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Isolation and screening of bacteria from Jeevamrutha and Ghanajeevamrutha against the pathogen *Sclerotium rolfsii* for their biocontrol activity and their biochemical characterization

[1]SAHANA S. BETAGERI[*], [2]SREENIVASA M.N.[**], [3]C.R. PATIL[***] AND [4]GURUDATT M. HEGDE[***]

** Part of the M. Sc. (Agri.) Thesis submitted by the first author to the Department of Agricultural Microbiology, College of Agriculture, Dharwad, UAS, Dharwad – 580005, India*

***Professor, Dept. of Agricultural Microbiology, College of Agriculture, Dharwad, University of Agricultural Sciences, Dharwad-580 005, Karnataka (India).*

****Professor and Head Institute of Organic Farming College of Agriculture, Dharwad, University of Agricultural Sciences, Dharwad-580 005, Karnataka (India).*

*****Principal Scientist (Plant Pathology), AICRP on Wheat and Barley, MARS, University of Agricultural Sciences, Dharwad-580 005, Karnataka (India).*

Abstract: An experiment was conducted to study the biocontrol activity of bacterial isolates from Jeevamrutha and Ghanajeevamrutha at the University of Agricultural Sciences, Dharwad. Jeevamrutha and Ghanajeevamrutha are cheaper and eco-friendly organic preparations made of desi cow products. These are efficient plant growth stimulants that enhance the biological efficiency of crops. Soil borne plant pathogens cause serious losses to crop plants reducing the yield to the extent of 75-80 per cent. In this context, bacteria were isolated from the Jeevamrutha and Ghanajeevamrutha (prepared at laboratory scale) and were subjected for dual culture plate assay on Potato Dextrose Agar medium against the plant pathogen *Sclerotium rolfsii*. Zone of inhibition was calculated by comparing to the pathogen growth on the control plate. The isolated bacteria showed different degrees of antagonism (28.75-47.78 per cent) against the pathogen, among them the isolates J1 (isolated from Jeevamrutha) and GJ1(isolated from Ghanajeevamrutha) have shown significantly the highest inhibition percentage of 47.78 per cent and 36.55 per cent respectively against the pathogen *Sclerotium rolfsii*. Further the isolates were subjected for morphological and biochemical characterization, the bacterial isolates were found to be a mixture of both Gram positive and Gram-negative bacteria with varying shapes. Most of them were positive for citrate utilization test indicating sodium citrate as the sole source of carbon used by the isolates, only few were positive for PAL test i.e., Phenylalanine ammonia lyase test.

Keywords: Jeevamrutha, Ghanajeevamrutha, *Sclerotium rolfsii*, per cent inhibition, biochemical characterization.

1. Introduction

Organic farming system in India is an age-old practice and is being followed in many parts of the country. It is a method of farming system which is principally aimed at cultivating the land and raising crops in such a way, as to keep the soil thriving and in good health by the use of organic residues/ manure (crop, animal and farm

wastes, aquatic wastes) and other biological materials along with beneficial microbes (biofertilizers) to release nutrients to crops for increased sustainable production in an eco-friendly, pollution free, environment.

Green revolution introduced hybrid seeds and replaced the organic manures with chemical fertilizers and pesticides, brought several hectares of land under cultivation which directly contributed to the increased food production in the country. However, in the long run this was not productive because the excessive use of chemical pesticides caused toxicity to the food grains, reduced the soil fertility, caused huge ecological imbalance, raised numerous health hazards; and in brief impaired the biodiversity.

India has tremendous biodiversity, genetic as well as of species and ecosystems. It contains over 7 per cent of the world's biodiversity on 2.5 per cent of the Earth's surface (Soni,2020). It is very much important to develop a strong workable and compatible package of nutrient management through organic sources for various crops based on scientific facts, local conditions and economic viability (Kannaiyan, 2000). Among indigenous technologies used by farmers, use of beejamrutha, jeevamruta, paanchagavya, ghanajeevamrutha etc., have been given importance since age old days. Presence of naturally occurring beneficial microorganisms predominantly bacteria, yeast, actinomycetes, photosynthetic bacteria and certain fungi detected in theses organic formulations proved to enhance the crop yield, disease management, improved crop health and many more things. Jeevamrutha, panchagavya and beejamrutha have become an inevitable part of natural farming, organic farming, zero budget natural farming, and other similar practises over time (Duraivadivel et al., 2022).

