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Isolation and Characterization of Entomopathogenic Symbiotic Bacterium, *Photorhabdus luminescens* of *Heterorhabditis indica* from Soils of Five Agro Climatic Zones of Karnataka

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The present study was undertaken at the Department of Agricultural Microbiology, College of Agriculture, University of Agricultural Sciences, Raichur. We selected Five agro-climatic zones of (covering one hundred and fifty locations) Karnataka to isolate, characterize for morphological and biochemical properties, to screen against some important crop pests and plant pathogens. All the five zones were found to harbor EPNs but with varying degree of occurrence in each cropping system. *Photorhabdus luminescens* was isolated by streaking the haemolymph of the dead larva of *Galleria* on nutrient agar and NBTA plates. A total of eight isolates were obtained. The colonies of *P. luminescens* from all zones were found to be smooth, small, circular, and glistering, convex with entire margin and white with shiny in colour and primary form was turned into secondary form due to prolonged static incubation of the culture. Both the forms of *P. luminescens* from all five zones were long to medium rod shaped, gram negative, motile, bioluminescent bacterium. Primary form was more bioluminescent than the secondary form. The broth culture of *P. luminescens* isolate, Z-8-1 caused 100% nymphal mortality of *Aphis gossypii* after 24 hr. of exposure while the isolate Z-3-1 caused 100% mortality of *T. macferlanei* after 36 hr.s of exposure. However, these two isolates failed to infect DBM and leaf worms - *S. litura* (Fabr.).The present study indicates that plant sap feeders are susceptible to *P. luminescens.*

Key words: Symbiotic bacterium, Crop post, plant pathogen, Karnataka.

Modern agriculture apart from improving the overall production and productivity has also caused destruction to the environment. The increased use of conventional chemical pesticides over the years has not only contributed to an increase in food production, but also resulted in adverse effects on the environment and non-target organisms.

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In view of these side effects, the necessity for sustainable crop production through eco-friendly pest management technique is being largely felt in the recent times. Of the several entomopathogenic microbial pathogens, *viz.*, bacteria, fungi, viruses, protozoans and entomopathogenic nematodes reported so far, only a few have been studied systematically for their commercial utility. A careful evaluation of these beneficial entomopathogenic microorganisms can lead to gainful exploitation in biocontrol programmes[1].

Nematodes, which are capable of killing, sterilizing or seriously hampering the development of insect and completing at least one stage of their life cycle in the host are called entomopathogenic nematode (EPNs). Poinar[2] listed nine families of

entomopathogenic nematodes (Allontone-matidae, Diplogasteridae, Heterorhabditidae, Mermithidae, Neotylenchidae, Rhabditidae, Sphaerularidae, Steinernematidae and Tetradonematidae) which attack insect and kill, sterilize or alter host development. Out of these only steinernematids (Steinernematidae: Rhabditidae) and heterorhabiditids (Heterorhabditidae: Rhabditida) are found effective due to their special qualities like quick action, wide host range and wide distribution. The added advantage is their symbiotic association with bacteria which enhance their efficacy. Due to their efficacy, they are commercially produced and sold in several countries. However, their efficacy mainly depends on the existence of specific conditions such as cool temperature, high relative humidity and compatibility with chemical pesticides.

Entomopathogenic bacterium *Photorhabdus luminescens* is a non spore forming, motile, bioluminescent, Gram negative and facultative anaerobic in nature belonging to the family *Enterobacteriaceae* in the gamma subdivision of the purple bacteria. Infective juveniles (IJs) stage of nematodes of the family *Heterorhabditidae* carry this bacterium in the internal tract[3]. Upon entry into an insect host, nematodes release the bacterium by regurgitation directly into the insect's heamolymph. Once the bacterium enters inside the heamolymph, it replicates rapidly and causes lethal sepsis in the host by producing different toxins that kill the insect within 48-72 hr. By time, the bacterial population reaches high level; the insect cadaver becomes red in colour and visibly bioluminescent. Antibiotics with antifungal and antibacterial properties are produced by *P. luminescens* and these substances prevent invasion of the cadaver by other microorganisms, resulting in ideal condition for the growth and reproduction of nematodes[4].

