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Research Article

Isolation and molecular identification of four culturable endophytes from TV22 clone of *Camellia sinensis* (L.)

Pranjal Pratim Das, Tapas Medhi*
Applied Biochemistry Laboratory, Department of Molecular Biology and Biotechnology, Tezpur University, Assam, India.

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Abstract: Endophytes are microorganisms presents within plant in asymptomatic manner and often act as a reservoir of novel bioactive secondary metabolites having antimicrobial, anti-insect and other beneficial properties. In absence of any reports on the presence of endophyte and their possible metabolic role in the process of infestation of tea plants (*Camellia sinensis* L.) by tea mosquito bug (*Halopeltis theivora* Waterhouse), the present study was undertaken to trace and isolate them from tea leaves for their molecular characterisation based on 16S rRNA sequencing. In this process, four endophytic bacteria have been identified as *Brachybacterium sp.* strain TMCS1, *Bacillus pumilus* strain TMCS2, *Moraxella osloensis* strain TMCS3 and *Moraxella osloensis* strain TMCS4 from the 2nd leaf of a young flash of *C. sinensis* (TV22 clone) which will enable us to study their culturable properties and role as biocontrol agents.

Key words: Endophytes, *C. sinensis*, 16S rRNA, sequencing, TV22, plant-microbe interaction.

Introduction

Phyllosphere, the above-ground portions of plants is a habitat of diverse microscopic organisms living on the epidermis as epiphytes and within the plant tissue as endophytes (Arnold, Maynard, Gilbert, Coley, & Kursar, 2000; Lindow & Brandl, 2003; Monier & Lindow, 2004). These include many important members of filamentous fungi and bacteria inhabit as endosymbionts in the phyllosphere. The endophytic bacteria do not visibly harm the bacteria and can be isolated from superficially disinfected healthy plant tissues (Compant, Duffy, Nowak, Clément, & Barka, 2005; Hallmann, Quadt-Hallmann, Mahaffee, & Kloepper, 1997). The population density of endophytic bacteria may differ from 10^2 to 10^9 (Bell, Dickie, Harvey, & Chan, 1995; Chi *et al.,* 2005; Jacobs, Bugbee, & Gabrielson, 1985; Misaghi & Donndelinger, 1990; Van Overbeek & Van Elsas, 2008) and relies on many factors like the plant being studied, the portion of the plant parts (Lamb, Tonkyn, & Kluepfel, 1996; Quadt-Hallmann & Kloepper, 1996), developmental stage of the plant (Hallmann *et al.,* 1997; Van Overbeek & Van Elsas, 2008), plant cultivar (genotype) (Compant *et al.,* 2005; Van Overbeek & Van Elsas, 2008) and the relation with other organisms, as well as further environmental-related factors (Hallmann *et al.,* 1997). The understanding of the interaction between endophytic bacteria and their host plants needs further study to understand further beneficial effects on their hosts (Ulrich, Ulrich, & Ewald, 2008) in details which include promotion of host growth and biological control of phytopathogens (Hallmann *et al.,* 1997; Hardoim, van Overbeek, & van Elsas, 2008).

Tea, the most popular, and oldest non-alcoholic caffeine containing beverage is prepared from processed young leaf of *C. sinensis* and consumed by two-thirds of the population around the world (Soni, Katoch, Kumar, Ladohiya, & Verma, 2015). Plant growth promoting endophytic fungi isolates from stem, root, and leaves of tea plant has been documented (R Nath, GD Sharma, & M Barooah, 2015). So far the presence of endophytic bacteria (unidentified) in tea leaf, root and stem and its population dependency on the management system (Ratul Nath, GD Sharma, & M Barooah, 2015) have been reported in terms of multiple plant growth promoting activities by the bacterial isolates (unidentified) from roots of tea plant (Nath, Sharma, & Barooah, 2013). The isolates of *Bacillus* strains with strong antagonism to pathogens of tea leaf spot diseases (Hong, 2007) has been reported till today.

Both seeds and cuttings are used to grow a tea plant (Unknown, 2012). Clonal seedlings are from cuttings, true to type and contain same qualities as that of their mother plants (Unknown, 2012). Since 1949, there is a number of clones commercialized by different tea research organization based on standard, yield, and quality (Unknown, 2012). On the other hand, endophytes profile may also vary according to the clone of a particular plant species.

