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Antimicrobial Activity and Bacterial Potency of Sphingomonas Paucimobilis as Endophytes Isolated from Leaf of Citrus Limon

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Abstract - Endophytes are to be found inside of the plant which truly matters all around on earth. These microorganisms live in the living tissues of the host plant and do as such in an assortment of connections, going from harmonious to somewhat pathogenic. It occupies such biotopes, to be specific, higher plants, which is the reason they are presently viewed as a wellspring of novel auxiliary metabolites offering the potential for therapeutic, horticultural, as well as mechanical abuse. Planting organic product trees likewise have numerous accommodating ecological advantages, from cleaner air to decreased vitality expenses and green occupations. *Citrus* variety is the most significant natural product tree crop on the planet and lemon is the third most significant *Citrus* species. This bacteria isolated from leaf of *Citrus limon*, selected from *in planta* experiment were subjected to antimicrobial activity by agar well method.

Key Words*: Antimicrobial activity, *Citrus limon, Endophytes, Sphingomonas paucimobilis.

1. INTRODUCTION

Plants have been utilized as a wellspring of new therapeutic mixes from the beginning of time and keep on filling in as the reason for a large number of the pharmaceuticals utilized today. [1] (Cragg GM, Grothaus PG, et. al., 2009) Antibacterial action of *Citrus* remove against nourishment borne decay microbes was explored by [2] Verma et al. (2012). Citrus Limon separates showed a strong antimicrobial activity against *Salmonella enteritidis, E. coli* and *S. aureus* yet in factor degree and with different MIC depending on the plant isolated and pathogenic living thing. [3];[4](Ahmad et al., 1998; Akinyemi et al., 2006) Basic oils are important common items utilized as crude materials in numerous fields, including aromas, beautifiers, fragrance based treatment, phytotherapy, flavors and nourishment [5] (Buchbauer, 2000).

All plants are important medically. The plant endosphere contains a diverse group of microbial communities. Endophytes are the microorganisms may be bacteria; fungi or actinomycetes. Endophytic organisms live within the plant tissues and can promote host species tolerance to different environmental stresses. Almost all plants species have been found to harbour endophytic bacteria or fungi [6] Sturz and Nowark, 2000).The term Endophytes was first coined by De Berry in 1866. They are also reported to supply essential vitamins to plants [7] Rodelas et al., 1993), confer protection against plant pathogenic microorganisms via production of antibiotics or synthesis of secondary metabolites [8] Long et al., 2005). Endophyte containing plants grow faster than the none containing ones [9] Cheplick et al., 1989). Endophytes would have enhanced the hosts' uptake of nutritional elements such as nitrogen and phosphorus [10] Gasoni and Gurfinkel, 1997; [11] Malinowski and Belesky, 1999). The beneficial effects that the endophytes can confer on plants have made their role highly significant in biological control of diseases in various crops [12] (Bargabus *et al*., 2004); [13] (Kloepper *et al*., 2004).

2. MATERIAL AND METHODS

2.1. Sample

The *Citrus limon* (Lemon) plant was collected from the Scientific Nursery, Department of Horticulture, AAU, Anand, Gujarat, India latitude of 22.56°N and longitude of 17.95°E by a random sampling method in the sterile zip lock poly bags.

2.2. Endophytes

Endophyte MSZLc isolated from leaf of *Citrus limon* plant on Nutrient agar and Tryptic soy agar medium [14] (Welington L. Araújo et al., 2002). Morphologically characters using Gram's staining and string test with 5% KOH.

2.2. Chemicals and reagents

Sodium hypochlorite, tween 20, sodium bicarbonate, sodium nitrite, hydrogen peroxide, hydroxide, hexane, chloroform, methanol, and ethanol all chemicals used from Sigma and SRL brand.

2.3. Instruments used

Electric microscope (Lawrence and Mayo), Laminar air flow (Bright), Refrigerator (Kelvinator Master cool Deluxe), Deep freezer (Blue star), Orbital shaker (Ssi) and Autoclave (EQUITRON).