MATERIAL AND METHODS

The present study was undertaken at the Department of Agricultural Microbiology, College of Agriculture, University of Agricultural Sciences, Raichur. Five agro-climatic zones of (covering one hundred and fifty locations) Karnataka i.e. North Eastern Dry Zone (Raichur), Southern Transition Zone (Belgaum), North East Transition Zone (Bidar), Hill Zone (Sirsi) and Coastal Zone (Honnavar) were sampled for the extraction of EPNs.

Sampling strategy

One hundred fifty locations covering five agro-climatic zones of Karnataka were sampled for the extraction of EPNs. All the locations were of agricultural fields under different cropping ecosystem. To our knowledge, no EPNs based biocontrol based treatment has ever been reported in any of the sampled sites. Locations consisted of 100 m^2 area in which each of the 15 sample points was 6.6 m distance from the nearest.

Isolation of EPNs

EPNs were directly isolated from the soil using a modified *Galleria* - trap technique consisting in place a last instar larvae of *Galleria mellonella* (Lepidoptera: Galleriidae) into plastic vials (2.5 ml capacity) in which circular holes were made on the flat surface and sealed with 80 mesh brass sheet. In such vials 2-3 grown up *G. mellonella* larvae were released along with a piece of wet blotting paper to facilitate easy entry of the nematodes. The traps were treated with 0.04 per cent chlorpyriphos 20 EC in order to prevent the entry of predatory ants. At each location, 30 *Galleria* - traps were placed at 25 cm depth. Traps were left in the soil for 72 h before removal. Once in the laboratory, each vial was opened and dead larvae were placed onto White's trap at 24^{o}C in the dark to collect emerging infective juveniles[5].

Maintenance of laboratory hosts

The laboratory host Greater wax moth larvae*, Galleria mellonella* L. was used for maintenance, multiplication of *H. indica* and isolation of its symbiotic bacterium *P. luminescens*.

Culturing of *G. mellonella* on bee wax comb and on artificial diet

Freshly fallen *Aphis dorsata* combs were collected from the field. Fully grown larvae and pupae of *G. mellonella* were separated from the infested comb and kept in glass containers (10 x 5 cm) for adult emergence. Uninfected combs were sprayed with 0.4 per cent formalin and stored under air tight condition in polythene covers for two days. Later treated combs were shade dried and used as food for the culture. Emerging moths were placed in a glass container with a piece of comb (3 x 3 cm) for egg laying. Cotton soaked in

sugar solution (20%), served as adult food. Combs with eggs were removed daily and kept in separate transparent glass containers for hatching (10 x 5 cm). From these containers third, fourth and fifth instars of *G. mellonella* larvae were separated and used for nematode multiplication and when natural honey comb was not available, *G. mellonella* was reared on its artificial diet .

Rearing of *Plutella xylostella* (L.) (Plutellidae: Lepidoptera)

Diamondback moth was cultured in the laboratory following the method[6]. The culture of the DBM was obtained from farmer's field at Askhyal, Raichur District and reared on leaves of cabbage plants grown in pots under net house condition. Upon pupation, they were collected in Petriplate and placed in mesh cage with glass top (25 cm × 25 cm × 25 cm) for adult emergence. Ten per cent honey solution was provided as food through cotton swab for adults, three days old mustard seedlings raised in sterilized vermiculite in ice cream cups (6 cm × 4.5 cm) were provided for oviposition. The seedlings were replaced at 24 hr. interval. Moths laid eggs on both sides of tender leaves and eggs hatched in 2 to 4 days; neonates mined the tender leaves and fed on it. Within 48 hr. of hatching when the seedlings were about to be completely consumed by the larvae, they were transferred to the cabbage leaves with petiole which are fully expanded and wet cotton swab was provided along with leaves to maintain turgidity of leaf. Raising of mustard seedlings and rearing of DBM larvae were done under laboratory condition, laboratory temperature varied from 25 + 10°C to 30 + 10°C and relative humidity ranged from 60 to 90 per cent.

