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Rhizospheric microbes associated with bioenergy crops with special reference to *Jatropha curcas*: A critical review on the prospects and future challenges for sustainable bio-energy production

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There is an ever increasing need for bio-fuels due to escalations in oil prices. The global biofuel production tripled in last decade and it is estimated that the demand for bio-ethanol and bio-diesel will further double by the end of this decade. Among the various bio-energy crops, *Jatropha curcas* L. is one of the most promising as it has been popularly exploited for the production of bio-diesel. The review discusses prevalence of important microbial groups involved in nutrient cycling such as phosphate-solubilizing bacteria, arbuscular mycorrhizal fungi, plant growth promoting rhizobacteria in the rhizosphere. Further, the review identifies limitations, and addresses research gap of application of genomic technologies to characterize rhizospheric microbiota relevant for sustainability of bioenergy crop *J. curcas* L.

Key words: Bioenergy, *Jatropha*, rhizosphere, microbial diversity, nutrient cycling.

INTRODUCTION

In the recent years, serious attention has been diverted from non-renewable fossil fuels towards renewable, bio-degradable and non-polluting bio-fuels that has ignited tremendous research in the field of bio-energy crops all over the world such as Brazil, the United States, European Union (EU), Asia, Australia and Africa who are vying to shift their dependency from fossil fuels to bio-fuels in the next few years due to inflating oil prices. The global biofuel production tripled during 2000 to 2007, although it accounted for less than 3% of global transportation fuel supply (Coyle, 2007). According to the OECD-FAO Agricultural Outlook 2007 to 2016 (OECD-

Organization for Economic Co-operation and Development, FAO- Food and Agricultural Organization of the United Nations), bio-fuel demand is going to augment further over the time period 2007 to 2016. They expect ethanol output to double in Brazil and the United States and growth of oilseeds to increase drastically in the EU. USA and Brazil are the largest producers of bio-ethanol, followed by EU. India promotes growth of bio-diesel crops and 317 million L of biodiesel was produced during 2007 to 2008 as estimated by OECD-FAO 2008 and it is proposed that the transport mix should contain 20% bio-fuels by the end of this decade.

The utilization of energy crops as a source of renewable fuels is a concept with great relevance to current ecological and economic issues at both national and global scales. Crops that are currently being adapted for bio-energy are limited because of their potential role as food for human or animal or animal consumption. There is need for identifying crops that are least consumable and can be grown in waste or degraded land with minimum input. Many bioenergy crops that are widely explored recently are *Jatropha curcas* L. grown for bio diesel production. The genus name *Jatropha* derives from the Greek jatrós (doctor), trophé (food), which implies medicinal uses. Curcas is the common name for physic nut in Malabar, India. *Panicum virgatum* commonly known as switch grass, is a perennial grass native to the United States that has been studied as a sustainable source of biomass biofuel. *Pongamia pinnata* L., known as pongam or karanja or karanj. It is indigenous to India and Burma/Myanmar. Manihot esculenta, that is, cassava also known as manioc, which is grown worldwide (particularly in Africa, South America and most of Southeast Asia) contains excellent source of carbohydrates, raising the possibility that it could be used globally to alleviate dependence on fossil fuels. Other well recognized bioenergy crops are Agave, *Miscanthus* sp., *Saccharum* sp. and *Sorghum* L. Among them, *J. curcas* L. and *P. pinnata* L. are responsible for the production of bio-diesel. The seeds of *J. curcas* L. are found to contain 31 to 37% extractable oil with high oil yields under optimum environmental conditions (Pan and Xu, 2011). Although, concerns have been raised regarding growth of bio-energy crops in agricultural land which could perturb a country's food security, persistent research has suggested that these crops grow well in marginal soils like arid and semi-arid regions and wastelands.

