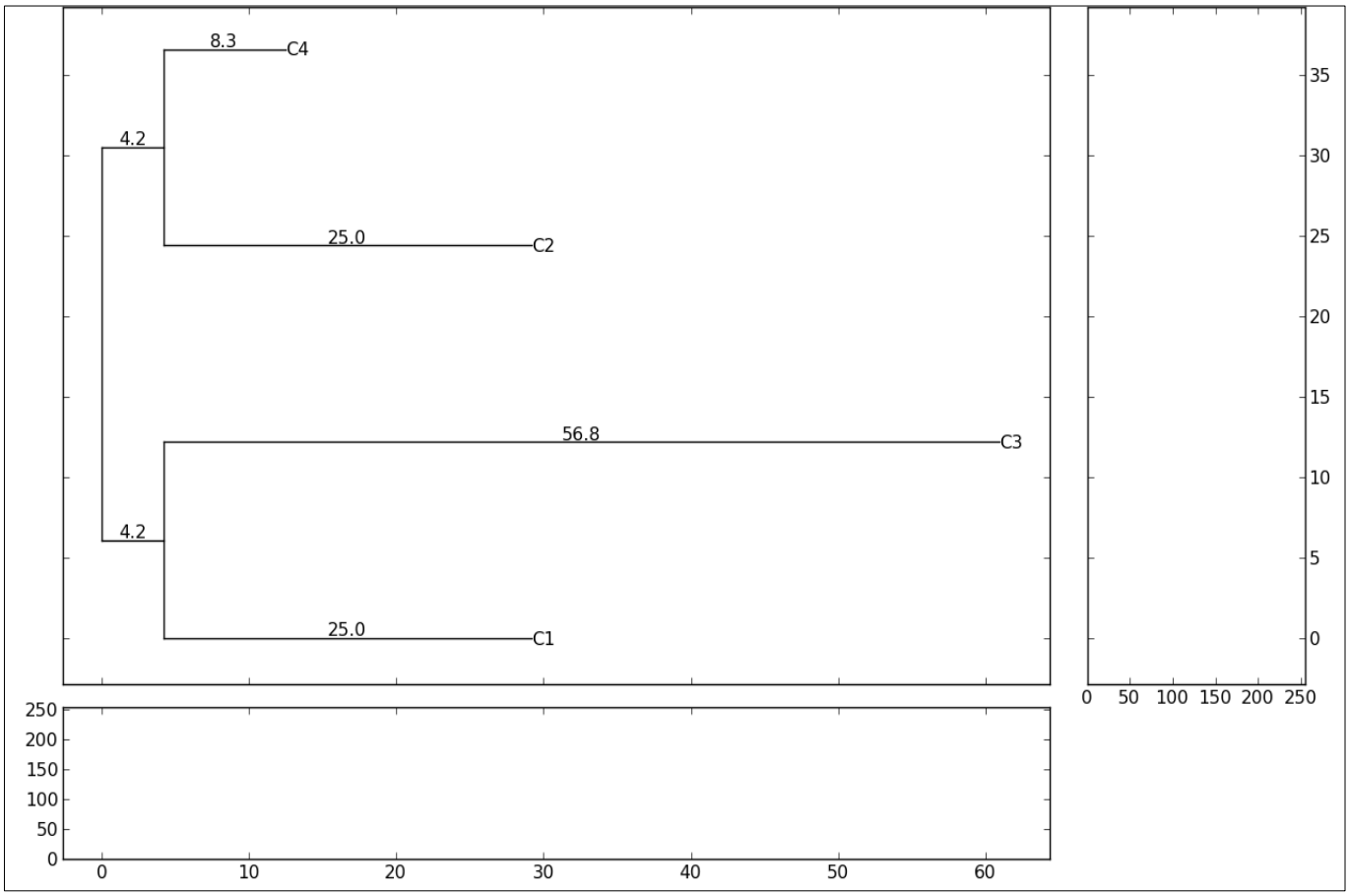
Biochemical test	C1	C2	C3	C4
Gram staining	-ve (rods)	-ve (cocci)	+ ve (rods)	- ve (rods)
Catalase test	+ve	+ve	+ve	+ve
Nitrate reductase test	+ve	+ve	+ve	+ve
Cellulase test	+ve	+ve	+ve	+ve
Urease test	+ve	+ve	+ve	+ve
IAA	+ve	+ve	+ve	+ve

The genomic DNA of four bacterium was isolated and purity was confirmed by Nanodrop. The nanodrop quantification showed the results as shown in table 4.