Jeevamrutha is a liquid biodynamic microbial organic formulation used since several decades by farmers across the country. In India, Jeevamrutha is widely used by farmers practising natural farming in the states of Andhra Pradesh, Karnataka, and Tamil Nadu, either alone or in combination with organic amendments such as vermicompost, farmyard manure, etc. Small and medium landholding farmers are more accepting Jeevamrutha than large landowning farmers (Kumar et al., 2020). Jeevamrutha uses local resources, has minimal input costs, can increase farm income, and favours chemical-free farming and subsistence farming.

Ghanajeevamrutha is an organic manure redeveloped for critical crop requirements by using desi cow products. This is prepared for supplying major and minor supplements to plants and supply food to earth worms and other useful micro flora and fauna in soil by improving aeration in root zone enhancing mineralization process in soil helps in sustained release of nutrients in the soil, which in turn makes available useful elements to plants.

Soil-borne plant pathogenic fungi cause heavy crop losses all over the world. With variable climate from region to region, most crops grown in India are susceptible to diseases caused by soil-borne fungal pathogens. *Sclerotium rolfsii* is a destructive soil inhabitant of worldwide significance which has a host range of over 500 species of plants (Susleendra Desai & Schlosser 1999). Management of *S. rolfsii* through soil application of fungicides is difficult because of its broad host range as well as its worldwide distribution which precludes such strategy. Once established in the soil, it is hard to eliminate the pathogen. Management through chemical methods leads to ill-effects like residual toxicity, environmental pollution and fungicide resistance. Although seed treatment with fungicides is recommended to minimize the infection at early stages, it does not give prolonged protection. Biological control has been proved to be a promising disease-management technology especially against soil-borne plant pathogens.

In the light of the above facts and this study was conducted to isolate bacteria from Jeevamrutha and Ghanajeevamrutha antagonistic to the plant pathogen *Sclerotium rolfsii* and studying their morphological and biochemical characteristics.

2. Material and methods

2.1 Preparation of Jeevamrutha and Ghanajeevamrutha

Jeevamrutha and Ghanajeevamrutha were prepared using local desi cow products, the ingredients were scaled down to laboratory set up (5 per centage of the original recommendations) from the standard protocol given by Palekar, (2006).

Jeevamrutha

Ingredients: Desi cow dung 0.5 Kg, desi cow urine 0.5 L, black jaggery 100 g, pulse flour 100 g, soil from the bund (the amount which can be held in a wrist), water 10 L.

Procedure: A container of capacity 10 litre was taken and was filled with some amount of water initially. Desi cow dung of 0.5kg was made into slurry and was added to the container, then 0.5L of desi cow urine was poured. Black jaggery of 100g was powdered and added to the mixture, similarly 100g of pulse flour was mixed with water and added to the above mixture finally some amount of soil taken from the bund was also added and stirred well and final volume was made up to 10 litres and was covered with a wet gunny bag and kept in shade. This mixture was stirred thrice a day for 7 days.

Ghanajeevamrutha:

Ingredients: Desi cow dung 5 Kg, desi cow urine 0.5 L, black jaggery 100 g, pulse flour 100 g, soil from bund (the amount which can be held in a wrist), water 10 L.

Procedure: Initially 5Kg desi cow dung was spread on dry floor, some amount of desi cow urine was sprinkled on to it to moisten it. Then 100g of powdered jaggery and 100g of pulse flour were added along with some amount of soil taken from the bund. finally, the remaining cow urine was added and the mixture was thoroughly mixed and covered with a wet gunny bag and left for 24hrs. After 24hrs time period the mixture was made into small balls kept for drying for 21 days in shade. After 21 days the balls were broken down to fine powder and filled in bags to be used later.

2.2 Isolation of the bacteria on R2A medium

A method given by Yang *et al.* (2017) modified for this study was employed. 10 g of Ghanajeevamrutha and 10 ml of Jeevamrutha were transferred separately to sterile Erlenmeyer flasks containing 90 ml sterile PBS solution (1.2g Na2HPO4(137mM), 0.18g NaH2PO4 (1.8mM),8.5g NaCl(2.7mM) made up to 1L with distilled water) of conc.10mM. The samples were shaken for 30 minutes at room temperature. The suspension was filtered through doubled sterile gauze prior to isolation of bacteria. The filtered solutions were serially diluted to 10^{-1} - 10^{-5} with sterile PBS solution and 0.1ml of each dilution was spread on R2A medium (Reasoner and Geldreich, 1985).