***Corresponding Author:**
Dr. Tapas Medhi,
Assistant Professor,
Department of Molecular Biology and Biotechnology,
Tezpur University, India.
E-mail: tmedhi@tezu.ernet.in

Therefore, to understand the profile of endophytes present in the young leaf of individual tea clone, the study was carried out to culture them from the 2nd leaf of Tocklai vegetative (TV) tea clone (TV22) of *C. sinensis* grown in field conditions for their molecular characterization through 16S rRNA sequencing method. To our knowledge, this is the first report on endophytic bacteria from the 2nd leaf of TV22 clone of *C. sinensis.*

Materials and Methods

Selection of tea plant and Isolation of endophytic bacteria

Around seven days old, fresh tea 2nd leaf of clone TV22 was collected from the premises Tezpur, Assam, India (26.7008°N, 92.8303°E) and washed for half an hour with tap water in a sterile beaker (borosil) covered with a muslin cloth. After consecutive three washes with double distilled water, the leaf was cross sectioned in to small pieces using a sharp sterile scalpel (Himedia) and exposed to $NaOCl_2$ (Himedia) solution (0.6%) for around one minute for surface sterilization inside the LAF chamber which was dipped into PBS buffer solution (PH-7.4) for around one minute. After incubation, samples were again sterilized in 70% ethanol (Merck) for one minute followed by washing thrice in PBS buffer and the third wash was taken as a negative control for cross checking of any surface contamination. The sterilized sample is crushed using a mortar/pestle (Sigma) and mixed properly with 1ml of NaCl (0.9%) solution (Himedia). 100µl of crude leaf extract is laid over the LB-agar (Himedia) plates using the standard spreading techniques and incubated for overnight at 37ºC in a static incubator (ORBITEK, Scigenics Biotech) for observing bacterial growth in the next day. Colonies having identical morphology and phenotypic characteristics were isolated by streaking method to develop pure culture of unknown bacterial strain which were used later, as sources for Colony PCR.

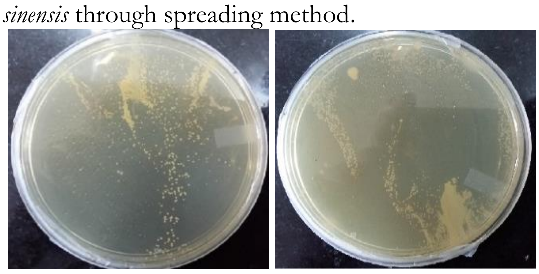
Ribotyping and Phylogenetic analyses

As described previously, genomic DNA isolation, PCR amplification and taxonomic identification were done through 16S rRNA gene sequencing method (ribotyping) (Rahman, Mahamad, Salleh, & Basri, 2007; Rai, Roy, & Mukherjee, 2010). Forward (27f) and reverse (1492r) primers are procured from Eurofins, PCR master mix from Himedia and gel extraction kit is used from Qiagen. To retrieve the homologous sequences in GenBank, the deduced sequence is subjected to blastn in National Centre of Biotechnology, Bethesda, MD, USA (http://www.ncbi.nlm.nih.gov). For each sequence, phylogenetic tree is created using CLC sequence viewer 7.7 of QIAGEN (https://www.qiagen.com/us/) and identified sequences are submitted to GenBank of NCBI for the tag of accession nos.

Results

Lack of microbial growth in the third PBS buffer-wash of the selected tea leaf indicated effective surface sterilization of the samples, already treated with NaOCl and ethyl alcohol respectively, which was then used as negative control. The extracts of sterilized crushed leaf, when used as inoculums, showed bacterial growth (Fig. 1) in LB agar media. Based on morphology and phenotypic appearance of the individual colony, four endophytic bacterial strains such as TMCS1, TMCS2, TMCS3, & TMCS4 were isolated with the help of streaking method (Fig. 2). The genomic DNA extraction for colony PCR was effective with 260/280 ratio of >1.5 for amplification of 16S rRNA gene (Fig. 3) as also evident from QC reports of eurofins (Fig. 3). All the amplified products were of about 900-1000 base pairs in size in 0.8% agarose gel as compared to the molecular marker of DNA ladder sequence. After removing the anomalies in all the four 16S rRNA sequences (using Pintail 1.1), the length of the sequences in base pairs are found to be 1002 bp for TMCS1, 1111 bp for TMCS2, 1109 bp for TMCS3, and 928 bp for TMCS4. Statistical significance of similarities of the four sequences (16S rRNA) of endophytes of *C. sinensis* through blastn of NCBI has shown TMCS1 as *Brachybacterium sp.*, TMCS2 as *Bacillus sp.,* and TMCS3/ TMCS4 as *Moraxella sp.* After phylogenetic tree analyses through CLC sequence viewer 7.7, the isolated bacterial strains were identified to be *Brachybacterium sp.* strain TMCS1, *Bacillus pumilus* strain TMCS2, (Fig. 4A) *Moraxella osloensis* strain TMCS3, and *Moraxella osloensis* strain TMCS4 (Fig. 4B) with GenBank accession nos KX641167, KX683004, KX646168, and KX687850 respectively.

