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Research Article

Assessment of Endophytic *Bacillus* spp. As Exopolysaccharide Producers with Reference of FTIR Analysis

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Abstract

The objective of this study was to evaluate quantitative and qualitative EPS production potency of endophytic *Citrus limon* (Lemon) leaf isolates D, N and A which are identified as Bacillus spp. according to their morphological characteristics. Endophytic Isolate D identified as *Bacillus subtilis*, Endophytic Isolate N identified as *Bacillus tequilensis* and Endophytic Isolate A identified as *Bacillus amyloliquifaciens*through16s rRNA nucleotide gene expression study. EPS production studied using different sugars 5% sucrose, 5% lactose and 5% dextrose. Fourier transform infrared spectroscopy (FTIR) was performed of EPS produced by isolates D, N and A in 5%sucrose to know functional group presents in it.

Keywords: Endophytes; Exopolysaccharide; FTIR

Introduction

Any organism occurring inside plant tissues are known as endophytes [34]. The term 'endophyte' was first coined by De Berry in 1866. Endophytes are able to colonize internal tissues of living plants without having any destructive effect on plants [2,15,23]. Almost all plants species have been found to harbour endophytic bacteria or fungi [31]. Endophytes are presumably ubiquitous in the plant kingdom with the population being dependent on host species and location. Several reports have shown that endophytes have important role in protecting their host plant against predators and pathogens [9]. The relationship between the endophytes and its host plant ay ranges from latent pathogenesis to mutual symbiosis [29]. Endophytes colonizing inside plant tissue usually get nutrition and protection from the host plant. In return they confer profoundly enhanced fitness to the host plant by producing certain functional metabolites. Endophytes containing plants grow faster than the non-containing ones [6]. Endophytes have been revealed to produce secondary metabolites for example polyphenol, indole, peptides, alkaloids, pyrroliziidines, terpenoids, quinines, steroids, isocoumarins derivatives etc. [1,30,33,35,38]. Different biopolymers are secreted by microbial cells for instance polysaccharide, polyester, polyamides into its surroundings. Endophytes are decent producers of bioactive EPS [5,11,18,25,26,28,36]. Exopolysaccharide (EPS) is high molecular weight compounds formed by polymerization of homo- and hetero- monomeric sugar residue [37]. EPS produced by microorganisms lead to formation of soil aggregates which forms immediate environment where plants can take up water and nutrients for their growth [10,17]. EPS produced by plant growth promoting bacteria makes slime which makes protective sheath around soil aggregates, when plants are inoculated

with EPS producing bacteria (Asharaf., *et al*. 2004; Naseem., *et al*. 2012). EPS produced by bacteria can improve plant productivity as they improve aggregate formation and physicochemical properties of soil [32].

FTIR (Fourier Transform Infrared Spectroscopy) is vibrational spectroscopic method which is simple, rapid, sensitive, accurate [12] and non-destructive to tissue and only small amount of material with minimum sample preparation are required. This type of analysis can be used for characterizing samples in forms of liquids, solutions, pastes, powders, films, fibres, and gases. This analysis is also possible for analysing material on the surfaces of substrate [8]. These techniques also provide molecular level information allowing investigation of functional groups [13], bonding types and molecular confirmation. Spectral bands in vibrational spectra are molecule specific and provide direct information about biochemical composition. These bands are relatively narrow, easy to resolve, and sensitive to molecular structure, confirmation and environment [20].

Materials and Methods

Collection of isolates

Isolates are endophytes and obtained from leaf of *Citrus limon* (Lemon) tree (Rathod Z., *et al*. 2020). Isolates were transferred on Nutrient agar media for further study [22].

Characterization of isolates

Isolates were characterized by morphological and cultural characterization. Morphological characterization was done by Gram staining with the use of Gram staining reagents like Crystal violet, Gram's iodine, Decolourizer (acetone), Safranin, etc. Organisms were observed under microscope in oil immersion lens. Cultural characterization was done by eye visualization of Nutrient agar media and note down results.

