Chapter 7
Function-Based Metagenomics to Reveal Rhizosphere Microbiome: A Glimpse

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Abstract To unravel the perplexity of cultivation methodologies, several technical efforts that involve molecular methods have been widely introduced. However, it is not feasible to elucidate the wide presence of soil microorganisms employing traditional approaches. Nowadays, a different approach has been employed, the so-called metagenome (MG), to know the functionality of bulk and rhizospheric soil, and later the result of comparative analysis on environmental MG was reported and suggested that comparative MG approach can be an extremely valuable tool for the characterization of complex microbial communities.

7.1 Introduction

Plant is a sessile organism which holds belowground with the help of roots per se, and upon seed maturity the spermosphere becomes a rhizosphere (RS). An area so-called RS is very close to the root with the range 1–10 mm and considered as a functional niche (Fig. 7.1) (Hiltner 1904). The plant rhizosphere exhibits similarity with animal intestine wherein root hairs and villus of intestine enhance the surface area of cells that helps in the nutrient uptake. Besides, in both areas, a large number of microorganisms play key roles in the decomposition of complex substances into simpler ones along with the production of vitamins and hormone-like compounds. The RS shows functional niche wherein quorum of microbes present and produce benign/detrimental secretion known as the RS effect. It has been reported that more than 99 % of microbial species present in soil are still refractory to cultivate (Amann 1995).

To unravel the perplexity of cultivation methodologies, several technical efforts that involve molecular methods have been widely introduced. However, it is not feasible to elucidate the wide presence of soil microorganisms employing

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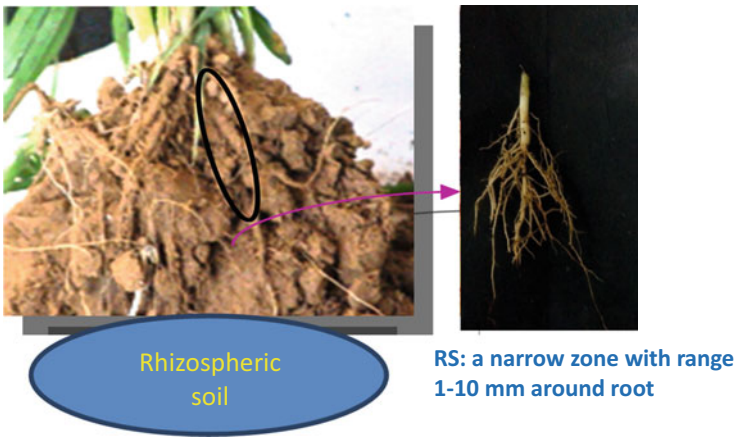

Fig. 7.1 Pictorial of RS

traditional approaches. Nowadays, a different approach has been employed, the so-called metagenome (MG), to know the functionality of soil and RS, and later the result of comparative analysis on environmental MG was reported and suggested that comparative MG approach can be an extremely valuable tool for the characterization of complex microbial communities (Edwards et al. 2006; Wegley et al. 2007; Krause et al. 2008; Schlüter et al. 2008; Diaz et al. 2009; Kröber et al. 2009).

For cultivation-independent analyses and exploitation of microbial communities present in complex ecosystems, MG has paved the way for characterization of microbes present in an ecosystem. In recent years, significant progress has been made in the deployment of MG wherein MG has been proven to be a powerful tool for the recovery of novel biomolecules. In most cases, functional MG comprising construction and screening of complex MG DNA libraries has been applied to isolate new enzymes and drugs of industrial importance. The developed MG technologies have been employed to replace culture-based approaches and allow the assessment and exploitation of the taxonomic and metabolic diversity of microbial communities on an ecosystem level (Handelsman 2004).

