

A review on plant-endophytic microbial interactions for enhancing natural flavours of tea (*Camellia sinensis*)

Jinu Medhi, M. C. Kalita

Department of Biotechnology, Gauhati University.

Recent molecular studies on endophytic bacterial diversity have revealed a large richness of species, which promote plant growth and yield, fight pathogens, help to solubilize phosphate, or contribute assimilable nitrogen to plants, and more importantly regulate aromas and flavours of plant products. This technological advance has now become so pervasive that it is being regularly applied to explore soils and plants of agricultural interest. Interestingly, many large companies are taking notice, with significant financial investment being used to exploring ways to manipulate the productivity, disease resistance and stress tolerance for crops and aroma enhancement by influencing the microbiome. It is important to understand which microbes one needs to manipulate to influence these valuable characteristics, this requires sequencing the microbiome and capture the genetic and hence functional metabolic information contained therein. For tea aroma-flavour industry where the tea is also associated to particular flavour properties that may also be manipulated, understanding how the endophytic bacteria and fungi influence the development and hence chemical makeup of the tea is essential.

Emerging molecular techniques such as high throughput sequencing is dramatically intensifying our knowledge of plant microbiome diversity, linking microbial ecology and the plant host's biology and functioning and viewing microorganisms as a reservoir of additional genes and functions for their hosts. Deciphering the various types of interaction between plants and their microbiome has shown that microbes can also be manipulated to produce aromas and flavours. Flavours are generally used as food additives and they are mostly produced through artificial means i.e. chemical synthesis or by extraction from plant and animal sources. Flavours and fragrances are widely used in food, beverage, feed, cosmetic, detergent, chemical and pharmaceutical formulations. Moreover, due to consumer's increased interest and health awareness in natural products, there has been more stress towards the use of natural fragrances and flavours obtained from natural sources. The worldwide demand of flavours and fragrances was estimated to be 16 billion US dollar in the year 2013 (Gupta et al., 2015). Most of these flavouring and fragrance compounds are prepared by chemical technology and only a small fraction of the demand is met from plant or through microbial sources (Bomgardner, 2012). However, there are events of plant-microbial associations in nature that result in formation of fragrant compounds, as in case of vetiver (Alifano et al., 2010) and agarwood (Supriyo et al., 2017). Moreover, it has been reported that the flavour of strawberries, furanoids are responsible for the typical fragrant (Zabetakis, 1997) where plant-associated methylobacteria enhanced the biosynthesis of furanoids bio-molecules and influence the quality and quantity of the flavour (Verginer et al., 2010). The production of natural

flavourings and perfumes partly arising from the metabolism by microbes in infected plants has also been reported (Dourtoglou, 1986). However, a considerable amount of information exists on the metabolic potential of endophytic fungi but bacteria can also thrive as endophytes in various plants and plant parts but are less investigated for their metabolic potential (Brader *et al.* 2014). For example, endophytic Actinobacteria suggests that large numbers of secondary metabolites produced by endophytic bacteria remain to be discovered. This is underlined by recently described multicyclic indolosesquiterpenes found in the endophytic *Streptomyces* sp. HKI0595 of the mangrove tree *Kandelia candel* (Ding *et al.*, 2011). However, endophytes have to be adapted to the specific plant environment where they colonize and therefore, the metabolic potential of endophytes is likely to differ from their soil dwelling counterparts. It has been demonstrated that the endophyte-derived compounds belong to diverse structural groups such as terpenoids, steroids, xanthenes, chinones, phenols, isocoumarins, benzopyranones, tetralones, cytochalasines and enniatines (Schulz *et al.*, 2002). In general, microorganisms are capable of producing remarkably wide array of flavour compounds, by *de novo* synthesis, such as monoterpenes and esters by *Saccharomyces cerevisiae* (Carrau *et al.*, 2010; Carrau *et al.*, 2005). Different classes of microorganisms have been described by relating their individual biosynthetic pathways to the production of specific flavour compounds. Scientists studying diverse plant-associated microbes for their potential roles in the production of volatile flavour compounds have achieved interesting results. For example, *Penicillium solitum*, isolated from the surface of kiwi (*Actinidia deliciosa*) has revealed a high tolerance for monoterpenes and can transform α -pinene into verbenone (Pescheck *et al.*, 2009).