Qualitative and Quantitative estimation of Exopolysaccharide (EPS) production by isolates

EPS production was estimated in the Nutrient broth containing 5% of different sugars like sucrose, maltose and dextrose [19]. Systematized 30 ml of media and inoculate them with the isolate; kept one tube uninoculated in each sugar and marked as a control. Tubes were incubated at 28 ± 2°C on shaker with 150 rpm. 2 ml of media was collected under aseptic condition at every 24 h for up to 5 d and extraction of EPS was done using chemical methods includes separation of cell biomass by centrifugation, followed by the addition of three volumes of chemical solvents like chilled acetone in the culture suspension to extract EPS by precipitation. Density of broth for precipitates was observed for qualitative estimation.

For quantitative estimation, Centrifuge the media at 10,000 rpm for 20 min, supernatant was discarded and pellet was collected on a pre weighed aluminium foil. Take wet weight of foil containing EPS and allow the EPS to dry in the oven at 70 ± 5°C and weigh dry weight at every 10 min duration till 40 min. Note the weight of EPS for up to 5 d.

FTIR of EPS produced by isolates

FTIR was done with OPUS Fourier Transform Infrared Spectrometer in laboratory of Department of Microbiology and Biotechnology, School of Sciences, Ahmedabad. FTIR was run of EPS produced by all three isolates on 6 d after incubation. FTIR analysis extracted EPS was registered in the region of 400-4000 cm^{-1}. The spectra obtained were analysed and results were recorded.

Results and Discussion

Collection of isolates

Isolates were isolated from leaf of *Citrus limon* (Lemon) tree (Rathod Z., *et al*. 2020) [22]. The isolates obtained were endophytes and they were coded as D, N and A.

Characterization of isolates

Morphological characterization was done by Gram Staining. For bacterial classification characteristics such as size, shape, arrangement and gram reaction were observed as shown in the table below.

Cultural characteristics were observed directly from the plate by cultural characteristics as shown in the table below.

Morphological characteristics	D	N	A
Size	Small	Small	Small
Shape	Rod	Rod	Rod
Arrangement	Single	Chain	Single, Chain
Gram's reaction	Gram positive	Gram positive	Gram positive

Table 1: Morphological Characteristics.

Cultural characteristics	D	N	A
Size	Medium	Big	Big
Shape	Circular	Circular	Irregular
Edge	Irregular	Entire	Irregular
Elevation	Flat	Raised	Flat
Texture	Rough	Smooth	Rough
Opacity	Opaque	Opaque	Opaque
Pigmentation	-	-	-

Table 2: Cultural Characteristics.

Based on the observations in the above tables, the isolates can be of *Bacillus* sp. And this has been derived from the help of Experimental Microbiology Volume 1, 9th edition (Rakesh P and Kiran P, 2004).

Isolated organism	3% KOH
D	-ve
N	-ve
A	-ve

Table 3: Confirmative test of bacterial isolates.

Key: (+) shows gel formation, (-) no gel formation.

Qualitative and Quantitative estimation of Exopolysaccharide (EPS) production by isolates

In qualitative estimation of EPS, growth was observed in media supplemented with different sugars to the isolates. Results are as the table below.

As seen in the table above, isolate D showed maximum growth at 96 h in all sugars, visible growth was observed at 120 h and viable growth was observed at 24 h, 48 h and 72 h. Isolate N showed maximum growth at 96 h in all sugars, visible growth was observed at 72 h and 120 h and viable growth was observed at 24 h and 48 h. Isolate A showed maximum growth at 96 h in all sugars, visible growth was observed at 48 h and 72 h and viable growth was observed at 24 h and 120 h.