2.4. Vitek Identification

Bacterial partial identification by BCD Card of Vitek machine by Supra tech Patho Lab Paldi, Ahmedabad, Gujarat, India [16](Rathod et.al 2018)

2.5. Antimicrobial Activity

Antimicrobial potentials of test cultures were carried out by agar well diffusion method. Antibacterial and Antifungal activity were screened using dual culture method in which both test endophytes and pathogens were inoculated in same media plates. Suspension of pathogenic bacteria and fungi was spread on Nutrient Agar and test cultures were inoculated in wells. By measuring the zone of inhibition antimicrobial activity was calculated. For antibacterial activity *Escherichia coli, Staphylococcus aureus, Salmonellatyphi, Proteus valgaris, Pseudomonas aeruginosa, Serratiamarcescens, Micrococcus luteusand Enterobacter aeruginosa standard strains* were used.

For antifungal activity *Macrofornina phaseolina, Fusarium oxysporium, Sclerotinum rolfsii, Tricoderma asperellum* and *Rhizoctonia solani* were used.

2.6. Inoculums preparation

The bacterial strain was sub cultured overnight at 37^{o}C ±2 in Nutrient agar slants. The bacterial growth was harvested using 5 ml of sterile saline water, its absorbance was adjusted at 580 nm and diluted to attain viable cell count of 10^{7} CFU/ml using spectrophotometer [15] (Rathod et. al 2020).

3. RESULTS AND DISCUSSION

3.1. Endophyte from *Citrus limon*

Isolation of Endophyte was done with surface sterilization [14] (Welington L. Araújo et al., 2002) under laminar air flow. MSZLc isolated from leaf of *Citrus limon* plant on Nutrient agar and Tryptic soy agar medium using streak plate and four flame method incubated at ±37°C for a week. Repeated it in thrice. The culture transferred for confirmation incubated at ±37°C for 24-48 h and preserved it below 4 ^{o}C in refrigerator on Nutrient agar slant.

Figure 1: Leaf of *Citrus limon* as a Sample

Table -1: Morphological characteristics of isolate

Characters	MSZLc
Size	Small
Shape	Thin rod
Arrangement	Single or chain
Gram's Reaction	Gram's Positive
KOH test	Negative

Table -2: Cultural characteristics of isolate

Characters	MSZLc
Size	Pinpoint
Shape	Punctiform
Margin	Entire
Elevation	Pulvisate
Texture	Punctate
Viscous	Moist
Opacity	Translucent
Pigment	Yellow

Morphological, cultural and physiological characteristics of the strain was studied using electric microscope with gram's staining (Table 1), (Table 2) and (Figure 1). Thread like string formation was not observed under KOH test. The isolates MSZLc had been identified as *Sphingomonas paucimobilis* from BCD Card of Vitek machine bacteriological identification (Table 3). Table 4 shows the results of bacterial potency.

Figure 2: Endophyte isolated from Leaf of *Citrus limon*

Table -3: Test Substrates on BCL Card and Results

Biochemical details	Amount/ Well (mg)	MSZLc
Ala-Phe-Pro-ARYLAMIDASE	0.0384	+
ADONITOL	0.1875	-
L-Pyrrolydonyl – ARYLAMIDASE	0.018	+
L-ARABITOL	0.3	-
D-CELLOBIOSE	0.3	-
BETA-GALACTOSIDASE	0.036	-
H2S PRODUCTION	0.0024	-
BETA-N-ACETYL GLUCOSAMINIDASE	0.0408	-
Glutamyl Arylamidase pNA	0.0324	-
D-GLUCOSE	0.3	-
GAMMA-GLUTAMYL-TRANSFERASE	0.0228	+
FERMENTATION/GLUCOSE	0.45	-
BETA-GLUCOSIDASE	0.036	+
D-MALTOSE	0.3	-
D-MANNITOL	0.1875	+
D-MANNOSE	0.3	-
BETA-XYLOSIDASE	0.0324	-
BETA-Alanine Arylamidase pNA	0.0174	-
L-Proline ARYLAMIDASE	0.0234	+
LIPASE	0.0192	-
PALATINOSE	0.3	-
Tyrosine ARYLAMIDASE	0.0276	+
UREASE	0.15	-
D-SORBITOL	0.1875	-
SUCROSE	0.3	-
D-TAGATOSE	0.3	-
D-TREHALOSE	0.3	-
CITRATE(SODIUM)	0.054	-
MALONATE	0.15	-
5-KETO-D-GLUCONATE	0.3	-
L-LACTATE alkalinisation	0.15	-
ALPHA-GLUCOSIDASE	0.036	+
SUCCINATE alkalinisation	0.15	-
BETA-N-ACETYL GALACTOAMINIDASE	0.0306	-
ALPHA-GALACTOSIDASE	0.036	-
PHOSPHATASE	0.0504	-
Glycine ARYLAMIDASE	0.012	-
ORNITHINE DECARBOXYLASE	0.3	-
LYSINE DECARBOXYLASE	0.15	-
L-HISTIDINE assimilation	0.087	-
COUMARATE	0.126	-
BETA-GLUCORONIDASE	0.0378	-
O/129 RESISTANCE (comp. vibrio)	0.0105	-
Glu-Gly-Arg- ARYLAMIDASE	0.0576	-
L-MALATE assimilation	0.042	-
ELLMAN	0.03	-
L-LACTATE assimilation	0.186	-
Identified as	***Sphingomonas* paucimobilis**	