7.2 Function-Based MG and Rhizosphere

The sequence-based MG has been used to characterize members of gene families wherein target genes are identified either by employing PCR-based or hybridization-based approaches and probes derived from conserved regions of known genes and gene products (Daniel 2005). It is not selective for full-length genes and functional gene products, and the advantage includes the independence on gene expression and production of foreign genes in the library host (Lorenz et al. 2002). Function-driven screening of MG libraries is free of sequence information/

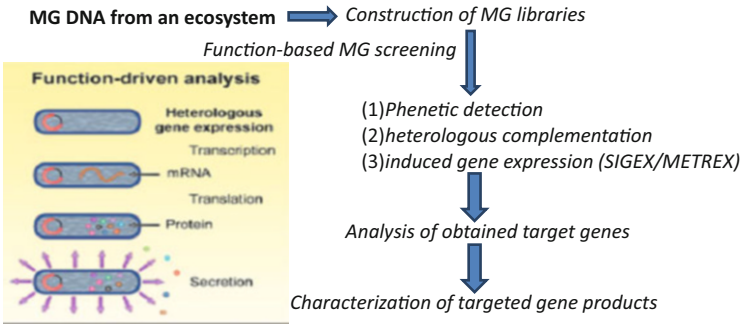

Fig. 7.2 Deployment of function-based MG approach

similarity to exist genes, and this is the approach that bears the potential to explore novel classes of functional genes (Heath et al. 2009). A major stringent of this technique is the use of foreign host *Escherichia coli* on the expression of the target genes and production of functional gene products. Hence, in function-based screening MG approach, only full-length genes and functional gene products have been detected. The three types of function-driven MG approaches have been employed to libraries that involve (1) detection of definite phenotypes of individual clones, (2) heterologous complementation of host strains and/or mutants and (3) induced gene expression (Fig. 7.2 and Table 7.1).

The functional MG libraries have been characterized by various researchers to identify enzymatic functions of individual clones by adding different substrates into the growth medium (Ferrer et al. 2009). This approach involves the detection of recombinant *E. coli* clones that showed protease activity in medium containing skimmed milk as protease substrate (Waschkowitz et al. 2009) and lipolytic activity in solid medium containing tributyrin/tricapryl as enzyme substrates (Heath et al. 2009). A varied MG approach has been used for host strains that require heterologous complementation by foreign genes for growth under selective conditions wherein a high selectivity of the screen is achieved. In the recent time the identification of DNA polymerase-encoding genes from metagenomics libraries derived from microbial communities present in glacier ice has been characterized (Simon et al. 2009). Furthermore, this approach has been employed for the detection of genes encoding Na^+/H^+ antiporters (Majernik et al. 2001), antibiotic resistance (Riesenfeld et al. 2004), enzymes involved in poly-hydroxybutyrate metabolism (Wang et al. 2006) and lysine racemases (Chen et al. 2009).

In the third type of function-based MG approach, Uchiyama et al. (2005) introduced a substrate-induced gene expression screening system (SIGEX) for the identification of novel catabolic genes wherein an operon-trap expression vector (a promoterless green fluorescent protein; gfp) was employed for cloning of environmental DNA. A similar screening strategy termed metabolite-regulated expression (METREX) has been developed together with fluorescence microscopy-mediated identification of positive fluorescent clones (Williamson et al. 2005). Now it is consensus that MG approach has been employed to characterize plant

Table 7.1 Function-based MG-derived biomolecules

Gene involved	Screening strategy	Source	Screened clones	References
Cyclodextrinase	Phenetic	Phagemids	200,000	Ferrer et al. (2005)
Beta-lactamase	Phenetic	Fosmids	8823	Song et al. (2005)
Quorum sensing inducer/inhibitor	METREX	BACs and fosmids	52,500 and 300	Williamson et al. (2005)
Aromatic hydrocarbon Catabolic operon fragments	SIGEX	Plasmids	152,000	Uchiyama et al. (2005)
Lysine racemase	Heterologous complementation	Plasmids	–	Chen et al. (2009)
Poly-3-hydroxybutyrate metabolism	Heterologous complementation	Cosmids	45,630	Wang et al. (2006)
Antibiotic resistance	Heterologous complementation	BACs and plasmids	28,200 and 1,158,000	Riesenfeld et al. (2004)
Na+/H+ antiporters	Heterologous complementation	Plasmids	1,480,000	Majernik et al. (2001)
DNA polymerase I	Heterologous complementation	Plasmids and fosmids	230,000 and 4000	Simon et al. (2009)
Antibiotics	Phenetic	Cosmids	–	Brady and Clardy (2004)
Xylanase	Phenetic	Phagemids	5,000,000	Lee et al. (2006)
Amidase	Heterologous complementation	Plasmids	193,000	Gabor et al. (2004)
Agarase	Phenetic	Cosmids	1532	Voget et al. (2003)
Protease	Phenetic Phenetic	Plasmids Fosmids	80,000 30,000	Waschkowitz et al. (2009) and Lee et al. (2007)
Cellulase	Phenetic Phenetic	Phagemids Cosmids	385,000 32,500	Rees et al. (2003) and Feng et al. (2007)
Esterase	Phenetic Phenetic	Fosmids Phagemids	5000 385,000	Rhee et al. (2005) and Rees et al. (2003)
Lipase/esterase	Phenetic	Plasmids	1,016,000	Henne et al. (2000)
Lipase	Phenetic Phenetic	Fosmids Cosmids	>7000 10,000	Hårdeman and Sjöling (2007) and Wei et al. (2009)