Table 4: Nanodrop spectrophotometer readings

Sr.No.	Colony	Concentration of DNA (ng/mL)	Ration of 260/280 nm
1	C1	674.2	1.76
2	C2	386.3	1.93
3	C3	287.2	1.93
4	C4	213.0	1.94

The isolated DNA was used for PCR studies and successful amplification of DNA using RAPD primer was performed. The dendogram was drawn by using PyElph version 1.4. From PCR studies of a single primer it was observed that DNA purity and quantity is adequate for PCR further PCR studies. It was observed that isolate C2 and C4 are closely related and form separate branch while isolate C3 and C1 are form another branch.



This experiment was conducted to assess the rizospheric bacteria of fig plant because fig is generally more prone to bacterial diseases which can be controlled by using biocontrolled agents by means of antagonistic reaction phenomenon. It not only minimizes the ill effects of chemical pesticides but also improves yield and nutrient quality of the fruit from fig rizospheric soil. In this experiment four colonies were selected which were grown on four different selective media. The purpose of isolation of these colonies is to assess the biopesticide and biofertilizer activity of bacteria. The colonies were subjected for various morphological, biochemical and genetic characterization. The organisms were isolated on different selective media so as to know the primary nutrient requirement of the same. It was found that colony number 1, 2 and 3 were grown on kings b, pikovaskays media and ashby's media whereas colony number 4 was grown on kings b media, pikovakays media, ashbys media and zinc solubilizing media. Colony no.1, 2, 3 did not

show growth on zinc solubilizing media. All the four colonies were subjected for morphological characteristics. It showed motile nature except C2 which was non-motile morphologically. Three colonies were found gram negative except C2 which was gram positive cocci. The same three colonies C1, C3 and C4 were showing catalase test positive indicating the resemblance with mobile nature and C3 stated negative. Moreover all the four colonies were positive in the production of nitrate reductase enzyme, cellulase enzyme, urease enzyme indicating that they can convert NO2 to NO3 able to degrade cellulose into glucose and total sugars as well as able to degrade urea and able to produce a growth hormone indole acetic acid for the growth and enhancement. All these characteristics of this isolate were resembling to the *Pseudomonas* species when compared to the literature for the confirmation.