Screening of the bacterial isolates for their antagonistic activity against the plant pathogen, *Sclerotium rolfsii*

2.3 Dual culture method

The antagonism of all the bacterial isolates was examined with respect to their ability to suppress the growth of *Sclerotium rolfsii* by the dual culture method (Dennis and Webster, 1971). Each bacterial isolate was streaked on one side of the Petri plate (PDA medium) and a mycelial disc of 8mm diameter obtained from the pure culture of *Sclerotium rolfsii* was placed on the opposite side of the same plate, all such plates were kept for incubation at 28±2 °C. After 5 days of incubation when the control plate was completely covered with the *Sclerotium rolfsii* zone of inhibition was measured in the test plates to know the per centage inhibition.

Observations on width of inhibition zone and mycelial growth of test pathogen were recorded and per cent inhibition of pathogen growth was calculated by using the formula proposed by Vincent (1947).

• Per cent disease inhibition (I) = C-T/C ×100

Where,

• C- mycelial growth of pathogen in control

• T- mycelial growth of pathogen in dual culture plate.

Then finally bacteria were selected based on their antagonistic activity against fungi (measurement of inhibition zones).

All the antifungal isolates were purified on nutrient agar media by following streak plate method and preserved for further analysis.

2.4 Characterization of the bacterial isolates

2.4.1 Morphological characterization of the bacterial isolates:

All the antagonistic bacterial isolates were streaked on Nutrient Agar medium and incubated at 28±2 °C for 24 hours. After incubation, isolated colonies obtained were characterized for morphological traits *viz*., colony shape, color, elevation (Vincent, 1970).

2.4.2 Gram staining and microscopic examination:

Gram staining was carried out according to the procedure of Graham and Parker (1964). A loop full of thin culture was uniformly spread on the glass slide and the smear was airdried and heat fixed. Few drops of crystal violet dye were dropped over the smear and left for 1 min. the stain was washed under running water and left the for drying. Then the smear was flooded with iodine solution for 1 min. Then the iodine solution was drained off and cells were decolorized with 95 per cent ethanol for 30 seconds. The smear was washed with water and blot dried carefully; counter stained with safranin. Lastly, the smear was rinsed with water, air dried and observed under microscope. The stained microscopic slides were examined through a bright field microscope under oil immersion for Gram reaction and cell morphology.

2.4.3 Biochemical characterization of selected isolates:

The selected bacterial isolates were subjected for biochemical characterization *viz*., Indole test, Methyl Red test, Citrate utilization test, Oxidase test, Catalase test, and Starch hydrolysis test (Cappuccino and Sherman, 2005).

2.4.3.1 Indole test

The tubes of tryptophan broth were inoculated with overnight grown cultures of the bacterial isolates followed by incubation at 28 °C for 24 h. After incubation, 1 ml of Kovac's reagent was added. The tubes were shaken gently after interval of 10-15 min and allowed to stand for some time. Tubes were examined for change of colour to cherry red.

2.4.3.2 Citrate utilisation test:

Overnight grown cultures of the bacterial isolates were inoculated into test tubes containing Simmons citrate agar and incubated for 72 hrs at 28 °C. The presence of growth and change of colour from green to blue due to pH change were taken as positive reaction.

2.4.3.3 Oxidase test:

Bacterial isolates were grown on Tributyrin agar medium in Petri plates at 30 °C. After 24h of incubation 1.0 ml of 1% tetramethyl-p-phenyl-diamine-dichloride solution was poured over the growth. A positive reaction was indicated by the appearance of a dark purple colour on the growth within a minute.

2.4.3.4 Catalase test:

Isolates were grown on Tributyrin agar medium in Petri plates at 30 °C. After 24h of incubation 1.0 ml of 3% hydrogen peroxide solution was poured over the growth. Liberation of gas bubbles due to decomposition of hydrogen peroxide was taken as the positive catalase reaction.

2.4.3.5 Starch hydrolysis test:

The isolates were streaked on the sterilised starch agar plates and incubated at 30 °C for 48 hrs. After incubation, the plates were flooded with Lugol's iodine solution. The formation of clear zone around the colony was taken as positive for the starch hydrolysis test.

2.4.3.6 Phenylalanine ammonia lyase activity: Chen *et al.* (2003)

Phenylalanine agar medium was prepared and poured as a slant into a tube. The bacterial isolates were streaked on phenylalanine slant, incubated at 37°C for overnight. Then 4-5 drops of 10% aqueous ferric chloride ($FeCl_3$) solution was poured over the culture. Change in color of the medium to green color is taken as positive result.