J. curcas L., a potential bio-energy crop, is a monoecious perennial shrub that grows best in tropical monsoon type of climate with mean annual rainfall above 944 mm/year and annual temperature range of 19.3 to 27.2°C (Maes et al., 2009). Hence, it is distributed mostly in the tropical and subtropical regions of Asia (Natarajan et al., 2010), Africa (Brittaine and Litaladio, 2010a; Ndong et al., 2009; Renner, 2007), Australia (Carels, 2009), and South. However, it can also grow in temperate conditions and arid and semi-arid soils (Jongschaap et al., 2007). This deciduous shrub belongs to the family Euphorbiaceae plants (Maes et al., 2009). *Jatropha* species secrete secondary metabolites like curcin, saponin, trypsin inhibitor, lectins and alkaloids (HCN) allowing it tolerate various types of environmental stresses and rendering them toxic (Benge, 2006; Jongschaap et al., 2007; Devappa et al., 2011).

The soil types ideal for growth of *J. curcas* L. are loamy and aerated soils of minimum 45 cm depth with pH 6.0 to 8.0/8.5. Any soil type resulting in waterlogged conditions such as clayey soils are not suitable for cultivation of the

crop as it may result in diseases such as collar rot, leaf spots, root rot and damping-off (Brittaine and Litaladio, 2010b). Approximately 15% of Earth's land area has been degraded through anthropogenic activities and water erosion, wind erosion, chemical degradation and physical degradation are considered to be the major factors of soil degradation (Becker and Francis, 2000; Francis et al., 2005). Hence, *Jatropha* known for its vigorous drought-tolerant property can be one of the potential crops to restore soil fertility (Becker and Francis, 2000). Phosphorus is one of the major limiting factors for crop production on many tropical and subtropical soils (Norman et al., 1995) as a result of high phosphorus fixation. Inorganic fertilizer may affect soil biological properties and this practice may further reduce the soil function including microbial mediated nutrient transformations particularly in the degraded or marginal lands.

It is often reported that *J. curcas* can grow rapidly and produce commercial oil yields on poor or marginal agricultural lands growth is likely to be slow unless appropriate beneficial organisms and balanced fertilizer applied (Openshaw, 2000; Pramanik, 2003; Rao et al., 2008). To enhance productivity of bioenergy crops like *J. curcas* L. in waste lands, amendment of organic fertilizers and biofertilizers will be the best practice to manage soil and crop (Dobson et al., 1997; Mbagwu, 1992). For instance, a study suggests nitrogen and phosphorus fertilization effectively promotes the growth of *Jatropha* (Sinha et al., 2011). Therefore, research on rhizospheric microbes can throw light on their various advantages in terms of enhancement of growth and oil yields, nutrient cycling and ecosystem restoration. This review elucidates rhizospheric microbes of *J. curcas* L. and other bioenergy crops those are having their own advantages in terms of growth conditions, ecological impact and oil yields. Further, it discusses the possible gaps in our knowledge that need to be worked upon with regard to the importance of rhizospheric microbial community.

PREVIOUS RESEARCH ON DIVERSITY OF RHIZOSPHERIC MICROBIAL COMMUNITY OF BIOENERGY CROPS

Microbial population dynamics in the rhizosphere of bioenergy crop *Jatropha curcas*

Abundance of total heterotrophs, N₂ fixers, phosphase solubilizers estimated in bulk soil, rhizospheric soil, rhizosphere and endorhizosphere. Microbial groups irrespective of nutrient cycling activity were highly populated in the rhizosphere followed by rhizosphere and bulk soil (Mohanty et al., 2012). In addition soil enzymatic activity analyzed to characterize the differential microbial activity in bulk and rhizospheric soil. Microbial enzymatic activity comprising fluorescent diacetate assay (FDA),

dehydrogenase (DHA) and phosphatase were more in the rhizospheric soil compared to the bulk soil irrespective of soil types. FDA ranged from 5 to 10 μg fluorescein released g^{-1} soil h^{-1} . Whereas DHA varied in the range of 0.79 to 2.18 μg TPF released g^{-1} soil day^{-1} (unpublished data). Results confirmed high microbial metabolism in the rhizosphere in the rhizosphere however there is need of further studies linking plant attributes like root exudates and the microbial activity.