Biochemical Tests	Results		
	D	N	A
Sugar Utilization Test			
Dextrose	-	-	AG
Sucrose	AG	-	-
Lactose	AG	-	AG
Maltose	AG	-	AG
Fructose	AG	-	AG
Mannose	AG	-	AG
Oxidative Fermentative Test	+	+	+
Methyl red Test	+	+	+
Voges Prosauer Test	-	-	-
Citrate utilization Test	+	-	-
Indol Production Test	-	-	-
Hydrogen sulphide productionTest	-	-	-
Phenyl alenine deamination Test	-	-	-
Urea hydrolysis Test	+	+	+
Nitrate reduction Test	+	+	+
Ammonia production Test	+	+	+
Starch hydrolysis Test	+	+	+
Casein hydrolysis Test	+	+	+
Gelatin hydrolysis Test	+	+	+
Lipid hydrolysis Test	+	+	+
Catalase Test	+	+	+
Dehydrogenase Test	+	+	+
Oxidase Test	-	-	-
Hemolysin production Test	+	+	+
Triple sugar iron test	AG	AG	A
Litmus milk Test	+	+	+

Table 4: Biochemical Characteristics.

Key: (+) Positive test, (-) negative test, (AG) acid and gas production, (A) acid production.

Sugars	24 h			48 h			72 h			96 h			120 h		
	D	N	A	D	N	A	D	N	A	D	N	A	D	N	A
Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5% Sucrose	+	+	+	+	++	+	+	++	+	++	++	+++	++	+	+
5% Lactose	+	+	+	+	+	+	+	++	+	++	++	+++	+	++	-
5% Dextrose	+	+	+	+	+	++	+	+	+++	++	++	+++	+	+	+

Table 5: Qualitative analysis of EPS production from 24 h to 120.

Key: - = No Growth, + = Viable Growth, ++ = Visible Growth, +++ = Optimum Growth.

For quantitative estimation, the weights of EPS produced by isolates were recorded up to 5 days which are as below.

As seen in the table and graph above, the maximum production of EPS has been observed at 96 h (4 d) by each of the isolates. Maxi-

Sugars	24 h	48h	72h	96h	120h
Control	00	00	00	00	00
5% Sucrose	0.1 ± 0.02 g%	0.1 ± 0.02 g%	0.1 ± 0.03 g%	0.6 ± 0.03 g%	0.5 ± 0.02 g%
5% Lactose	0.05 ± 0.02 g%	0.1 ± 0.03 g%	0	0	0
5% Dextrose	0.05 ± 0.02 g%	0.1 ± 0.02 g%	0.1 ± 0.03 g%	0.5 ± 0.02g%	0.05 ± 0.03 g%

Table 6a: Amount of EPS produced by isolate D in g% (w/v) from 24 h to 120 h.

Note: Standard deviation and standard error run by using statistical tool ANOVA.

Sugars	24 h	48h	72h	96h	120h
Control	00	00	00	00	00
5% Sucrose	0.05 ± 0.02 g%	0.3 ± 0.02 g%	0.4 ± 0.02 g%	0.85 ± 0.03 g%	0.05 ± 0.02 g%
5%Lactose	0.1 ± 0.02 g%	0.1 ± 0.03 g%	0.3 ± 0.02 g%	0.5 ± 0.02 g%	0.2 ± 0.02 g%
5% Dextrose	0.05 ± 0.03 g%	0.05 ± 0.03 g%	0.1 ± 0.02 g%	0.15 ± 0.03 g%	0.1 ± 0.03 g%

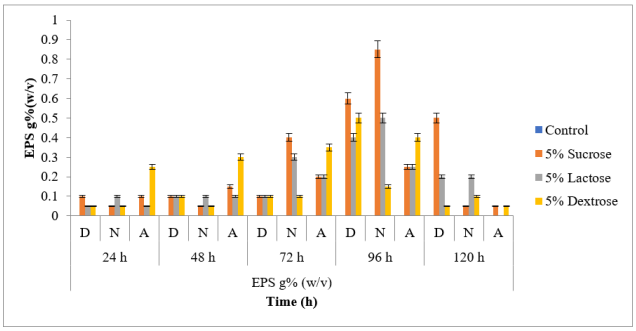
Table 6b: Amount of EPS produced by isolate N in g% (w/v) from 24 h to 120 h.

Note: Standard deviation and standard error run by using statistical tool ANOVA.