Fig. 1: Isolation of endophytic bacteria from *C. sinensis* through spreading method.



NaOCl (0.6%) assisted isolation of culturable endophytic bacteria from 2nd leaf of TV22 clone of *C. sinensis* in LB agar plate. Mechanical method (morter/pestle) was used to crushing of leaf material.

Fig. 2: Preparation of pure culture from isolated endophytes through streaking method.

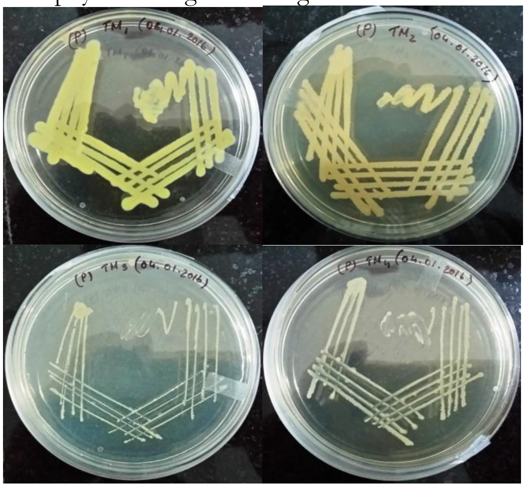
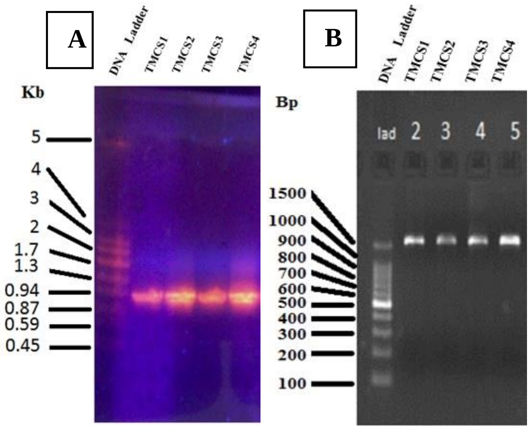
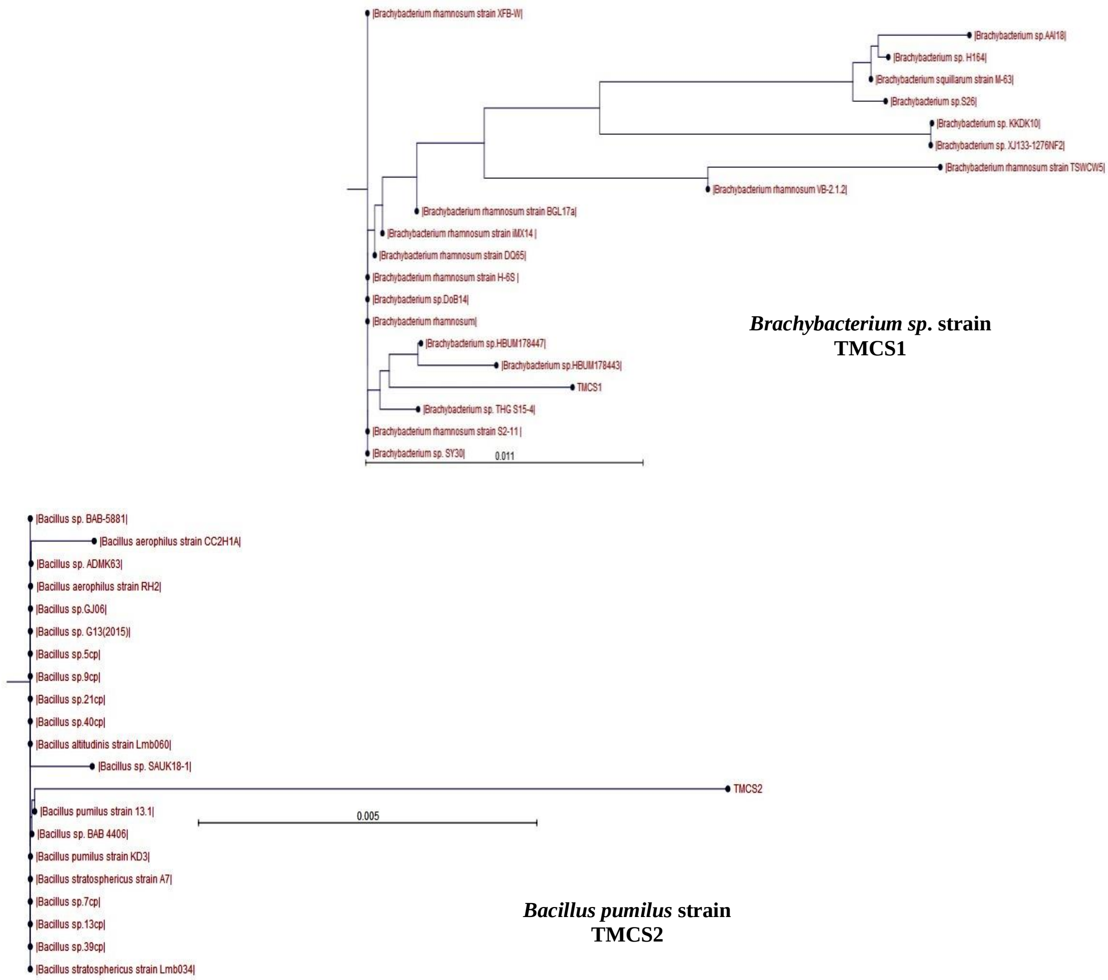