One of the most prized and amazing characteristics of tea (*Camellia sinensis*) is the dizzying array of natural flavours and aromas that can be coaxed from this single leaf by variations in climate, geography and processing. Tea is a very economically important, non-alcoholic caffeine-containing beverage crop cultivated for its leaf. India is currently the foremost producer, consumer and exporter of commercial tea. Tea is grown in over 36, 91,938 ha with an annual production of 4066.60 million kg globally, and India contributes 28% of world tea production with 21% of the area covered under tea cultivation (Majumder *et al.*, 2010; Dutta *et al.*, 2017). Though India stands second place next to China in terms of tea production and consumption but less research input has been implemented to improve and understand this crop. Moreover, the microbial interaction with tea plant is one of the neglected areas of research which have a tremendous scope in future. The major tea growing areas in India are Assam, Darjeeling and it also cultivated few places in South India. The Assam, Darjeeling and the tea cultivation in South India have distinct geographical disparity due to which the flavour of tea from each region has their unique signature. It has been observed that tea leaves from many countries having different climatic conditions including Indonesia, Kenya and Sri Lanka showed different flavour profiles of tea essence (Goodner and Wampler, 2009). However, apart from environmental factors, the role of endophytic microbes in natural flavours production in tea leaves is not addressed yet. Therefore, the purpose of present study is to answer these questions and to reveal the interaction of endophytic microbes-tea leaf to understand the hidden mystery of tea leaf natural flavour production. Moreover,

there is no such study is conducted so far in tea plant which makes this project proposal more challenging and will possibly give a novel insight into the tea leaf chemistry of flavour production. It was reported that there are four main catechins found in tea leaves, including (-)-epicatechin (EC), 3-(-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG) and (-)-epigallocatechin gallate (EGCG) and EGCG is the major catechin in tea leaves. Moreover, theaflavins are another group of polyphenol pigment found in both black and oolong tea (Thakur et al., 2011). However, these components of tea were mainly detected in the made tea to measure the quality of tea. Moreover, it was observed that the same tea clone cultivated in different geographic locations shows variation in natural flavour of leaf. Therefore, in this study the TV-1 tea clones which is commercially cultivated in geographically different regions of India would be evaluated to analyse the variation in flavours. Previously, Gohain et al., 2012 studied the genes/transcriptomics responsible for the typical flavour of Darjeeling tea. However, no such studies focused on the tea plant-microbe interaction previously. Therefore, proposed study projects for first time the metagenomic and metabolomic approaches across two different platforms of interaction *viz.* fresh natural tea leaves and callus from tender leaf tissues, to evaluate the endophytic microbes-tea leaf interaction in natural flavours production. It aims to find chemometric signatures that can explain natural flavour formation in tea leaves and also scan the microbial interaction landscape for new flavour compounds. Apart from this, it also gives the broad picture of endophytic microbiome dwelling inside the root and leaves of tea.

The factors that drive plant microbiome composition are now better understood, a major challenge for future research is to link microbiome composition to function. Tea is an economically important crop, massively cultivated in India and it is one of the most popular beverages due to its unique flavour. There are several instances in nature where it was observed that the microbes play a significant role in flavour and aroma production in plant. However, the recent advances of molecular and omics techniques give us a better opportunity and understanding to look more closely into the natural phenomenon. The metagenomic analysis of the plant microbiome explored greater insights beyond the genome information of individual bacterial strains into functional information. Moreover, due to the worldwide demand of natural flavours and fragrances in food and cosmetic industry, now researchers are shifting from chemical to natural resources for the flavour and aroma production. In this context, the microbes are considered as a potential candidate for the natural flavour and aroma production. In addition, the microbes prove themselves as a key player in industry due to its wide array of secondary metabolite production ability. Besides, it will also enhance the understanding of metagenomic endophytic microbes in tea crop which will open up new ventures in tea crop research for future. The flavour of tea formed as a result of plant-microbial interactions is a potential option for the future.