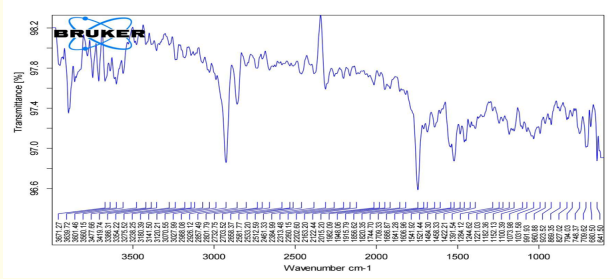
Sugars	24 h	48h	72h	96h	120h
Control	00	00	00	00	00
5% Sucrose	1.0 ± 0.02 g%	1.5 ± 0.02 g%	2.0 ± 0.02 g%	2.5 ± 0.03 g%	0.5 ± 0.02 g%
5% Lactose	0.5 ± 0.02 g%	0.1 ± 0.03 g%	2.0 ± 0.02 g%	2.5 ± 0.02 g%	0
5% Dextrose	2.5 ± 0.03 g%	3.0 ± 0.03 g%	3.5 ± 0.02 g%	4.0 ± 0.03 g%	0.5 ± 0.03 g%

Table 6c: Amount of EPS produced by isolate A in g% (w/v) from 24 h to 120 h.

Note: Standard deviation and standard error run by using statistical tool ANOVA.



Graph 1: Production of EPS in g%(w/v).

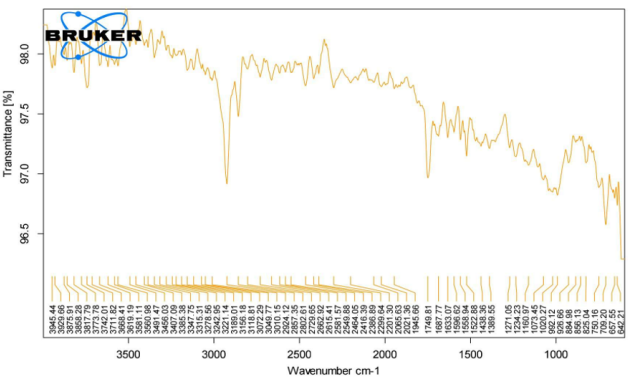


Graph 3: FTIR spectrum of EPS produced by isolate N.

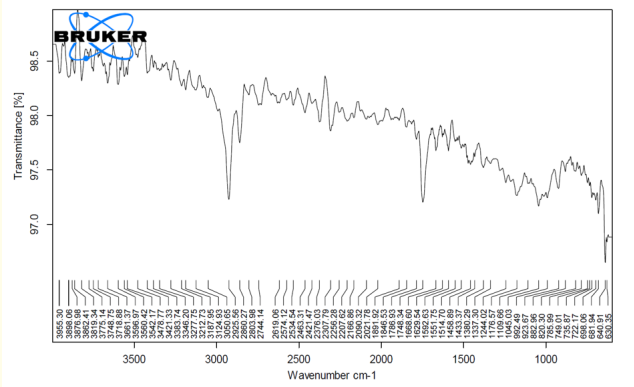
mum EPS production arise during early stationary phase than in the late stationary phase of culture [19]. The highest EPS production was recorded in *Pseudomonas aeruginosa* (226 µg/ml), *Streptococcus mutans* (220 µg/ml) and *Bacillus subtilis* (206 µg/ml) in N-free medium after 7 days of incubation at 28 ± 2°C [3].

FTIR of EPS produced by isolates

After incubation for 6 d, FTIR was performed of the EPS produced by the isolates and the results are as below.



Graph 2: FTIR spectrum of EPS produced by isolate D.



Graph 4: FTIR spectrum of EPS produced by isolate A.

An FTIR spectrum was recorded from 4000 to 600 cm^{-1} to identify functional groups of EPS of *Bacillus subtilis*. In EPS of isolate D which is *Bacillus subtilis*, peak was observed at 1749.81 cm^{-1}, 2201.36 cm^{-1} and 2802.61 cm^{-1} (Graph 2 and Table 5). Peak observed at 1749.81 cm^{-1} is attributed to functional group C=O, it indicates presence of functional group ester or ketone. The peak at 2201.36 cm^{-1} is assigned to functional group alkyl. The peak observed at 2802.61 cm^{-1} corresponds to the presence of aldehyde functional group.