Fig. 3: PCR amplified product of 16S rRNA of four endophytes (A), Quality Control (QC) report of the four gel extracted amplicons (B).



A: PCR amplification of the four endophytic bacteria in agarose gel (0.8%), B: QC report of the amplified products before sequencing as received from eurofins laboratory. Kb: Kilobase pair. Bp: Base pair.

Fig. 4A: Phylogenetic relationships of two endophytes of *C. sinnensis* with other closely related species based on 16S rRNA sequencing.



Phylogenetic tree of two strains out of four endophytes with GenBank accession no (KX641167 & KX683004) are generated using CLC sequence viewer 7.7. The methods used are Neighbor Joining algorithm, Jukes-Cantor for distance measure, and Bootstrapping in 100 replicates.

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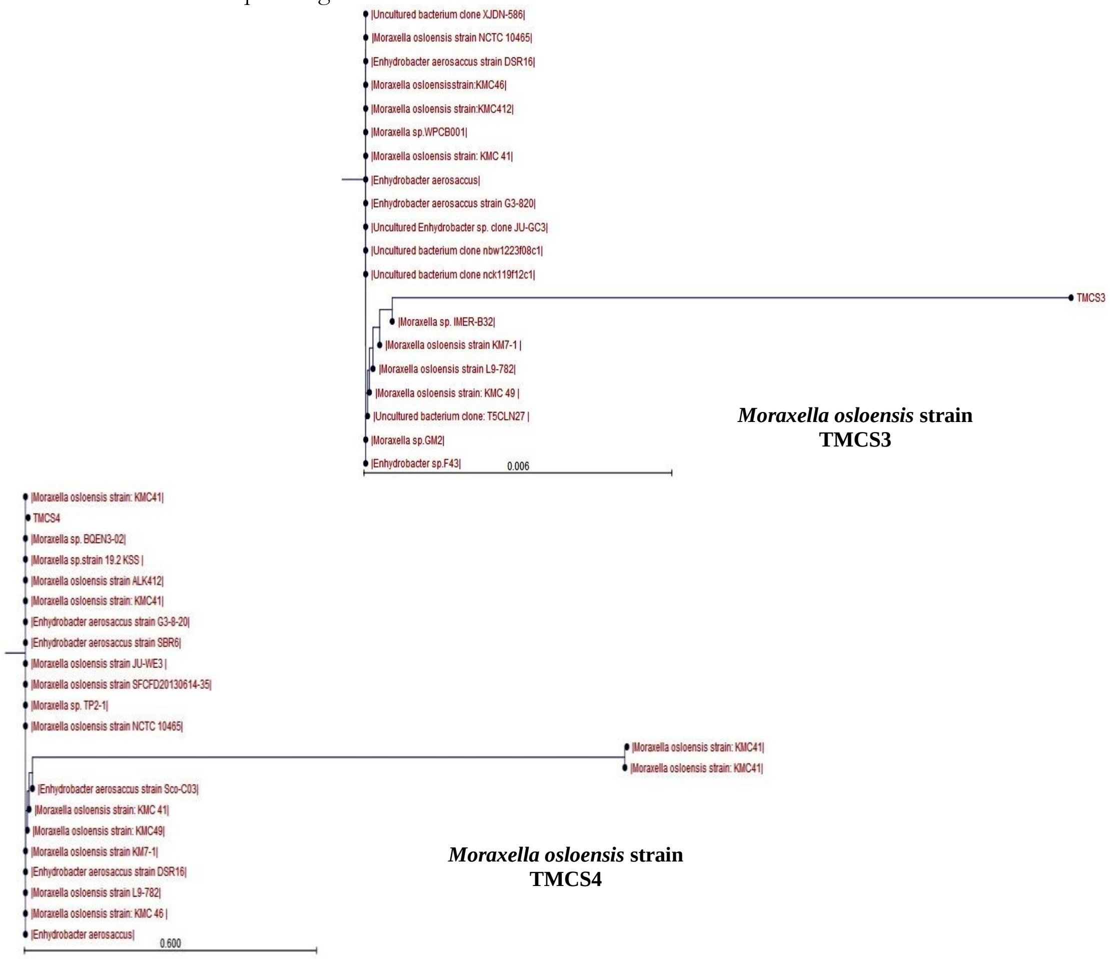
Fig. 4B: Phylogenetic relationships of two endophytes of *C. sinnensis* with other closely related species based on 16S rRNA sequencing