Rearing of *Spodoptera litura* (F)

Egg massses of *Spodaptera litura* collected from castor field were surface sterilized with 10 per cent formaldehyde, washed 3-4 times with distilled water and kept in petriplates (5 cm diameter) for hatching on a moist filter paper. Freshly hatched larvae were provided with castor leaves in transparent plastic rearing container and covered with muslin cloth. Food was changed twice a day till pupation. Before pupation, larvae were transferred to another container containing sterilized sawdust. Pupae were kept for moth emergence in cages (35 x 25 x 45 cm). Diluted honey (10%) was provided as adult food in small vials with cotton wad. Groundnut plants kept in conical flask with water placed inside the cage for egg laying. Egg masses collected every day were sterilized, washed and incubated to get disease free larvae. First and second instar larvae were reared in small plastic containers (15 cm dia x 20 cm ht) on fresh castor leaves provided twice a day. As the caterpillar grew, they were transferred to slightly bigger containers (35 x 15 cm ht), for continuous supply of third instar larvae to take up further laboratory studies.

Collection of dead larva of *Galleria mellonella* for extraction of infective juveniles from different agroclimatic zone. The dead larvae collected from the trap were transferred to White's traps containing sterile distilled water. The infective juveniles that emerged from the dead larva of *Galleria mellonella* were collected in sterile distilled water.

Purification of infective juveniles

The infective juveniles (100 nos. approximately) were collected from the distilled water using a sterile hypodermic syringe and transferred to Petri plate containing Whatman No. 71 filter paper. Two to three larvae of *Galleria mellonella* were released on to the moist filter paper. After three days, dead larva were collected and placed in White's trap containing sterile distilled water. The infective juveniles that emerged from the dead larva into sterile distilled water were collected and stored as a pure culture of nematodes in 250 ml conical flask containing sterile water.

Maintenance of *H. indica* culture in the laboratory

The infective juveniles (IJs) of *H. indica* harvested from the soil were used as stock culture for further multiplication and maintenance of nematode. The stock solution of nematode suspension was serially diluted in such a way that each one ml contained 100 infective juveniles per five larvae. One ml of the nematode suspension was spread evenly on a filter paper and kept in a Petri dish of 10 cm diameter. Five healthy larvae were released in each Petri dish covered with a lid after inoculation with the nematodes. The Petri dishes were kept in polythene covers and secured with rubber band. The insect cadavers from each Petri dish were shifted to individual modified White's trap to harvest the nematodes by following the methodology given by Prabhuraj[7].

General laboratory procedure for isolation of *P. luminescens*

Media preparation

The selected media like Nutrient agar and broth, NBTA (Nutrient Bromothymol blue Agar) were used for the isolation, maintenance and characterization of the bacterium *P. luminescens.*

Isolation of *P. luminescens*

Five last instars larvae of *G. mellonella* were placed in Petri plates lined with moist filter paper and inoculated with pure culture nematodes @ 100 IJs per larvae. After 72 hr. of inoculation, the dead larval cadavers were surface sterilized by dipping in 95 per cent of ethanol, followed by igniting and plunging in to sterile water. The cadavers were dissected immediately using a pair of sterile scissors and needle in a watch glass. A loopful of haemolymph was streaked on to nutrient agar plates and incubated at 28 ^{o}C for 24-48 hr. in a B.O.D incubator. The plates were observed for the appearance of colonies of *P. luminescens*.

Preparation of pure culture of *P. luminescens*

Later 24 hr. of incubation, a well developed colony was picked up from the plates and streaked on NA plates. The discrete colonies of *Photorhabdus* that appeared on NA plates were picked up and transferred to NA slants and maintained as pure culture for further studies.