In experiment work done by Ravneet Chug and Vinod Singh in 2016, EPS by *Bacillus subtilis* showed peaks at 1060 cm^{-1} and 1170 cm^{-1} correspond to the presence of carboxyl group and phosphate group [7]. FTIR spectra were recorded from 4000 to 600 cm^{-1} suggests that the EPS of isolate N which is *Bacillus tequilensis*, peaks were observed at 1521.44 cm^{-1}, 1744.7 cm^{-1}, 2857.49 cm^{-1}, 2926.12 cm^{-1}, 3601.46 cm^{-1}, 3639.72 cm^{-1} and 3560.15 cm^{-1} (Graph 3 and

D		N		A	
Functional group	**Wavelengths**	**Functional group**	**Wavelengths**	**Functional group**	**Wavelengths**
Ester, Ketone	1749.81	Benzene ring	1521.44	Ester	1748.34
C=O		C=C		-C=O	
Alkyl	2201.36	Ester, Ketone	1744.7	C-H	2307.19
Aldehyde	2802.61	C=O		Alkane	
		Aldehyde	2857.49	Aldehyde	2860.27
		Carboxylic acid	2926.12		2925.56
		O-H			2744.14
		Alcohol	3601.46	Alcohol	3661.37
		OH	3639.72	O-H	3819.34
			3560.15		

Table 7: Wave number (cm^{-1}) of dominant peak obtained from EPS produced by isolates.

Table 5). The peak at 1521.44 cm^{-1} is attributed to Benzene ring. The band at around 1542.52 cm^{-1} and 1332.25 is attributed to the symmetrical stretching vibration of carboxyl groups and reveals the complexity of EPS due to occurrence of various functional groups [27]. The peak 1744.7 cm^{-1} is assigned to C=O which indicate the presence of ketone or ester group. The peak at 2857.49 cm^{-1} indicates aldehyde. Presence of peak at 2926.12 cm^{-1} indicates carboxylic acid. Peaks observed at 3601.46 cm^{-1}, 3639.72 cm^{-1} and 3560.15 cm^{-1} corresponds to alcohol functional groups. In this, alcohol functional group was frequent. In experimental work done by Rizwan P Rani., *et al*. 2016, EPS of *Bacillus tequilensis* Broad and intensely stretched peak was observed at 3414.3 cm^{-1} due to presence of hydroxyl group (O-H) which confirms polysaccharide characteristics of *Bacillus tequilensis* [21,24,37]. The carboxyl and hydroxyl groups serve as binding sites for divalent cations [4]. An absorption peak at 1658.8 cm^{-1} shows presence of carboxylate group and characteristic. Table 5 and graph 4 show that EPS of isolate A which is *Bacillus amyloliquefaciens*. FTIR spectra were recorded from 4000 to 600 cm^{-1} to identify functional groups of *Bacillus amyloliquefaciens*. EPS of *Bacillus amyloliquefaciens* showed peaks at 1748.34 cm^{-1}, 2304.19 cm^{-1}, 2860.27 cm^{-1}, 2925.56 cm^{-1}, 2744.14 cm^{-1}, 3661.37 cm^{-1} and 3819.34 cm^{-1}. Peak observed at 1748.34 cm^{-1} attributed to functional group ester. The peak at 2304.19 cm^{-1} is assigned to functional group alkane. The peaks at 2860.27 cm^{-1}, 2925.56 cm^{-1} and 2744.14 cm^{-1} indicate functional group aldehyde. The peaks at 3661.37 cm^{-1} and 3819.34 cm^{-1} are attributed to functional group alcohol. The IR spectrum of EPS displayed a broad stretching intense peak at around 3420 cm^{-1} characteristics for hydroxyl group [14]. Band at 2893.92 was ascribed to C-H stretching vibration [16].