3.Results

3.1 Isolation of bacteria from Jeevamrutha and Ghanajeevamrutha

The bacteria were isolated from Jeevamrutha and Ghanajeevamrutha on R2A medium by standard serial dilution and spread plate method. The bacterial isolates were purified on nutrient agar medium by four-way streak method.

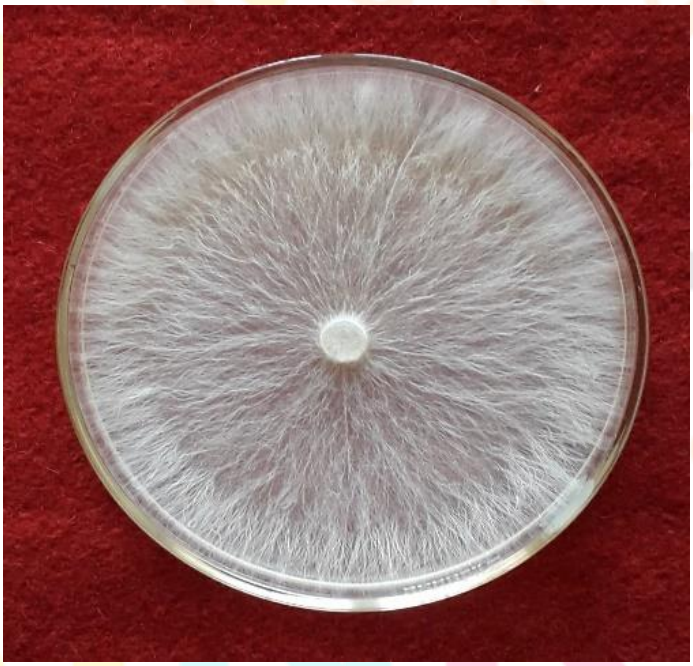
3.2 Screening of the bacterial isolates for their antagonistic activity against the plant pathogen, *Sclerotium rolfsii*

All the bacterial isolates were subjected for dual culture method to test their antagonistic activity against the plant pathogen *Sclerotium rolfsii* on PDA medium.

All the isolates tested have shown zone of inhibition against plant pathogen *Sclerotium rolfsii.* For different antagonistic bacterial isolates percentage zone inhibition varied significantly at 1% level of significance under *in vitro* condition against the pathogen *Sclerotium rolfsii.* The highest zone of inhibition was shown by the isolates J1and GJ1 at 47.78 per cent and 36.55 per cent respectively. The lowest percentage of inhibition zone was shown by the isolates J11 and GJ15 (28.75 per cent and 28.99 per cent respectively). Among the bacterial isolates the isolates from Jeevamrutha have illustrated comparatively higher zone of inhibition as that of the isolates from Ghanajeevamrutha (Table 1 and 2).

(J1) Jeevamrutha

(GJ1) Ghanajeevamrutha



Control (*Sclerotium rolfsii*)

Plate 1: Antagonistic activity of the bacterial isolates against *Sclerotium rolfsii*

3.3 Morphological and biochemical characterization of the isolates

The morphological and biochemical results showed that all the bacterial isolates were positive for oxidase and catalase test with few isolates being positive for starch hydrolysis and PAL (Phenylalanine Ammonia Lyase) test and most of the isolates were positive for citrate utilization test. Gram reactions showed that the isolates were a mixture of both gram positive and gram negative, with cocci and rod-shaped bacteria forming creamy white, pale-yellow colonies on the Nutrient Agar medium (Table 3).

4.Discussion

4.1 Isolation of bacteria from Jeevamrutha and Ghanajeevamrutha

Jeevamrutha and Ghanajeevamrutha were prepared using desi cowdung, desi cowurine, black jaggery, gram flour and bund soil by following the standard procedure given by Palekar (2006) and quantities of the ingredients were reduced to 5 percent of their original recommendation. Bacteria were isolated from these organic preparations on the R2A medium and the cultures were purified on nutrient agar medium by four-way streak method and further maintained in 50% glycerol stock. Several scientists earlier have successfully isolated the microorganisms from the liquid organic formulations like panchagavya, Beejamrutha (Nagaraj and Sreenivasa, 2009) Jeevamrutha (Devakumar *et al.*, 2014).

