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Research Article

Analysis about Seasonal Dissimilarity of Endophytic Bacteria Isolated from different Indigenous Plants

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Abstract: Bacterial endophyte infect host plant without causing any detectable symptoms, these groups of bacteria are generally persistent. In all fifteen Indigenous plants were selected for isolation of endophytic bacteria. About 250 total bacterial endophytes were isolated as, 74 were from root, 88 from stem, and 88 from leaf tissues. Information obtained was evaluated and analysis proves that endophytes are not much affected by climatic conditions and are capable to survive inside host plants.
Keywords: Bacterial endophytes, standard deviation, Value box plot

INTRODUCTION

Microbes living within plant tissues without instigating any visible symptoms of their presence are known as endophytes, [1,2]. Studies on microbial endophytes over the past 25 years indicate that they occupy a distinctive ecological niche and are supposed to influence plant distribution, physiology and biochemistry. Endophytic bacteria are omnipresent in most plant species, through their active colonization of plant tissues and their latent residence. It is worthy to note, that practically 300,000 plant species that exist on the earth, each plant is host to one or more bacterial endophytes. They reside in several tissues, seeds, roots, stems and leaves [3]. Host plants get benefit by sheltering these microorganisms as they promote plant growth[4] and confer improve resistance to various pathogens, [5,6,7] .Question arise why do plants quay such a load of bacteria internally? Would it be an unnecessary stress on plant metabolism? These questions are needed to be answered satisfactorily. The purpose of this investigation is to find out the influence of change in season with evident to tissue specificity of visibly occurring bacterial endophytes in selected host plants.

MATERIALS AND METHODS

The number of plant species in the world is so great, creative strategies used to quick search for isolation of endophytic bacteria. Several reasonable theories direct plant selection strategies such as, plants that have ethano botanical history, have occupied a certain original land mass having biodiversity with prospects of accommodating endophytes. Mature healthy plant materials were collected from Jalgaon, India (geographical elevation about 209 meters) and immediately brought to laboratory within 8 hrs, during three different seasons such as monsoon, winter and summer. Bacterial endophytes were isolated from roots and rhizomes of *Aloe vera* (L.) Burm.f. and *Curcuma longa* (L.) correspondingly. Plant tissues such as leaves, stem and roots were broadly screened for presence of endophytic microbes from *Azadirachta indica* A. Juss., *Coriandrum sativam* L., *Eucalyptus globules* Dehnh, *Hibiscus rosa sinensis* L., *Ixora coccinea* L., Murrayo *koenginii* (L.) Sprengel, *Musa paradiasica* L., *Ocimum sanctum* (L.), *Pongamia glabra* Vent., *Sphaeranthus indicus* Linn, *Vinca rosea* (L.) G. Don., *Vitex nigundo* (L.) and W*ithania somniphera* (L.) Dunal.

Samples collected from these plants were clean with distilled water to remove adhered unwanted debris. Sub sections were prepared from each sample for further isolation of endophytes and immersed in 70% ethanol for 1-3 min and 4% aqueous solution of sodium hypochlorite 1.5 min, l min with 70 % ethanol again and finally rinsed 4-5 times with sterile distilled water [8,9]. By aseptic cutting using sterile blade and mechanical instruments, inner tissues were excised. Later the segments were blotted on sterile blotting paper in the laminar air flow using. Sections divided in pieces of 1 cm long and inoculated aseptically on to sterile nutrient agar with 50mg/l cyclohexamide and incubated at 30^0C for 24-96 hrs. Pure endophytic cultures were isolated from crowded plates and maintained on fresh nutrient agar medium, [10,9].

Colonization frequency

% Colonization frequency of an endophyte equals to the number of segments colonized by a single endophyte divided by the total number of segments observed **X** 100, [10].

$$\% \text{ Colonization frequency} = \frac{\text{Number of segments colonized endophyte}}{\text{Total number of segments}} \text{ X } 100$$

RESULTS

In experimental attempt, we used 15 plants of different families about 250 bacteria were isolated, 74 were from roots, 88 from stems, and 88 from leaves, isolated endophytes then distributed in different groups according to their colonization frequency as shown below in Table 1.