Characterization of *P. luminescens*

The characterization of primary and secondary forms of the bacterium *P. luminescens* was done on the basis of cultural characters, morphological characters and biochemical tests as per the standard microbiological procedures[8].

Cultural characteristics

The cultural characteristics such as colony shape, edge, elevation, consistency, surface and color were recorded.

Morphological characteristics

The Morphological characteristics such as Gram's staining , Motility test, Test for bioluminescence

Biochemical characteristics

After observing morphological characteristics both primary and secondary forms were subjected to biochemical tests. The following biochemical characters such as Amylase production test , Hydrolysis of gelatin , Casein hydrolysis, Urease Test, Fermentation of carbohydrates, IMVic tests were studied as per the methods described by Salle[9].

RESULTS AND DISCUSSION

Entomopathogenic bacteria naturally and efficiently restrict the build up of insect pests and plant pathogen, without any interference. This part of the 'Law of Natural Balance' is being inadvertently destroyed by the indiscriminate use of chemical pesticides. This has led to the notion that, all forms of pest control should be integrated and environmentally acceptable to the agricultural ecosystem. It is certain that biocontrol will continue to increase their share very rapidly in the integrated pest management.

Crop pests and diseases are major constraints in food production. Chemical insecticides proved to be effective means of control, but are costly and possess residual problems besides, resulting in development of resistance and resurgence in pests and diseases due to their repeated applications. Hence, there is a strong need for developing eco-friendly, cost effective, biodegradable, easily accessible and target specific alternate methods to control crop pests and diseases. Microbial pesticides are known to possess

Table 1. Details of sampling sites and recovery percentage of entomopathogenic nematodes from different agroclimatic zones and crop ecosystem

Agroclimatic Zones	District/ Taluk	Location	Cropping Ecosystem	Recovery of Entomopathogenic nematodes (%)
North Eastern Dry Zone	Raichur	College of Agriculture	Sapota	-
		College of Agriculture	Mango Orchard	13.3
Southern Transition Zone	Belgaum	Cindoli	Vegetables	-
		Kagawad	Sugar cane	13.3
North East Transition Zone	Bidar	Vadagi	Sugar cane	6.6

Table 2. Morphological and biochemical characteristics of primary forms of *P. luminescens*

S. No	Isolates	Morphological characters		Biochemical test											
		Colony characters													
		NA	NBTA	Gram reaction & cell shape	Motility	1	2	3	4	5	6	7	8	9	10	11
1.	Z-1-1	Convex white, circular, and opaque	Red overlaid by blue colour, circular, raised and opaque	-ve, rod	+ ve	-	-	+	-	-	-	+	+	-	+	+
2.	Z-3-1	White, convex circular, and opaque	Red overlaid by blue colour, circular, raised and opaque	-ve, rod	+ ve	-	-	+	-	-	-	+	+	-	+	+
3.	Z-3-2	White, convex circular, and opaque	Brick red overlaid by blue colour, circular, raised and opaque	-ve, rod	+ ve	-	-	+	-	-	-	+	+	-	+	+
4.	Z-8-1	White, convex circular, and opaque	Brick red overlaid by blue colour, circular	-ve, rod	+ ve	-	-	+	-	-	-	+	+	-	+	+
5.	Z-8-2	Circular, white, convex and opaque	Deep red colour, circular, raised and opaque	-ve, rod	+ ve	-	-	+	-	-	-	+	+	-	+	+
6.	Z-9-1	Circular, white, convex and opaque	Red overlaid by blue colour, circular, raised and opaque	-ve, rod	+ ve	-	-	+	-	-	-	+	+	-	+	+
7.	Z-9-2	White, smll circular, convex and opaque	Red overlaid by blue colour, circular, raised and opaque	-ve, rod	+ ve	-	-	+	-	-	-	+	+	-	+	+
8.	Z-10-1	White, circular, convex and opaque	Deep light red overlaid by blue colour, circular, raised and opaque	-ve, rod	+ ve	-	-	+	-	-	-	+	+	-	+	+