4.2 Screening of the isolates for their antagonistic activity against the plant pathogen *Sclerotium rolfsii*

The percentage of inhibition of the plant pathogen *Sclerotium rolfsii* by the isolates was exhibited in the range 28.75 per cent to 47.78 per cent. The isolate form Jeevamrutha *i.e.*, J1(47.78 per cent) showed highest zone of inhibition among all the isolates followed by the isolate rom Ghanajeevamrutha *i.e.*, GJ1 36.55 per cent. The results are in concurrence with the findings of Sreenivasa *et al.* (2009) who studied the antagonistic activity of the microorganisms isolated from beejamrutha against the plant pathogen *Sclerotium rolfsii* and reported the percentage inhibition of the pathogen in the range of 88 to 66 per cent in *in vitro.* Production of siderophores and chitinases are two factors that may be involved in biological control activity. Mahesh (2007) opined that fungal growth is mainly repressed by HCN production and siderophore production by the inhibiting microorganism. All these earlier results give support to the present findings.

5. Conclusion:

The results of the study showed that the bacteria isolated from the organic preparations *viz,* Jeevamrutha and Ghanajeevamrutha were efficient in suppressing the growth of the pathogen *Sclerotium rolfsii* in *in vitro* indicating the bacterial isolates as efficient biocontrol agents. The enzymes released by bacteria convert the complex insoluble organic compounds to simpler soluble form. This may be helpful for plants to uptake nutrients through the action of these microorganisms that are enzymatically active.

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References

Cappuccino J G and Sherman N, 2005, Microbiology: a Laboratory Manual. Benjamin Cummings Publishing Company.

Chen K, Feng H, Zhang M and Wang X, 2003, Nitric Oxide alleviates oxidative damage in the green algae Chlorella pyrenoidosa caused by UV-B radiation. *Folia Microbiologica*, 48(3): 389–393.

Dennis C and Webster J, 1971, Antagonistic properties of species-groups of Trichoderma: II. Production of volatile antibiotics. *Transactions of the British Mycological Society*, 57(1): 41-48.

Devakumar N, Shubha S, Gowder S B and Rao G G E, 2014, Microbial analytical studies of traditional organic preparations beejamrutha and jeevamrutha. Building organic bridges, 2:639-642.

Duraivadivel P, Kongkham B, Satya S and Hariprasad P, 2022, Untangling microbial diversity and functional properties of Jeevamrutha. *Journal of Cleaner Production*, 369:133218.

Graham P H and Parker C A, 1964, Diagnostic features in the characterisation of the root-nodule bacteria of legumes. *Plant and soil*, 3:383-396.

Kannaiyan, 2000, Bio-fertilizers- Key factor in organic farming. The Hindu Survey of Indian Agriculture, 165-173.

Kumar R, Kumar S, Yashavanth B S, Meena P C, Ramesh P, Indoria A K, Kundu S and Manjunath M, 2020, Adoption of Natural Farming and its Effect on Crop Yield and Farmers' Livelihood in India.

Mahesh G M, 2007, Growth promotion and disease suppression ability of Pseudomonas fluorescens on acid lime. M.Sc. (Ag.) Thesis, Akola.

Nagaraj N and Sreenivasa M N, 2009, Influence of bacteria isolated from panchagavya on seed germination and seed vigour in wheat. *Karnataka Journal of Agricultural Sciences*, 22(1): 231-232.

Palekar S, 2006, *Shoonya Bandavalada Naisargika Krushi*, Swamy Anand publisher, Agri Prakashana, Bangalore, Karnataka.

Reasoner D J and Geldreich E, 1985, A new medium for the enumeration and subculture of bacteria from potable water. *Applied and Environmental Microbiology*, 49(1): 1-7.

Soni V K, 2020, Effects and values of biodiversity in India. *International Journal of Creative Research Thoughts*, 8(10):497-501.

Sreenivasa M N, Nagaraj N and Bhat S N, 2009, Beejamrutha: A source for beneficial Bacteria. *Karnataka Journal of Agricultural Sciences*, 22(5): 1038-1040.

Susleendra Desai, Schlosser E, 1999, Parasitism of *Sclerotium rolfsii* by Trichoderma. *Indian Phytopathology*, 52(1):47-50.

Vincent J M, 1947, Distortion of fungal hyphae in the presence of certain inhibitors. *Nature*, 159(4051): 850.

Vincent J M, 1970, A manual for practical study of root nodule bacteria. I.B.P. Handbook Nr. 15. Blackwell, Oxford Edinburgh

Yang J H, Zhang W W, Zhuang Y Q and Xiao T, 2017, Biocontrol activities of bacteria from cowdung against the rice sheath blight pathogen. *Journal of Plant Diseases and Protection*, 124(2):131-141.