Table-1: Colonization frequency by endophytic bacteria in different groups

Groups	Host Plant	Range	% CF
I	*A.vera*	1-5	5
II	*S.indicus*	5-10	6
	P.glabra		6
	H.sinesis		8
	W. somniphera		9
III	*I. coccinea*	10-15	11
	C.longa		14
IV	*E.globules*	15-20	16
V	*M. paradiasica*	20-25	20
	C.sativum		21
	O.sanctum		23
VI	*M.koenginii*	25-30	26
	A.indica		27
	V.nigundo		28
	V.rosea		30

% CF: per cent colonization frequency

Results depicted in groups shows that frequency of colonization by endophytic bacteria is low in plant *A.vera*. Other host plants *S.indicus, P.glabra, H.sinesis, W. somniphera, I. coccinea, C.longa, E.globules, M. paradiasica, C.sativum, O.sanctum, M.koenginii, A.indica, V.nigundo* have more/less or same number of colonization by bacterial endophytes. It was remarkable to note that *V.rosea* is the richest source of bacterial endophytes (30%).

Endophytic bacteria were isolated from roots of selected plants. The occurrence and variation in number is shown below in Figure 1. Colonization frequency ranges from 1-5%, rhizomes of *C.longa* were highly colonized by endophytic bacteria during all seasons of the year, followed by *V.rosea* (3%). Generally bacterial endophytes were not much affected by seasonal variations, they were consistently present in all seasons in plants like *I.coccinea, M.koenginii, V.rosea, W.somniphera*. From plants like *E.globules, C.sativum, M.paradiasica* and *O.sanctum*, bacterial flora was isolated regularly in all seasons.

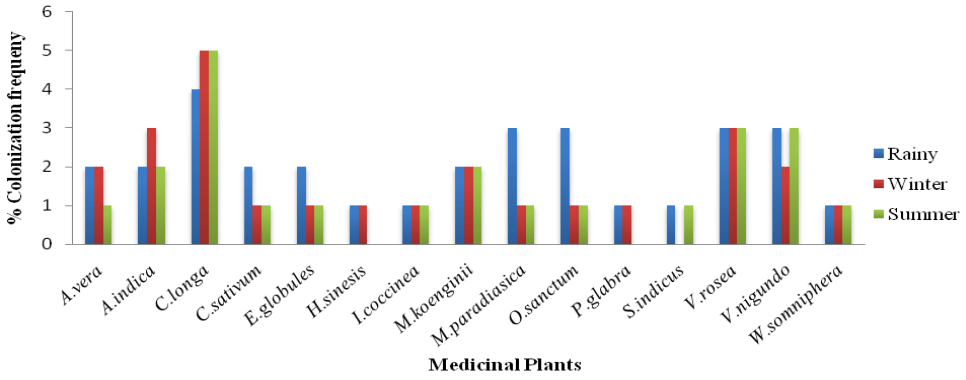

Fig-1: Colonization frequency by endophytic bacteria from root tissues

Stem tissues of host plant represents colonization frequency from 1-5%. In concern with plants like *A.indica*, *E.globules*, *H.sinesis*, *O.sanctum*, and *W.somniphera* bacterial endophytes were isolated frequently through out the year. From *P.glabra* and *S.indicus* bacteria were not grown during winter season. Maximum flora isolated during winter season from *M.koenginii* (5%), followed by *A.indica* (4%) and *C.sativum* (3%). Stem tissue of *V.nigundo* harbors rich bacterial endophytes, as it is evident from Figure 2 below.