1 - Amylase production test 2 - Hydrolysis of gelatin 3 - Catalase test 4 - Casein hydrolysis 5 - Urease test
6 - Fat hydrolysis 7 - Indole production test 8 - Methyl red test 9 - Voges Proskauer test 10 - Citrate utilization test
11 - Carbohydrate fermentation

Table 3. Morphological and biochemical characteristics of secondory forms of *P. luminescen*

S. No	Isolates	Morphological characters		Gram reaction & cell shape	Biochemical test											
		Colony characters			Motility	1	2	3	4	5	6	7	8	9	10	11
		NA	NBTA													
1.	Z-1-1	Irregular transparent and colourless	Dark red colour	-ve, rod	+ ve	-	-	+	-	-	-	+	+	-	+	+
2.	Z-3-1	Light transparent and not shiny	Light red colour	-ve, rod	+ ve	-	-	+	-	-	-	+	+	-	+	+
3	Z-3-2	Transparent Irregular and colourless	Dark red colour	-ve, rod	+ ve	-	-	+	-	-	-	+	+	-	+	+
4.	Z-8-1	Irregular Not Shiny colonies	Brick red color	-ve, rod	+ ve	-	-	+	-	-	-	+	+	-	+	+
5.	Z-8-2	Irregular transparent and colourless	Brick red color	-ve, rod	+ ve	-	-	+	-	-	-	+	+	-	+	+
6.	Z-9-1	Irregular transparent and colourles	Light red colour	-ve, rod	+ ve	-	-	+	-	-	-	+	+	-	+	+
7.	Z-9-2	Colourless Irregular small transparent	Green colour	-ve, rod	+ ve	-	-	+	-	-	-	+	+	-	+	+
8.	Z-10-1	Irregular transparent and colourless	Dark red color	-ve, rod	+ ve	-	-	+	-	-	-	+	+	-	+	+

1 - Amylase production test 2 - Hydrolysis of gelatin 3 - Catalase test 4 - Casein hydrolysis 5 - Urease test
6 - Fat hydrolysis 7 - Indole production test 8 - Methyl red test 9 - Voges Proskauer test 10 - Citrate utilization test
11 - Carbohydrate fermentation

Table 4. Bioefficacy of *P. luminescence* isolates on *Aphis gossypii*

Treatments	Per cent mortality at different time intervals			
	12 hr	24 hr	36 hr	48 hr
T_1: Z-1-1	35.00 (36.50)	57.50 (48.49)	63.75 (52.99)	71.25 (57.59)
T_2 : Z-3-1	58.75 (50.07)	78.75 (62.57)	86.25 (68.44)	100.00 (90.00)
T_3 : Z-3-2	55.00 (47.88)	70.00 (55.26)	75.00 (60.06)	82.50 (65.55)
T_4 : Z-8-1	70.00 (56.83)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T_5 : Z-8-2	46.25 (42.84)	72.50 (58.49)	72.50 (58.39)	78.75 (62.58)
T_6 : Z-9-1	47.50 (43.24)	62.50 (52.24)	66.25 (54.49)	72.50 (58.39)
T_7: Z-9-2	40.00 (39.22)	68.75 (56.04)	72.50 (58.39)	76.25 (60.86)
T_8 : Z-10-1	50.00 (45.84)	76.25 (60.85)	82.50 (65.32)	87.50 (69.39)
T_9 : Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
S.Em±	1.21	0.89	1.10	1.09
CD at 1%	4.76	3.50	4.30	4.28