Table 1: *In vitro* evaluation of bacterial isolates of Jeevamrtha against *Sclerotium rolfsii*

Sl. No.	Isolates	Per cent zone inhibition
1	J1	47.78
2	J2	40.33
3	J3	36.8
4	J4	37.84
5	J5	36.57
6	J6	35.8
7	J7	33.88
8	J8	33.72
9	J9	31.48
10	J10	33.44
11	J11	28.75
12	J12	30.43
13	J13	31.62
14	J14	30.43
15	J15	31.62
S. Em. ±		0.55
CD @ 1%		2.17

J- Isolates from Jeevamrutha

Table 2: *In vitro* evaluation of bacterial isolates of Ghanajeevamrtha against *Sclerotium rolfsii*

Sl. No.	Isolates	Per cent zone inhibition
1	GJ1	36.55
2	GJ2	34.27
3	GJ3	32.73
4	GJ4	32.38
5	GJ5	31.14
6	GJ6	31.75
7	GJ7	31.19
8	GJ8	30.16
9	GJ9	30.37
10	GJ10	30.99
11	GJ11	30.52
12	GJ12	29.18
13	GJ13	30.66
14	GJ14	30.74
15	GJ15	28.99
S. Em. ±		0.69
CD @ 1%		2.69

GJ- Isolates from Ghanajeevamrutha

Table 3: Morphological and biochemical characterization of the bacterial isolates of Jeevamrutha and Ghanajeevamrutha

Sl. No.	Isolates	Colony color	Elevation	Cellular morphology	Gram reaction	Indole test	Citrate utilization test	Oxidase test	Catalase test	Starch hydrolysis test	PAL
1	J1	Creamy white	Flat	Rod	+ve	-	+	+	+	+	-
2	J2	Creamy white	Flat	Rod	+ve	-	-	+	+	-	+
3	J3	Creamy white	Convex	Cocci	+ve	+	+	+	+	-	+
4	J4	Creamy white	Flat	Rod	-ve	-	-	+	+	-	-
5	J5	Creamy white	Flat	Rod	+ve	-	+	+	+	+	-
6	J6	Creamy white	Flat	Cocci	-ve	-	+	+	+	-	-
7	J7	Pale yellow	Convex	Cocci	-ve	-	+	+	+	-	-
8	J8	Creamy white	Convex	Rod	-ve	+	+	+	+	-	-
9	J9	Pale yellow	Convex	Rod	-ve	+	+	+	+	-	-
10	J10	Whitish	Flat	Rod	-ve	+	+	+	+	+	-
11	J11	Creamy white	Flat	Rod	+ve	-	+	+	+	-	-
12	J12	Creamy white	Convex	Rod	-ve	+	-	+	+	-	+
13	J13	Whitish	Flat	Rod	+ve	+	+	+	+	-	-
14	J14	Whitish	Flat	Cocci	+ve	-	+	+	+	-	+
15	J15	Whitish	Convex	Cocci	+ve	+	+	+	+	-	-
16	GJ1	Pale yellow	Flat	Rod	-ve	+	+	+	+	-	+
17	GJ2	Pale yellow	Convex	cocci	+ve	+	+	+	+	-	-
18	GJ3	Creamy white	Flat	Rod	+ve	+	+	+	+	-	-
19	GJ4	Pale yellow	Convex	Cocci	+ve	-	+	+	+	-	-
20	GJ5	Creamy white	Convex	Rod	+ve	+	+	+	+	-	+
21	GJ6	Pale yellow	Convex	Cocci	+ve	-	+	+	+	-	-
22	GJ7	Pale yellow	Flat	Cocci	-ve	-	+	+	+	-	-
23	GJ8	Whitish	Flat	Rod	-ve	-	+	+	+	-	-
24	GJ9	Creamy white	Convex	Rod	+ve	-	+	+	+	-	-
25	GJ10	Creamy white	Flat	Rod	-ve	-	+	+	+	-	-
26	GJ11	Pale yellow	Convex	Rod	+ve	-	+	+	+	-	-
27	GJ12	Pale yellow	Flat	Cocci	+ve	-	+	+	+	-	-
28	GJ13	Creamy white	Flat	Rod	+ve	-	+	+	+	-	-
29	GJ14	Creamy white	Flat	Rod	+ve	-	+	+	+	-	+
30	GJ15	Pale yellow	Convex	Rod	+ve	-	+	+	+	-	-

PAL- Phenylalanine Ammonia Lyase