Phylogenetic tree of two strains out of four endophytes with GenBank accession no (KX646168 & KX687850) are generated using CLC sequence viewer 7.7. The methods used are Neighbor Joining algorithm, Jukes-Cantor for distance measure, and Bootstrapping in 100 replicates.

Discussion

The presence of above-mentioned genera of endophytes had been previously reported in several other plant species such as *Brachybacterium* in the leaf of Rice (Bertani, Abbruscato, Piffanelli, Subramoni, & Venturi, 2016) and in the roots of *Chlorophytum borivilianum* L. (Barnawal *et al.,* 2016), *Bacillus* in the root, stem and leaf of *Solanum nigrum* L. (Guo *et al.,* 2010) and in the roots of *Platycodon grandifloram* (Jacq.) A. DC. (Islam *et al.,* 2010), *Phytolacca acinosa* Roxb. (Luo *et al.,* 2012) and also in other plant species (Bacilio-Jiménez *et al.,* 2003; Bacon & Hinton, 2002; Berg & Hallmann, 2006; Cho, Lim, Hong, Park, & Yun, 2003; Cho *et al.,* 2002; Luo *et al.,* 2012; Misaghi & Donndelinger, 1990; Shin *et al.,* 2012; Wang *et al.,* 2009). The presence of Moraxella genera as endophytes was also reported within a number of plant species (Araújo *et al.,* 2001; Araújo *et al.,* 2002; Bacon & Hinton, 2007; de Almeida *et al.,* 2009; Di Fiore & Del Gallo, 1995; Miller, Qing, Sze, Roufogalis, & Neilan, 2012; Reiter & Sessitsch, 2006).

Medicinal plants and their endophytes contribute to more than 80% of the natural drugs available in the market and are an important source of precious bioactive compounds and secondary metabolites (Gouda, Das, Sen, Shin, & Patra, 2016; Singh & Dubey, 2015). Although *C. sinensis* is not considered as a medicinal plant, it has been reported to have the attributes of preventing cancer, diabetes and heart disease, encouraging weight loss, lowering cholesterol, enhancing mental alertness to human and antimicrobial property (Edgar, 2018). The endophytes are often considered as reliable sources of novel bioactive compounds useful for developing drugs and antimicrobial agents (Gouda *et al.,* 2016; Jalgaonwala, Mohite, & Mahajan, 2017; Joseph & Priya, 2011; Omojate Godstime, Enwa Felix, Jewo Augustina, & Eze Christopher, 2014; Parthasarathi *et al.,* 2012), Therefore, the endophytes reported in this study would facilitate determining variables as an outcome of plant-microbe interaction which may be helpful in quality control, value addition and diversification of different products of tea industry. The bioactive metabolites as a result of such interaction in a single plant species can be applied in agriculture, cosmetics and food industries (Jalgaonwala *et al.,* 2017; Strobel & Daisy, 2003) for which further scientific evaluation is needed in order to understand the probable roles endophytes in *C. sinensis*. The importance of the identified bacterial profiles can also be linked to areas of interactions including pathogenic, symbiotic and associative – all of which impact plant productivity, stress tolerance and disease and insect resistance.

Conclusion

The authors are first to report the presence of endophytes in tea 2nd leaf up to clone (TV22) level which are of different genus group suggesting their potential of a diverse role and/or bioactive products. The result of a limited study on 2nd leaf of young flash is indicative of a possible increase in the profile of endophytes in the tea plant. In future, such studies on *C. sinensis* will improve our understanding of the role/s of endophytes in maintaining plant health and to harness useful bioactive compounds for human consumption.