Conclusion

In this study, from the morphological and cultural characteristics, it was observed that the given all of three isolates are *Bacillus* spp. Further study of biochemical tests expression study showed that the isolate D is *Bacillus subtilis*, isolate N is *Bacillus tequilensis* and isolate A is *Bacillus amyloliquefaciens*. Observation showed from the EPS production test that isolate A produced highest EPS in comparison to isolate D and isolate N. FTIR results shows that EPS produced by isolate N shows frequent alcohol group (-OH) in comparison to isolate D and isolate A and presence of benzene ring (-C=C), which is not present in EPS produce by isolate D and isolate A. Our results shows that the all three isolates have potential for EPS production. The important of EPS has long been recognized and a variety of functions have been qualified to EPS.

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Conflict of Interest

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

Bibliography

1. Abdalla M A and Matasyoh J C. "Endophytes as producers of peptides: an overview about the recently discovered peptides from endophytic microbes". *Natural Products and Bioprospecting* 4.5 (2014): 257-270.

2. Aly A H., *et al*. "Cytotoxic metabolites from the fungal endophyte Alternaria sp. and their subsequent detection in its host plant Polygonum senegalense". *Journal of Natural Products* 71.6 (2008): 972-980.

3. Borgio J Francis., *et al*. "Exopolysaccharide production by Bacillus subtilis NCIM 2063, Pseudomonas aeruginosa NCIM 2862 and Streptococcus mutans MTCC 1943 using batch culture in different media". *African Journal of Biotechnology* 8.20 (2009).

4. Bramhachari P V., *et al*. "Isolation and characterization of mucous exopolysaccharide (EPS) produced by Vibrio furnissii strain VB0S3". *Journal of Microbiology and Biotechnology* 17.1 (2007): 44-51.

5. Chen Y., *et al*. "Structural characterization and antioxidant properties of an exopolysaccharide produced by the mangrove endophytic fungus Aspergillus sp. Y16". *Bioresource Technology* 102.17 (2011): 8179-8184.

6. Cheplick G P., *et al*. "Interactions between infection by endophytic fungi and nutrient limitation in the grasses Lolium perenne and Festuca arundinacea". *New Phytologist* 111.1 (1989): 89-97.

7. Chug R., *et al*. "Optimization of extracellular polymeric substances production using Azotobacter beijreinckii and Bacillus subtilis and its application in chromium (VI) removal". *Bioresource Technology* 214 (2016): 604-608.

8. Fan M., *et al*. "Fourier transform infrared spectroscopy for natural fibres". *Fourier Transform-Materials Analysis* 3 (2012): 45-68.

9. Freeman S and Rodriguez R J. "Genetic conversion of a fungal plant pathogen to a nonpathogenic, endophytic mutualist". *Science* 260.5104 (1993): 75-78.

10. Gouzou L., *et al*. "Effect of inoculation with Bacillus polymyxa on soil aggregation in the wheat rhizosphere: preliminary examination". In Soil Structure/Soil Biota Interrelationships (1993): 479-491.

11. Guo S., *et al*. "Galactomannan with novel structure produced by the coral endophytic fungus Aspergillus ochraceus". *Carbohydrate Polymers* 105 (2014): 325-333.

12. Jaggi N and Vij DR. "Fourier transform infrared spectroscopy". In Handbook of Applied Solid State Spectroscopy (2006): 411-450.

13. Kirk R E and Othmer D F. "Encyclopedia of Chemical Technology: Pentacene to polymethine dyes". Interscience Encyclopedia, Incorporated 10 (1953).

14. Kumar C G., *et al*. "Purification and characterization of an extracellular polysaccharide from haloalkalophilic Bacillus sp. I-450". *Enzyme and Microbial Technology* 34.7 (2004): 673-681.

15. Kusari S., *et al*. "Biotechnological potential of plant-associated endophytic fungi: hope versus hype". *Trends in Biotechnology* 32.6 (2014): 297-303.

16. Liu J., *et al*. "Medium optimization and structural characterization of exopolysaccharides from endophytic bacterium Paenibacillus polymyxa EJS-3". *Carbohydrate Polymers* 79.1 (2010): 206-213.