CHARACTERIZATION OF RHIZOSPHERE ARBUSCULAR MYCORRHIZA FUNGI

Growing bioenergy crop *J. curcas* L. in degraded land can be challenging to achieve the projected global demand unless we know the associated microbes and use them for enhancing the nutrient uptake ability. Rhizospheric microbes play critical role in plant nutrient use for growth, and its adaptation to ecosystem. The plant has direct influence on the rhizosphere microbial community because of root exudates and residue chemistry which act as nutrients for the microbes distinct from those present in the bulk soil; and in turn, the microbes break down complex forms of macronutrients which become readily available to the plants and aid their growth (Barea et al., 2005; Matilla et al., 2007). This review is based on existing literatures on soil and rhizospheric microbial community of *Jatropha* and other bioenergy crops. It was found that rhizosphere of *Jatropha* comprise of specific microbes that usually play relevant role during nutrient availability for plants. Prevalent microbial groups were arbuscular mycorrhizal fungi (AMF), actinomycetes, nitrogen-fixing bacteria, and plant growth-promoting rhizobacteria (PGPR). In a study AMF associated with rhizosphere of *J. curcas* L. from different ecological regions of India were identified by taxonomical key features of spores as described by Pérez and Schenck (1990) (Table 1). A total of 20 AMF species were recorded, which consisted of two species of *Acaulospora* and 18 species of *Glomus*. Among the AMF, the *Acaulospora* species were detected from the rhizosphere of *J. curcas* L. and *P. pinnata*. *Glomus* species were more abundant in the soil samples than *Acaulospora* and other AMF species (Kamalvanshi et al., 2010a). Similar screening of arbuscular mycorrhizal (AM) fungi on *J. curcas* were carried out which identified altogether, 21 AM fungal spores. *Glomus* sp were the most predominant among the recorded AM fungal spores. A small number of *Sclerocystis* species were recorded in all rhizospheric samples. In a study on the AMF distribution in rhizospheres of *J. curcas* and *P. pinnata* from different soil types of southern India revealed that black soil contained highest number of AMF than red soil. Soils from hill region had higher AMF population than dry and transitional zone.

In *J. curcas*, *Acaulospora* sp., were more abundant followed by *Gigaspora* sp, *Glomus* sp, *Sclerocystis* sp,

and *Scutellospora* sp. In other hand soil samples from *P. pinnata* were predominantly occupied by *Glomus* sp followed by *Acaulospora* sp, *Gigaspora* sp, *Sclerocystis* sp, and *Scutellospora* sp. (Venkatesh et al., 2009). In a study by (Lakshman, 2009), AMF root colonization and spore number in *J. curcas* were calculated and found that Spore number was negatively correlated to percent root colonization. *Glomus* species was the most predominant among the recorded AM fungal spores although a small number of *Sclerocystis* species were recorded in all rhizospheric samples. In a study to trap AMF by *J. curcas* and its use to enhance productivity of other crops, ten species of AMF that were trapped, two species, *Scutellospora heterogama* (CMU33) and *Entrophospora colombiana* (CMU05) produced abundant spores (>50 spores/100 g soil) and heavily colonized the roots of the *Jatropha* plant (Charoenpakdee et al., 2010). Impact of land use on the AMF diversity pattern in the rhizosphere of *J. curcas* has been investigated at the Guantanamo (Cuba). The AM fungal small sub-unit (SSU) rRNA genes were subjected to PCR, cloning, sequencing and phylogenetic analyses. Twenty AM fungal sequence types were identified: 19 belong to the Glomeraceae and one to the Paraglomeraceae (del Mar Alguacil et al., 2012). In another experiment, taxonomic identification of AMF spores were identified by matching with the description provided by the International Collection of Vesicular Arbuscular Mycorrhizal Fungi (Morton et al., 1993). Soil samples were collected from rhizosphere of 120 plants of *J. curcas* and 120 plants of *P. pinnata* located at different agro-ecological regions of India. Study revealed that there were 5 different species AMF belonging to *Acaulospora* were recorded (Table 1). Species richness and frequency of occurrence (%) of AM fungi associated with different studied plant species were also calculated (Sharda and Rodrigues, 2008). In *J. curcas*, the highest frequency of AMF occurrence was recorded for *A. scrobiculata* (100%), followed by *A. denticulata* (33%), and *A. mellea* (17%).