Note: Figures in the parentheses are angular transformed values

Table 5. Bioefficacy *P. luminescence* on *Tetranychus macferlanei*

Treatments	Per cent mortality at different time intervals			
	12 hr	24 hr	36 hr	48 hr
T_1: Z-1-1	20.00 (26.48)	42.50 (40.68)	55.00 (47.88)	65.00 (53.25)
T_2 : Z-3-1	55.00 (47.88)	85.00 (67.36)	100.00 (90.00)	100.00 (90.00)
T_3 : Z-3-2	42.50 (40.68)	66.75 (54.49)	75.00 (60.06)	76.25 (60.86)
T_4 : Z-8-1	40.00 (39.22)	63.33 (52.99)	67.50 (55.26)	90.00 (71.86)
T_5 : Z-8-2	32.50 (34.74)	58.75 (50.07)	71.25 (57.59)	73.75 (59.25)
T_6 : Z-9-1	27.50 (31.60)	48.75 (44.06)	61.25 (51.51)	71.25 (57.63)
T_7: Z-9-2	33.75 (35.48)	62.75 (44.28)	63.75 (52.99)	80.00 (63.62)
T_8 : Z-10-1	37.50 (37.75)	62.50 (52.24)	73.75 (59.20)	86.25 (68.44)
T_9 : Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
S.Em±	1.06	1.10	0.84	1.38
CD at 1%	4.15	4.32	3.36	5.40

Note: Figures in the parentheses are angular transformed values

all these qualities and are now encouraged against several pests and diseases. *Bacillus thuringensis* (*Bt*) is one of the oldest and an effective bacterium employed against several crop pests. However, due to over use of *Bt*, several insects are reported to develop resistance. A similar toxin producing bacterium *P. luminescens* has been isolated from an entomopathogenic nematode, *H. indica* which is known to possess antibiotic (antibacterial and antifungal), insecticidal and nematicidal property. The novel toxin groups from this bacterium have shown insecticidal property against *G. mellonella*[10] and secondary metabolites have shown antifungal property against *Botrytis cinera*[11]. With the view of exploiting this bacterium for pest management program, the present investigation was under taken to isolate, characterize for morphological and biochemical properties, to screen against some important crop pests and plant pathogens and the results obtained are discussed in this chapter in the light of earlier reports.

Extraction of entomopathogenic nematodes *Heterorhabditis indica* from soils of five agro - climatic zones of Karnataka

All the five zones were found to harbor EPNs but with varying degree of occurrence in each cropping system. For example, the sugar cane cropping system of both southern transition zone and north eastern dry zones recorded a nematode recovery percentage of 13.33 and 13.33 respectively, but no recovery was recorded in vegetable gardens and ginger cropping system, respectively. In Multicropping system of both hilly zone and coastal zone, the per cent recoveries of EPNs were 13.33 and 6.6 per cent, respectively.

Table 6. Effect of *P. luminescence* isolates on defoliators

Treatments	Per cent mortality of *Plutella xylostella* at different time intervals				Per cent mortality of *Spodoptera litura* at different time intervals			
	12 hr	24 hr	36 hr	48 hr	12 hr	24 hr	36 hr	48 hr
T_1: Z-1-1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T_2: Z-3-1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T_3 : Z-3-2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T_4 : Z-8-1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T_5 : Z-8-2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T_6 : Z-9-1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T_7: Z-9-2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T_8 : Z-10-1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T_9 : Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
S.Em±	-	-	-	-	-	-	-	-
CD at 1%	-	-	-	-	-	-	-	-

Isolation of *P. luminescens* from *H. indica*

P. luminescens was isolated by streaking the haemolymph of the dead larva of *Galleria* on nutrient agar and NBTA plates. Those colonies with red core over laid by dark blue were scored as the primary form of *P. luminescens* and those which were blue after a prolonged incubation are scored as the secondary form of *P. luminescens.* A total of eight isolates were obtained. Isolation of *Photorhabdus* has been reported by A (4, 12, 13) from *H. indica*.

Cultural and morphological characters of *P. luminescens*

Colony characteristics of *P. luminescens* on NA media

The colonies of *P. luminescens* from all zones were found to be smooth, small, circular, and glistering, convex with entire margin and white with shiny in colour. After a prolonged incubation (10 days), all the isolates turned into secondary forms.