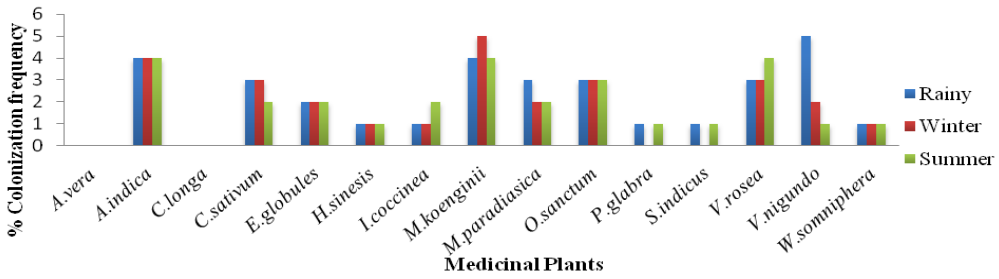

Fig-2: Colonization frequency by endophytic bacteria from stem tissues

Colonization frequency by bacterial endophytes from leaf tissues of selected plants ranges from 1-4%. Leaf tissues of *C.sativum*, *O.sanctum*, *E.globules*, *H.sinesis* and *W.somniphera* found rich source of bacterial endophytes throughout all seasons. During rainy season, good flora isolated from *V.rosea*, *M.koenginii*, *A.indica*, *V.nigundo*. From plants like *P.glabra* and *S.indicus* bacterial endophytes were isolated during rainy and winter seasons this is not the case in summer. Good number of isolates found in winter and summer seasons from *I.coccinea*, *M.koenginii* and *M.paradiasica*. Highest bacteria found for the duration of summer from host plant *V.nigundo* as shown below in Figure 3.

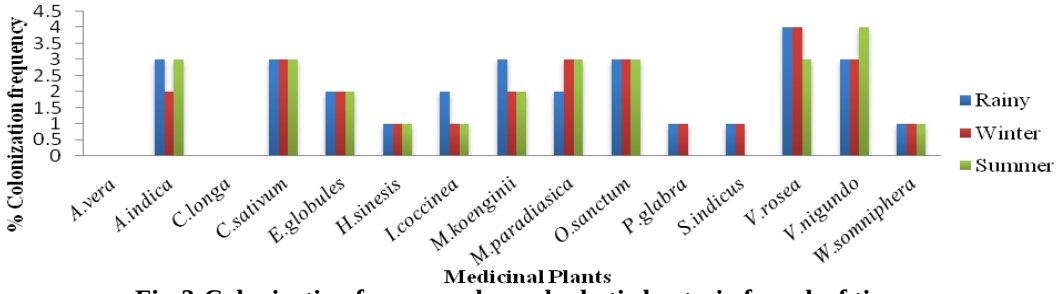

Fig-3:Colonization frequency by endophytic bacteria from leaf tissues

Table 2 and 3 indicates analysis of variance and summary statistics of root endophytic bacteria (one way ANOVA).

Similarly, Table 4 and 5 indicates analysis of variance and summary statistics of stem endophytic bacteria (one way ANOVA)

Table-2: Analysis of Variance for root endophytes

Source	DF	SS	MS	F	P
Factor	2	2.31	1.16	0.84	0.440
Error	42	58.00	1.38		
Total	44	60.31			

DF:degree of freedom; **SS**:sum of squares; **MS**:mean sum of square; **F**:ratio; **P**:value

Table-3: Summary statistics of bacteria from root tissues

Level	N	Mean	StDev
Rainy	15	2.067	0.961
Winter	15	1.667	1.234
Summer	15	1.533	1.302

Pooled Stdev = 1.175 *n:number of observation; Stdev:standard deviation

H0=M1=M2=M3 Vs H1= M1 ≠ M2 ≠ M3
Here, Ho: For all three seasons bacteria in roots are same
H1: For all three seasons bacteria in roots are not same, (M=Mean)
(If P value< 0.05, then reject H0).Since, P value=0.004<0.05 (Table 2. and 3.). Hence,
Ho is rejected, Therefore, bacteria in root for three seasons (rainy,winter,summer) not same. This is better explained using box plot graph (figure 4) .