Microbial communities associated with rhizosphere and bulk soils of switchgrass (*Panicum virgatum* L.) and *jatropha* (*J. curcas* L.) using phospholipid fatty acid (PLFA) and length heterogeneity PCR (LH-PCR) has been investigated (Chaudhary et al., 2011). PLFA and PLFA profile of rhizospheric and bulk soil of bioenergy crops revealed that microbial community was more dependent to plant type than soil characteristics. A comparative prevalence of PLFA in rhizospheric and bulk soil has been summarized in Table 1. Both plants exhibited higher abundance of saturated (16:0, 18:0, 20:0), actinomycetes (10Me17:0), and fungal (18:2 ω 6, 9c) PLFAs in rhizospheric soil than bulk soil; whereas bulk soil is abundant with gram negative PLFAs (16:1 ω 9c, 16:1 ω 5c, 16:1 ω 7c). *Jatropha* had a higher abundance of fungal PLFAs (18:2 ω 6,9c), 18:1 ω 9c, 20:1 ω 9c) compared to switchgrass rhizosphere soil; whereas Switchgrass soil contained a significantly

Table 1. Microbial groups associated in the rhizosphere of of bio-energy crop *Jatropha curcas* L.

Microbial groups	Method of microbial characterization	Species / biosignature identified	Selected references
Arbuscular mycorrhiza fungi (AMF)	Spore morphological characterization : (Pérez and Schenck, 1990)International Culture Collection of Vesicular and Arbuscular Endomycorrhizal Fungi [http://invam.caf.wvu.edu/Myc_Info/Taxonomy/species.htm]) (Trap and Schenck, 1982),	<i>Acaulospora scrobiculata</i>	Charoenpakdee et al. (2010), Kamalvanshi et al. (2011, 2010a, b), Lakshman (2009), Del Mar Alguacil et al. (2012), Morton et al. (1993), Sharda and Rodrigues (2008)
		<i>Acaulospora denticulata</i>	
		<i>Acaulospora mellea</i>	
		<i>Glomus intraradix</i>	
		<i>Glomus etunicatum</i>	
		<i>Glomus 1</i>	
		<i>Glomus aggregatum</i>	
		<i>Glomus arborensis</i>	
		<i>Glomus cerebriforme</i>	
		<i>Glomus diaphanum</i>	
		<i>Glomus hoi</i>	
		<i>Glomus microaggregatum</i>	
		<i>Glomus microcarpum</i>	
		<i>Glomus occultum</i>	
		<i>Glomus segmentatum</i>	
		<i>Glomus vesiculiforme</i>	
		<i>Glomus 2, 3</i>	
		<i>Glomus 4, 5, 6</i>	
		<i>Gigaspora sp.</i>	
<i>Sclerocystis sp.</i>			
<i>Scutellospora sp.</i>			
<i>Entrophospora colombiana</i>			
<i>Scutellospora heterogama</i>			
<i>Entrophospora colombiana</i>			
Partial small subunit (SSU) ribosomal RNA clone library		<i>Glomeraceae</i>	
		<i>Paraglomeraceae</i>	
Microbial diversity	Phospholipid fatty acid (PLFA) analysis	16:0,18:0,20:0	Chaudhary et al. (2011)
		18:2w6,9c; Fungi 18:1w9c; Fungi 20:1w9c; Fungi 10Me17:0; Actinomycetes	
	Length heterogeneity PCR (LH PCR)	DNA fragments : 310, 315, 321, 326, 340, 346, 348, 349, 352	
Plant growth promoting rhizobacteria (PGPR)	Enrichment culturing	<i>Enterobacter cancerogenus</i> MSA2	Jha et al. (2011)
		<i>Pseudomonas, Azotobacter and Rhizobium</i>	Nanda and Abraham (2011)

higher abundance of Gram positive (i14:0, i15:0, a15:0), Gram negative negative(16:1w5c, 16:1w7c, 18:1w5c), and saturated (14:0, 15:0) PLFAs compared to *jatropha* rhizosphere soil. LH-PCR using the primer set 27f- 5'-AGA GTT TGA TCC TGG CTC AG-3' and 338R- 5'GCT GCC TCC CGT AGG AGT - 3' resulted in 17 to 19 fragments per rhizosphere and bulk soil of *Jataropha*. Rhizosphere contained significant amount of 331, 337, 349, 352 and 357 fragnents while the bulk soil had 310, 313, 321, 326, 344 and 348 bp length fragments (Chaudhary et al., 2011).