Similar observations were made by Nagesh[12] who described colony characters of *P. luminescens* (PDBC isolate). However, they recorded the pigmentation in the colonies in which primary form was yellowish brown and secondary form was orange to red which is contradictory to the present observation where both the primary and secondary forms were white and colorless,

respectively. The variation in the pigmentation might be due to variation in the strains of *P. luminescens.*

Colony characteristics of *P. luminescens* on NBTA media

On NBTA plates, all the morphological characters of primary and secondary forms remained same except the pigmentation in which primary form had red core over laid by dark blue and the secondary form produced dark red colony.

As per the earlier reports by Akhurst[4] the primary form absorbed bromothymol blue and reduced TTC producing colonies with red core over laid by dark blue and surrounded by clear zone. The secondary form failed to absorb bromothymol blue and hence produced dark red colonies without clear zone around them.

In the present study, it was observed that the primary form was turned into secondary form due to prolonged static incubation of the culture. Similar observation was made by Bleakely[14]. The change of primary form into secondary form over a period of time might be due to depletion in the nutrient content of the medium which not only leads to change in the morphological character

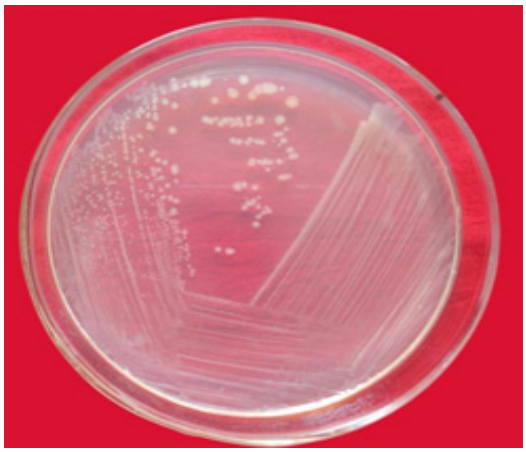

Plate 1. Primary from of *P. luminescens* Z-8-1 on nutrient agar plate

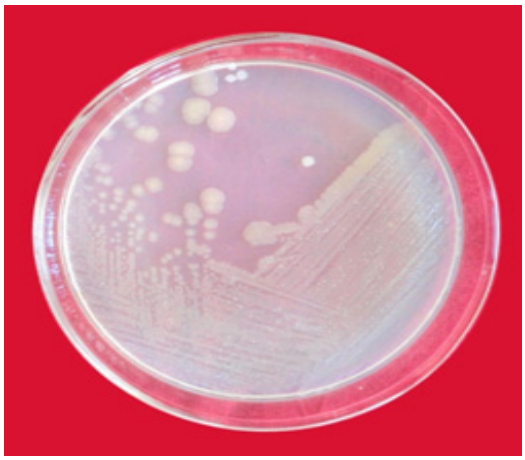

Plate 2. Secondary from of *P. luminescens* Z-8-1 on nutrient agar plate

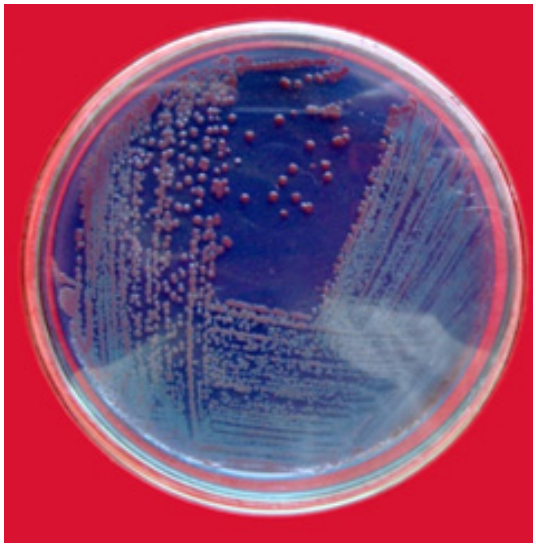

Plate 3. Primary from of *P. luminescens* Z-8-1 on NBTA plate

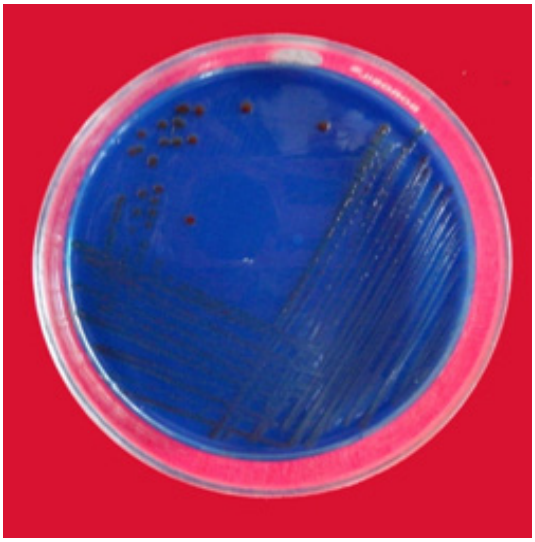

Plate 4. Secondary from of *P. luminescens* Z-8-1 on NBTA Plate

but also affect the biochemical, antibiotic and antagonistic activity. Similar observation was made by Akhurst[4].

Morphological character

Both primary and secondary forms of *P. luminescens* from all five zones were long to medium rod shaped, gram negative, motile, bioluminescent bacterium. Primary form was more bioluminescent than the secondary form. Similar observations were also made by previous researchers[14].

Biochemical characters of *P. luminescens*