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References

1. Araujo, Welington Luiz, Walter Maccheroni Jr, Carlos I Aguilar-Vildoso, Paulo AV Barroso, Halha O Saridakis, and Joao Lucio Azevedo. Variability and interactions between endophytic bacteria and fungi isolated from leaf tissues of citrus rootstocks. *Canadian journal of microbiology*,47.3, (2001): 229-236. Print.

2. Araujo, Welington Luiz, Joelma Marcon, Walter, van Elsas Maccheroni, Jan Dirk van Vuurde, Jim WL, and Joao Lucio Azevedo. Diversity of endophytic bacterial populations and their interaction with Xylella fastidiosa in citrus plants. *Applied and environmental microbiology*,68.10, (2002): 4906-4914. Print.

3. Arnold, A Elizabeth, Zuleyka Maynard, Gregory S Gilbert, Phyllis D Coley, and Thomas A Kursar. Are tropical fungal endophytes hyperdiverse? *Ecology letters*,3.4, (2000): 267-274. Print.

4. Bacilio-Jimenez, Macario, Sara Aguilar-Flores, Elsa Ventura-Zapata, Eduardo Perez-Campos, Stephane Bouquelet, and Edgar Zenteno. Chemical characterization of root exudates from rice (Oryza sativa) and their effects on the chemotactic response of endophytic bacteria. *Plant and Soil*,249.2, (2003): 271-277. Print.

5. Bacon, Charles W, and Dorothy M Hinton. Endophytic and biological control potential of Bacillus mojavensis and related species. *Biological Control*,23.3, (2002): 274-284. Print.

6. Bacon, Charles W, and Dorothy M Hinton. Bacterial endophytes: the endophytic niche, its occupants, and its utility. *Plant-associated bacteria*. (2007): 155-194. Print.

7. Barnawal, Deepti, Nidhi Bharti, Arpita Tripathi, Shiv Shanker Pandey, Chandan Singh Chanotiya, and Alok Kalra. ACC-deaminase-producing endophyte Brachybacterium paraconglomeratum strain SMR20 ameliorates Chlorophytum salinity stress via altering phytohormone generation. *Journal of plant growth regulation*,35.2, (2016): 553-564. Print.

8. Bell, CR, GA Dickie, WLG Harvey, & JWYF Chan. Endophytic bacteria in grapevine. *Canadian journal of Microbiology*,41.1, (1995): 46-53. Print.

9. Berg, Gabriele, and Johannes Hallmann. Control of plant pathogenic fungi with bacterial endophytes. *Microbial root endophytes*. (2006): 53-69. Print.

10. Bertani, Iris, Pamela Abbruscato, Pietro Piffanelli, Sujatha Subramoni, and Vittorio Venturi. Rice bacterial endophytes: isolation of a collection, identification of beneficial strains and microbiome analysis. *Environmental microbiology reports*,8.3, (2016): 388-398. Print.

11. Chi, Feng, Shi-Hua Shen, Hai-Ping Cheng, Yu-Xiang Jing, Youssef G Yanni, and Frank B Dazzo. Ascending migration of endophytic rhizobia, from roots to leaves, inside rice plants and assessment of benefits to rice growth physiology. *Applied and environmental microbiology*,71.11, (2005): 7271-7278. Print.

12. Cho, Soo Jeong, Woo Jin Lim, Su Young Hong, Sang Ryeol Park, and Han Dae Yun. Endophytic colonization of balloon flower by antifungal strain Bacillus sp. CY22. *Bioscience, biotechnology, and biochemistry*,67.10, (2003): 2132-2138. Print.

13. CHO, Soo Jeong, Sang Ryeol Park, Min Keun KIM, Woo Jin LIM, Sung Kee RYU, Chang Long AN, Su Young HONG, Young Han LEE, Seon Gi JEONG, Yong Un CHO and Han Dae YUN. Endophytic Bacillus sp. isolated from the interior of balloon flower root. *Bioscience, biotechnology, and biochemistry*,66.6, (2002): 1270-1275. Print.

14. Compant, Stephane, Brion Duffy, Jerzy Nowak, Christophe Clement, and Essaid Ait Barka. Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and

15. future prospects. *Applied and environmental microbiology*,71.9, (2005): 4951-4959. Print.

16. Almeida, CV de, Fernando Dini Andreote, Ricardo Yara, Francisco Andre Ossamu Tanaka, Joao Lucio Azevedo, and Marcilio de Almeida. Bacteriosomes in axenic plants: endophytes as stable endosymbionts. *World Journal of Microbiology and Biotechnology*,25.10, (2009): 1757-1764. Print.