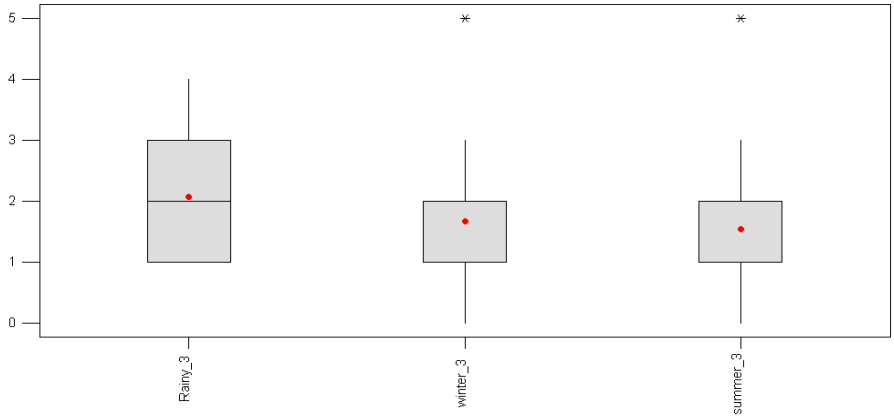

Fig-4: Box plot of root endophytic bacteria in different seasons

Table 4.Analysis of variance for stem endophytes

Source	DF	SS	MS	F	P
Factor	2	1.08	0.54	0.29	0.753
Error	36	67.85	1.88		
Total	38	68.92			

DF: degree of freedom; **SS: sum** of squares; **MS**: mean sum of square; **F**: ratio; **P**: value

Table-5: Summary statistics of bacteria from stem tissues

Level	N	Mean	St Dev
Rainy	13	2.462	1.391
Winter	13	2.077	1.498
Summer	13	2.154	1.214

Pooled Stdev = 1.373 *n:number of observation; Stdev:standard deviation

H0=M1=M2=M3 Vs H1= M1 ≠ M2 ≠ M3
Here, Ho: For all three seasons bacteria in stem are same;
H1: For all three seasons bacteria in stem are not same, (M=Mean)
(If P value < 0.05 , then reject H0), P value=0.753>0.05. Here, Ho is not rejected (as shown by box plot of stem endophytes in Figure 5.)

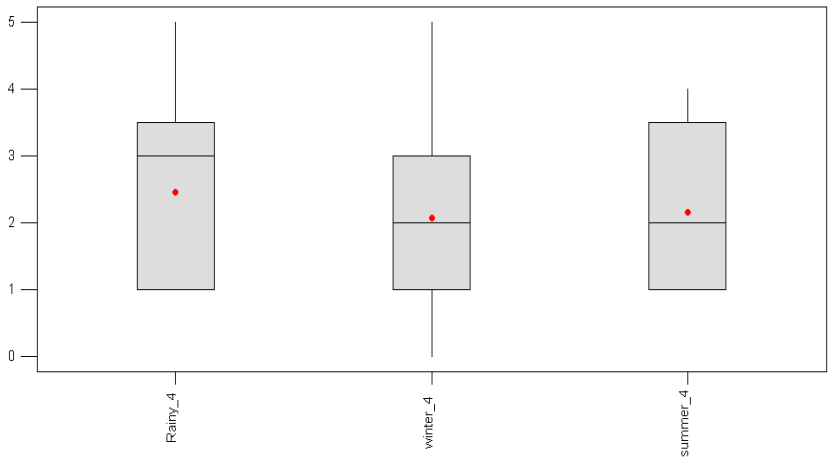

Fig-5: Box plot of stem endophytic bacteria in different seasons

Following table (6,7) also explains variance and statistics of endophytic bacteria isolated from leaf (one way ANOVA).