growth-promoting rhizobacteria (PGPRs) in RS. There are two steps for RS wherein MG involves in finding out the exploration of PGP genes and their products and the characterization of non-cultivable PGPRs. The MG approach has been employed to define bulk and RS soil with the same challenges (Daniel 2005). The relative low accessibility of initial sample may be the major obstruction in the construction of a MG library from RS soil DNA. To obviate this problem, a sample

should contain 1–10 g of soil recovered from 50 to 500 cm of root material (Jacobsen 2004). Researchers have developed methodologies to recover endophytic DNA for MG analysis, e.g. Jiao et al. (2006) employed enzymatic hydrolysis of plant tissues to recover DNA for cloning and deployed to the exploration of microbes for MG in the RS. The MG used for in vitro activity assays to exploit gain-of-function screenings of MG library from RS DNA, e.g. antibiosis to phytopathogens by testing whole-cell library clones. Several researchers have employed this assay to soilborne pathogens, e.g. *Erwinia*, *Xanthomonas*, *Fusarium*, *Rhizoctonia*, *Phytophthora* and *Pythium* (Emmert et al. 2004; Rangarajan et al. 2003; Chin-A-Woeng et al. 1998; Kim et al. 2006). Amongst functional attributes of PGPRs, researchers have employed MG library to characterize indole-3-acetic acid (IAA), cytokinins and their metabolites, genes for nitrogen fixation, exploration of RS exudates, 1-aminocyclopropane-1-carboxylate (ACC) deaminase, chitinase, solubilization of mineral phosphate, halotolerant cellulose and siderophore production (Glick et al. 1998; Timmusk et al. 1999; Rodriguez et al. 2000; Lee et al. 2003; Leveau and Lindow 2005; Tejera et al. 2005; Leveau et al. 2006; Voget et al. 2006).

The MG has been employed to understand the key metabolic processes that involved in soil phytic acid utilization. The MG analysis revealed changes in the relative abundance of the bacterial strains that enhance plant growth and phytic acid utilization together with gene clusters, namely, alkaline phosphatase and citrate synthase, with the phytic acid utilization status of the plant (Unno and Shinano 2013). In addition, MG approach has employed to assess microbial community composition and function in a constructed receiving surface water. Upon use of MG, it was estimated that the diversity of the microbial community of rhizosphere soil was found to be significantly greater than that of the wetland influent water. Functional analysis revealed a number of biodegradation pathways associated with 14 xenobiotic compounds that were identified in soil. Bai et al. (2014) found that the biological oxidation of Mn^{2+} in the influent water yielded insoluble Mn^{4+}, which subsequently precipitated and was incorporated into the wetland soil. These data showed that the use of MG analysis provided important new insights for the study of wetland ecosystems wherein how biologically mediated transformation used to reduce contamination of waste water (Bai et al. 2014). The MG approach was applied to investigate the bacterial diversity associated with the rice rhizosphere from a paddy field ecosystem in Kerala. Sequence analysis of 16S rRNA clones indicated a high diversity in the rhizosphere bacterial community with the majority of microbes being closely related to the *Proteobacteria*. Only a small fraction of the 16S rRNA sequences were highly similar to rRNA sequences from *Acidobacteria, Firmicutes* and *Bacteroidetes* groups. Since rhizosphere-associated microbes possess diverse metabolic capabilities and play a crucial role in plant health, knowledge on their community structure is imperative for the proper understanding of their individual roles, and metagenomics holds the promise to reveal several important questions regarding the unculturable fraction of the rhizosphere community (Arjun and Harikrishnan 2011).

7.3 Conclusions

Hence, MG approach is worthwhile to characterize functional microbial diversity in an environmental sample recovered from RS along with bulk soil with a particular function of interest.

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