17. Fiore, S.Di, and M Del Gallo. Endophytic bacteria: their possible role in the host plant Azospirillum VI and related microorganisms. 37, (1995): 169-187. Print.

18. Edgar Julie. Types of Teas and Their Health Benefits. Retrieved 01/02/2018, 2018, from https://www.webmd.com/diet/features/tea-types-and-their-health-benefits#1. Online.

19. Gouda, Sushanto, Gitishree Das, Sandeep K. Sen, Han-Seung Shin, and Jayanta Kumar Patra. Endophytes: a treasure house of bioactive compounds of medicinal importance. *Frontiers in microbiology*,7, (2016): 1538. Print.

20. Guo, Hanjun, Shenglian Luo, Liang Chen, Xiao Xiao, Qiang Xi, Wanzhi Wei, . . . and Jueliang Chen. Bioremediation of heavy metals by growing hyperaccumulaor endophytic bacterium Bacillus sp. L14. *Bioresource technology*,101.22, (2010): 8599-8605. Print.

21. Hallmann, J, A. Quadt-Hallmann, WF Mahaffee, and JW Kloepper. Bacterial endophytes in agricultural crops. *Canadian Journal of Microbiology*,43.10, (1997): 895-914. Print.

22. Hardoim, Pablo R, Leo S van Overbeek, and Jan Dirk van Elsas. Properties of bacterial endophytes and their proposed role in plant growth. *Trends in microbiology,*16.10, (2008): 463-471. Print.

23. Hong, Y C. Study on Endophytic Bacteria (Bacillus Subtilis) From Tea Plant. (2007): Retrieved 19/01/2018, 2018, from http://www.globethesis.com/?t=1103360185980073. Online.

24. Islam, Shah Md Asraful, Renukaradhya K Math, Jong Min Kim, Myoung Geun Yun, Ji Joong Cho, Eun Jin Kim, Young Han Lee, and Han Dae Yun. Effect of plant age on endophytic bacterial diversity of balloon flower (Platycodon grandiflorum) root and their antimicrobial activities. *Current microbiology,*61.4, (2010): 346-356. Print.

25. Jacobs, Mark J, William M Bugbee, and David A. Gabrielson. Enumeration, location, and characterization of endophytic bacteria within sugar beet roots. *Canadian Journal of Botany*,63.7, (1985): 1262-1265. Print.

26. Jalgaonwala, Ruby Erach, Bhavna Vishwas Mohite, and Raghunath Totaram Mahajan. A review: natural products from plant associated endophytic fungi. *Journal of microbiology and biotechnology research*,1.2, (2017): 21-32. Print.

27. Joseph, Baby, and R Mini Priya. Bioactive Compounds from Endophytes and their Potential in. *American Journal of biochemistry and Molecular biology*,1.3, (2011): 291-309. Print.

28. Lamb, Thomas G, David W Tonkyn, and Daniel A Kluepfel. Movement of Pseudomonas aureofaciens from the rhizosphere to aerial plant tissue. *Canadian Journal of Microbiology*,42.11, (1996): 1112-1120. Print.

29. Lindow, Steven E, and Maria T Brandl. Microbiology of the phyllosphere. *Applied and environmental microbiology*,69.4, (2003): 1875-1883. Print.

30. Luo, Shenglian, Taoying Xu, Liang Chen, Jueliang Chen, Chan Rao, Xiao Xiao and Chengbin Liu. Endophyte-assisted promotion of biomass production and metal-uptake of energy crop sweet sorghum by plant-growth-promoting endophyte Bacillus sp. SLS18. *Applied microbiology and biotechnology*,93.4, (2012): 1745-1753. Print.

31. Miller, I Kristin, Chen Qing, Daniel Man-Yuen Sze, Basil D Roufogalis, and Brett A Neilan. Culturable endophytes of medicinal plants and the genetic basis for their bioactivity. *Microbial ecology*,64.2, (2012): 431-449. Print.

32. Misaghi, IJ, and CR Donndelinger. Endophytic bacteria in symptom-free cotton plants. *Phytopathology*,80.9, (1990): 808-811. Print.

33. Monier, J-M, and SE Lindow. Frequency, size, and localization of bacterial aggregates on bean leaf surfaces. *Applied and environmental microbiolo*gy,70.1, (2004): 346-355. Print.

34. Nath, Ratul, GD Sharma, and M Barooah. Plant growth promoting endophytic fungi isolated from tea (Camellia sinensis) shrubs of Assam, India. *Appl Ecol Environ Res*,13, (2015): 877-891. Print.