Fig 1: Isolation of Bacteria

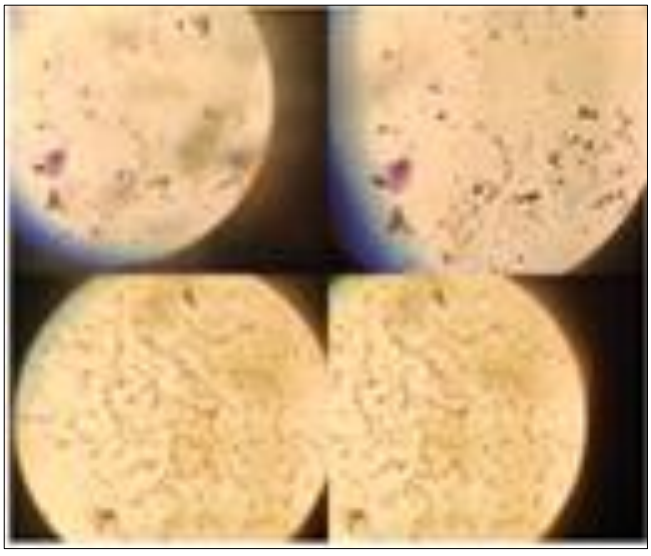


Fig 2: Grams staining of isolates

Fig 3: Biochemical Test Catalase

Fig 4: Biochemical Test Cellulose degradation

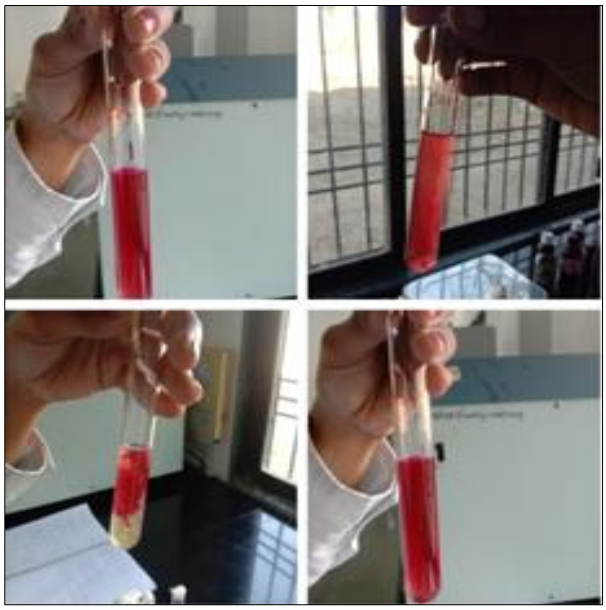


Fig 5: Biochemical Test Nitrate Reduction

Fig 6: Biochemical Test IAA formation

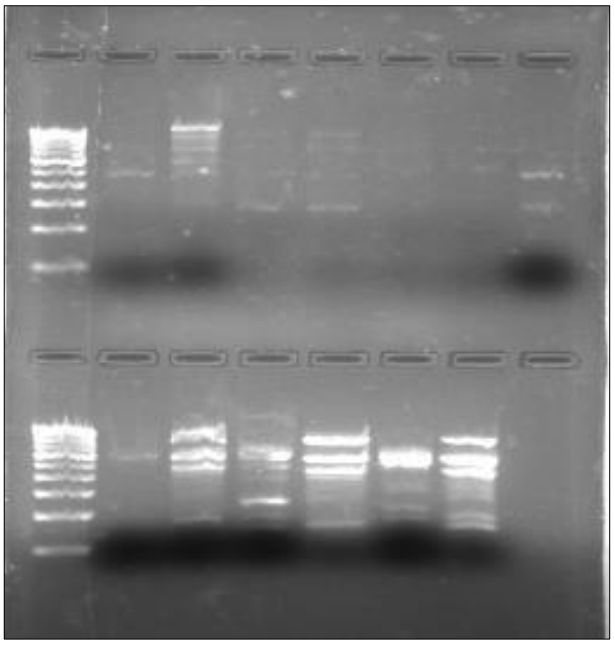


Fig 7: PCR reaction for RAPD analysis

Acknowledgement
Authors are thankful to Management of Mahatma Gandhi Mission Trust, Aurangabad. Authors are also thankful to MGM College of Agricultural Biotechnology, Gandheli for providing all necessary material for this research work. Authors has no conflict of interest.

References

1. Dueñas M, Pérez-Alonso JJ, Santos-Buelga C, Escribano-Bailón T. Anthocyanin composition in fig (*Ficus carica* L.). Journal of Food Composition and Analysis. 2008;21(2):107-115.
2. Dick R. Lecture on Soil Bacteria in Soil Microbiology, Personal collection of R. *Dick*, The Ohio State University School of Environment and Natural Resources, Columbus, OH. Lecture; c2009.
3. Ingham ER. Soil biology primer, Chapter 4: Soil fungus. Soil and Water Conservation; Soil & Water Conservation Society: Ankeny, IA, USA; c2009. p. 22-23.
4. Islam KR. Lecture on Soil Physics, Personal Collection of K. Islam, Ohio State University School of Natural Resources, Columbus, OH; c2008.
5. Jeong WS, Lachance JA. Phytosterols and fatty acids in fig (*Ficus carica* var. mission) fruit and tree components, Food Chemistry and Toxicology. 2001;66(2):278-281.
6. Slatnar A, Klancar U, Stampar F, Veberic R. Effect of drying of figs (*Ficus carica* L.) on the contents of sugars, organic acids, and phenolic compounds, Journal of Agricultural and Food Chemistry. 2011;59(21):11696-11702.
7. Veberic R, Jakopic J, Stampar F. Internal fruit quality of figs (*Ficus carica* L.) in the Northern Mediterranean Region, Italian Journal of Food Science. 2008;20(2):255-262.
8. Vinson JA, Hao Y, Su X, Zubik L. Phenol antioxidant quantity and quality in foods: vegetables, Journal of Agricultural and Food Chemistry. 1988a;46(9):3630-3634.
9. Vinson JA. The functional food properties of figs, Cereal Foods World. 1999b;44(2):82-87.
10. Vinson JA, Zubik L, Bose P, Samman N, Proch J. Dried fruits: excellent *in vitro* and *in vivo* antioxidants, Journal of the American College of Nutrition. 2005;24(1):44-50.
11. Dueñas M, Pérez-Alonso JJ, Santos-Buelga C, Escribano-Bailón T. Anthocyanin composition in fig (*Ficus carica* L.). Journal of Food Composition and Analysis. 2008;21(2):107-115.