17. Lynch J M and Bragg E. "Microorganisms and soil aggregate stability". In Advances in soil science (1985): 133-171.

18. Mahapatra S and Banerjee D. "Production and structural elucidation of exopolysaccharide from endophytic Pestalotiopsis sp. BC55". *International Journal of Biological Macromolecules* 82 (2016): 182-191.

19. Mody B., *et al*. "Extracellular polysaccharides of cowpea rhizobia: compositional and functional studies". *Archives of Microbiology* 153.1 (1989): 38-42.

20. Movasaghi Z., *et al*. "Fourier transform infrared (FTIR) spectroscopy of biological tissues". *Applied Spectroscopy Reviews* 43.2 (2008): 134-179.

21. Rani R P., *et al*. "Physiochemical and biological characterization of novel exopolysaccharide produced by Bacillus tequilensis

FR9 isolated from chicken". *International Journal of Biological Macromolecules* 96 (2017): 1-10.

22. Rathod Zalak R., *et al*. "Antimicrobial Activity and Bacterial Potency of Sphingomonas Paucimobilis as Endophytes Isolated from Leaf of Citrus Limon". *International Research Journal of Engineering and Technology* 7.8 (2020).

23. Reinhold-Hurek B., *et al*. "Living inside plants: bacterial endophytes". *Current Opinion in Plant Biology* 14.4 (2011): 435-443.

24. Saravanan C., *et al*. "Isolation and characterization of exopolysaccharide from Leuconostoc lactis KC117496 isolated from idli batter". *International Journal of Biological Macromolecules* 90 (2016): 100-106.

25. Serrato R V., *et al*. "Structural studies of an exopolysaccharide produced by Gluconacetobacterdiazotrophicus Pal5". *Carbohydrate Polymers* 98.1 (2013): 1153-1159.

26. Singh R P., *et al*. "Isolation and characterization of exopolysaccharides from seaweed associated bacteria Bacillus licheniformis". *Carbohydrate Polymers* 84.3 (2011): 1019-1026.

27. Singh R P., *et al*. "Isolation and characterization of exopolysaccharides from seaweed associated bacteria Bacillus licheniformis". *Carbohydrate Polymers* 84.3 (2011): 1019-1026.

28. Smol'Kina O N., *et al*. "Capsular and extracellular polysaccharides of the diazotrophic rhizobacterium Herbaspirillumseropedicae Z78". *Microbiology* 81.3 (2012): 317-323.

29. Strobel G A. "Endophytic microbes embody pharmaceutical potential". *Asm News* 5 (1998): 263-268.

30. Strobel G A. "Endophytes as sources of bioactive products". *Microbes and Infection* 5.6 (2003): 535-544.

31. Sturz A V., *et al*. "Bacterial endophytes: potential role in developing sustainable systems of crop production". *Critical Reviews in Plant Sciences* 19.1 (2000): 1-30.

32. Subair H. "Isolation and Screening Bacterial Exopolysaccharide (EPS) from potato rhizosphere in highland and the potential as a producer Indole Acetic Acid (IAA)". *Procedia Food Science* 3 (2015): 74-81.

33. Tan R X and Zou W X. "Endophytes: a rich source of functional metabolites". *Natural Product Reports* 18.4 (2001): 448-459.

34. Vega F E., *et al*. "Entomopathogenic fungal endophytes". *Biological Control* 46.1 (2008): 72-82.

35. Venugopalan A and Srivastava S. "Endophytes as in vitro production platforms of high value plant secondary metabolites". *Biotechnology Advances* 33.6 (2015): 873-887.

36. Yeh C W., *et al*. "Quantitative and morphologic analysis on exopolysaccharide and biomass production from a truffle endophytic fungus Hypocreales sp. NCHU01". *Journal of the Taiwan Institute of Chemical Engineers* 45.1 (2014): 108-114.

37. Yu L., *et al*. "Preparation and partial structural characterization of the exopolysaccharide from Bacillus mucilaginosus SM-01". *Carbohydrate Polymers* 146 (2016): 217-223.

38. Zhou X., *et al*. "A review: recent advances and future prospects of taxol-producing endophytic fungi". *Applied Microbiology and Biotechnology* 86.6 (2010): 1707-1717.

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