Table- 6: Analysis of Variance for leaf endophytes

Source	DF	SS	MS	F	P
Factor	2	0.36	0.18	0.14	0.867
Error	36	45.23	1.26		
Total	38	45.59			

DF:degree of freedom; **SS**:sum of squares; **MS**:mean sum of square; F:ratio; P:value

Table-7: Summary statistics of bacteria from leaf tissues

Level	N	Mean	StDev
Rainy	13	2.231	1.013
Winter	13	2.077	1.038
Summer	13	2.000	1.291

Pooled Stdev = 1.121 *n:number of observation; Stdev:standard deviation

H0=M1=M2=M3 Vs H1= M1 ≠ M2 ≠ M3
Here, Ho: For all three seasons bacteria in leaf are same;
H1: For all three seasons bacteria in leaf are not same, (M=Mean)
(If P value< 0.05, then reject H0), P value=0.867 >0.05, Hence, Ho is not rejected. (also according to Figure 6. given below)

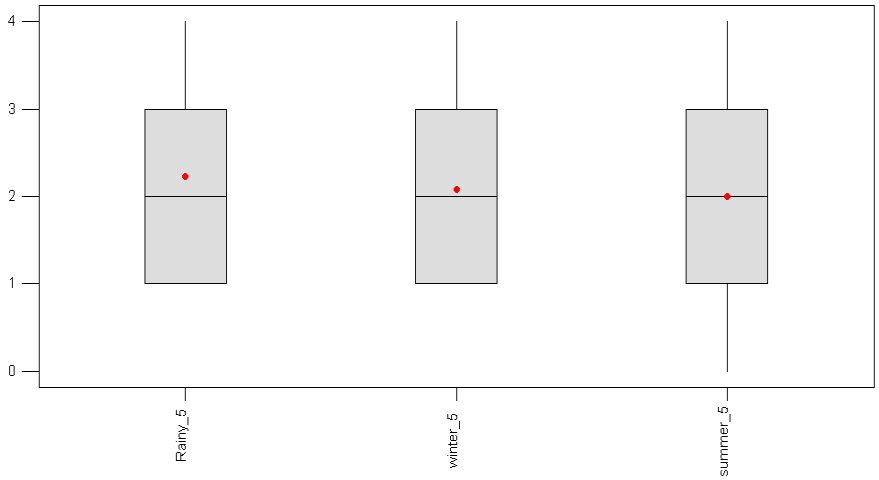

Fig-6: Box plot of leaf endophytic bacteria in different seasons

Massive endophytic bacterial flora was isolated and per cent colonization frequency found nearly same for leaf and stem tissues, while less number of endophytes isolated from root tissues. To determine that whether endophytic bacteria had the ability to inhabit and persevere in plant hosts, studies were done. The obtained was statistical, analysis of variance, pooled standard deviation and value box plot studies. Indicating that occurrence of endophytic bacteria was tissue specific. Our research goals were to select indigenous plants for the presence of endophytic bacteria and to determine their variation with respect to tissue specificity, host specificity and change in seasons. Investigation verified that multiple endophytes occurred in single plants.

DISCUSSION

Endophytic bacteria also known as important component of biodiversity, [12,13] and are pretentious by deviation in climatic conditions. During the course of investigation endophytic bacteria were frequently isolated during all seasons indicating non specific for temperature variations, as demonstrated. Fluctuation in colonization has been observed during the showery season compared to dry seasons. These may be due to alteration in environmental factors that should be affecting the bacterial populations. Endophytes are not much affected by climatic conditions and variation in their occurrence is shown statistically. Endophytic bacteria are capable to survive inside host plants and are not host specific. It is important to point out that individual plants comprise communities of

microorganisms and plants growing in this region (Jalgaon) harbor diverse species of endophytic microbes. Such observations are in good agreement with result stated by Suryanarayan [14]. By studying multiple endophytes within individual host and multiple host plant within a single habitat, we found that diverse group of endophytes are present among single host plant species.

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