The primary form was found to be positive for fermentation of carbohydrates, methyl red and citrate utilization test indicating its capability to utilize sugar as a source of energy, produce organic acid from glucose and to utilize citrate as sole source of carbon by citrate enzyme, respectively. However, it failed to liquefy gelatin, amylase production, hydrolyze casein, and utilize urea and to produce indole indicating the absence of *gelatinase, protease, urease* and *tryptophanase* enzymes, respectively. Similar observations were also made by Akhurst[4].

Screening of *P. luminescens* isolates on sucking and defoliators

The broth culture of *P. luminescens* isolate, Z-8-1 caused 100% nymphal mortality of *Aphis gossypii* after 24 hr. of exposure while the isolate Z-3-1 caused 100% mortality of *T. macferlanei* after 36 hr.s of exposure. However, these two isolates failed to infect DBM and leaf worms - *S. litura* (Fabr.).The present study indicates that plant sap feeders are susceptible to *P. luminescens*. Further, the present findings are in agreement with the report of Bussman[15] who recorded the susceptibility of mushoom mite to *P. luminescens*. However, the susceptibility of *H. armigera, S. littorali, S. litura* and *P. solani* to *P. luminescens* have been reported[13, 16-17]. The variation in the susceptibility might be due to the difference in the nematode species from which the bacteria were isolated.

CONCLUSIONS

a) *Heterorhabdus indica – P. luminescens* symbiosis is well documented. Nevertheless, a very few reports with scanty information is available in Indian literature on the diversity and bioefficacy of *P. luminescens*. Therefore, to work out the information gap on the diversity of *P. luminescens*, an attempt was made to isolate *P. luminescens* from different agroclimatic zones of Karnataka to select efficient strains of *P. luminescens* for the control of sucking pests and defoliators and also for the control of plant pathogens.

b) A total of eight isolates of *P. luminescens* were isolated from different agro – climatic conditions of Karnataka. All the isolates confirmed their pathogenicity against sucking pests as well as plant pathogens under *in vitro* conditions.

c) Pathogenicity of these isolates against insect pests and plant pathogens varied with the isolates.

d) *In vitro* screening of these isolates yielded two efficient strains, Z-8-1 and Z-3-1 for the control of sucking pests such as aphids and red spider mite, respectively.

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