Key: (+) test positive; (-) test negative

Table -4: Bacterial Potency

Isolates	MSZLc	Control
% NaCl tolerance	10.5	-
% Bile salt tolerence	1.5	-
6th day zone of PO4 solubilizing	24mm	-
Ammoniya production of 8th day	+++	-
IAA Results as per Salkowsky's Reagents	+++	-
IAA Results as per SIM's Medium	+ Highly motile	-
Qualitative Analysis of EPS Growth	+++	-
1% Glucose	++	-
1% Sucrose	-	-
1% Arabinose	-	-
1% Dextrose	+	-
1% Fructose	-	-
1% Galactose	+	-
1% Lactose	-	-
1% Maltose	+	-
1%Mannitol	++	-
1% Xylose	-	-
1% Ribose	+	-
1% Mannose	+++	-
Catelase test	Positive	-

Key: (+) visible growth; (++) viable growth; (+++) maximum growth; (-) no growth

3.2. Antimicrobial activity

Antimicrobial assay performed using Nutrient agar by agar well diffusion method adding 1ml of MSZLc culture suspension and incubated at ±37°C for 24-48 h. observing the zone of inhibition of bacterial growth against MSZLc to standard pathogenic bacteria strains listed in Table 5. It is evident that the bacterial strains isolated from agricultural soil were able to grow in the presence of pesticides. High concentrations of the pesticides in soils have already been reported by Nawab et al. (2003) [17].

Table -5: Antibacterial activity of endophyte

Sr. No.	Pathogenic bacteria strains	Zone of inhibition (mm)
1	*E.coli*	22mm
2	*Staphylococcus aureus*	27mm
3	*Salmonella typhi*	23mm
4	*Proteus valgaris*	24mm
5	*Pseudomonas aeruginosa*	26mm
6	*Serratia marcescens*	28mm
7	*Micrococcus luteus*	21mm
8	*Enterobacter aeruginosa*	20mm

Table -6: Antifungal activity of endophyte

Sr. No.	Pathogenic bacteria strains	Zone of inhibition (mm)
1	Macrofornina phaseolina	22mm
2	Fusarium oxysporium(White)	27mm
3	Fusarium oxysporium(pink)	23mm
4	Sclerotinum rolfsii	24mm
5	Tricoderma asperellum	26mm
6	Rhizoctonia solani	28mm

Endophytic bacteria are attracting increasing attention not only for their promotion of plant growth and control of plant diseases [18] Backman, P. Avet al., (2008), but also for their stress tolerance and improvement of plant growth in an extreme environments [19] Jha *et al.* 2011. Endophytes play a major role in physiological activities of host plants influencing enhancement of stress tolerance, nematode and disease resistance; [20] Hallmann and Siora 1996; [21] Azevedo et al 2000).

4. CONCLUSIONS

In view of these studies, the endophyte isolated from Leaf of *Citrus limon* for the antibacterial strength of *Citrus limon* plant. The present study indicates that endophyte of the leaves showed higher antibacterial activity against some of plant and human pathogenic bacteria. Apart from fruit, the leaf also contains the high quality of antimicrobial agents having good endophyte itself. Along with vitamin C the different potency also present in leaf of *Citrus limon* which is beneficial for agriculture to increase soil fertility and also in pharmaceutical as good antimicrobial property it have. From the present study it can be concluded that endophytes are metabolically active within their hosts and play a vital role in maintaining the host endophytic mutualistic balance.

CONFLICT OF INTEREST

The authors have no conflict of interest in preparing of this article.

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