35. Nath, Ratul, GD Sharma, and M Barooah. Impact of agrochemicals on endophytic bacterial population in tea (Camellia sinensis L.) shrubs. *Int. J. Pure App. Biosci,*3.2, (2015): 542-546. Print.

36. Nath, Ratul, GD Sharma, and Madhumita Barooah. Screening of endophytic bacterial isolates of tea ('Camellia sinensis' L.) roots for their multiple plant growth promoting activities. *International Journal of Agriculture, Environment & Biotechnology*,6.2, (2013): 211. Print.

37. Omojate, Godstime C, Enwa Felix, O, Jewo Augustina O, and Eze Christopher O. Mechanisms of antimicrobial actions of phytochemicals against enteric pathogens–a review. *J Pharm Chem Biol Sci,*2.2, (2014): 77-85. Print.

38. Parthasarathi, S, S Sathya, G Bupesh, R Durai Samy, M Ram Mohan, G Selva Kumar and K Balakrishnan. Isolation and characterization of antimicrobial compound from marine Streptomyces

hygroscopicus BDUS 49. *World J Fish Mar Sci*,4.3, (2012): 268-277. Print.

39. Quadt-Hallmann, A, and JW Kloepper. Immunological detection and localization of the cotton endophyte Enterobacter asburiae JM22 in different plant species. *Canadian Journal of Microbiology*,42.11, (1996): 1144-1154. Print.

40. Soni, Rajju Priya, Mittu Katoch, Ashish Kumar, Rajesh Ladohiya, and Parmod Verma. Tea: Production, Composition, Consumption and its Potential an Antioxidant and Antimicrobial Agent. *International Journal of Food and Fermentation Technology*, 5.2, (2015): 95. Print.

41. Rahman, Raja Noor Zaliha Raja Abd, Shalihah Mahamad, Abu Bakar Salleh, and Mahiran Basri. A new organic solvent tolerant protease from Bacillus pumilus 115b. *Journal of industrial microbiology & biotechnology,*34.7, (2007): 509-517. Print.

42. Rai, Sudhir Kumar, Jetendra Kumar Roy, and Ashis Kumar Mukherjee. Characterisation of a detergent-stable alkaline protease from a novel thermophilic strain Paenibacillus tezpurensis sp. nov. AS-S24-II. *Applied microbiology and biotechnology*,85.5, (2010): 1437-1450. Print.

43. Reiter, Birgit, and Angela Sessitsch. Bacterial endophytes of the wildflower Crocus albiflorus analyzed by characterization of isolates and by a cultivation-independent approach. *Canadian journal of microbiology*,52.2, (2006): 140-149. Print.

44. Shin, Mi-Na, Jaehong Shim, Youngnam You, Hyun Myung, Keuk-Soo Bang, Min Cho, . . . and Byung-Taek Oh. Characterization of lead resistant endophytic Bacillus sp. MN3-4 and its potential for promoting lead accumulation in metal hyperaccumulator Alnus firma. *Journal of hazardous materials*,199, (2012): 314-320. Print.

45. Singh, Radha, and A.K. Dubey. Endophytic actinomycetes as emerging source for therapeutic compounds. *Indo Global J. Pharm. Sci*,5, (2015): 106-116. Print.

46. Strobel, Gary, and Daisy Bryn. Bioprospecting for microbial endophytes and their natural products. *Microbiology and molecular biology reviews*,67.4, (2003): 491-502. Print.

47. Kristina, Ulrich, Andreas, and Dietrich Ewald. Diversity of endophytic bacterial communities in poplar grown under field conditions. *FEMS microbiology ecology*,63.2, (2008): 169-180. Print.

48. Unknown. CLONAL PROPAGATION OF TEA. (2012): Retrieved 19/01/2018, 2018, from http://www.kvkmokokchung.in/index.php/2012-04-03-12-21-17/2012-04-03-12-34-32/leaflets/34-clonal-propagation-of-tea.

49. Van Overbeek, Leo, and Jan Dirk Van Elsas. Effects of plant genotype and growth stage on the structure of bacterial communities associated with potato (Solanum tuberosum L.). *FEMS microbiology ecology*, 64.2, (2008): 283-296. Print.

50. Wang, Huili, Kai Wen, Xiuyun Zhao, Xuedong Wang, Aiying Li, and Huazhu Hong. The inhibitory activity of endophytic Bacillus sp. strain CHM1 against plant pathogenic fungi and its plant growth-promoting effect. *Crop Protection*,28.8, (2009): 634-639. Print.

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