The AMF form a huge part of the microbial kingdom that boost plant growth, yield and augment its tolerance to environmental stress conditions like drought (Jeffries et al., 2003; Ruiz-Lozano et al., 1995), salinity (Al-Karaki, 2006), nutrient limitations (Pfleger and Linderman, 1994) and pathogens (Hussey and Roncadorl, 1982). The realm of fungal association lies with the fact that fungal communities are comparatively more efficient in nutrient mobilization than bacteria in nutrient deficient soils. Typically, in mycorrhizal association, the fungus acquires carbon from the plant, while the plant obtains otherwise

unavailable mineral nutrients via the fungal hyphae (threads) which extend the effective absorptive area of the root system. The hyphae are narrower than roots and can extend further to explore a greater volume of soil, thus having a much higher surface to volume ratio for absorption of nutrients and water. Additionally, the fungi secrete enzymes which break down organic matter and tightly bound micronutrients, such as phosphorus, zinc and copper, enabling the plant to absorb the required minerals from the surrounding soil. Also mycorrhizal fungi are known to be important for soil aggregation under well-watered conditions (Miller and Jastrow, 2000). Apart from plant nutrition, AMF contribute to soil structure stability. The fungal extra radical hyphae act as a skeleton to the soil structure holding the soil particles together that control plant growth and act as source of organic nutrients for the bacteria (Andrade et al., 1998; Jastrow, 1996; Wright et al., 2007). The microaggregates that enmesh into macroaggregates have high amount of microbial activity leading to accumulation of bacterial by-products that are beneficial to plants (Andrade et al., 1998). AMF are well documented for their potential in enhancing plant growth and yield, resistance to drought and salinity and tolerance to pathogens (Smith and Gianinazzi-Pearson, 1988).

CHARACTERIZATION OF PLANT GROWTH PROMOTING RHIZOTOBACTERIA (PGPR) IN RHIZOSPHERE OF BIOENERGY CROPS

The Plant growth promoting rhizotobacteria (PGPR) are a group of bacteria that can actively colonize plant roots and increase plant growth. PGPR produce plant growth-promoting compounds, including phytohormones (auxins, cytokinins and gibberellins) and siderophores (Saikia et al., 2006), and antibacterial peptides that inhibit pathogenic strains. Positive response of many bioenergy crops to microbial inoculants has been observed.

Supplementing bioenergy crop *P. pinnata* grown in nutrient-limiting conditions with AMF, *Azospirillum* sp., *Azotobacter* sp. and *P. fluorescens* by applying consortium approach appeared to be beneficial to the crop after two years. Few papers have acknowledged the presence of nitrogen-fixing *Azospirillum* sp. in the roots of biomass biofuel crop *Miscanthus*. For instance, Eckert et al. (2001) detected a new species, *Azospirillum doebereineriae* sp. nov. and Kirchhof et al. (1997) found *A. lipoferum* and *Herbaspirillum* sp. In a study at Guantanamo (Cuba) to verify if a change of land use to biofuel plant production had any effect on the AMF communities. Rhizospheric AMF community of *Jatropha curcas* and *Ricinus communis* were analyzed along with soil properties related to the soil fertility (total N, Organic C, microbial biomass C, aggregate stability percentage, pH and electrical conductivity). The AM fungal small sub-unit (SSU) rRNA genes were subjected to PCR, cloning,

sequencing and phylogenetic analyses. This is the first study using molecular techniques to derive extent of AMF diversity under changing the land use to biofuel plant production. Twenty AM fungal sequence types were identified where 19 belong to the *Glomeraceae* and one to the *Paraglomeraceae*. Two AMF sequence types related to cultured AMF species (Glo G3 for *Glomus sinuosum* and Glo G6 for *Glomus intraradices*-*G. fasciculatum*-*G. irregulare*) did not occur in the soil cultivated with *J. curcas* and *R. communis*.

Plant growth promoting gamma-proteobacterium, *Enterobacter cancerogenus* MSA2 were isolated from the rhizosphere of *J. curcas* L. (Table 1). *E. cancerogenus* produced growth hormone ACC (1-amino-cyclopropane-1-carboxylate) deaminase, phytases, auxin IAA (indole acetic acid), siderophores, ammonia and it solubilized phosphate. On sowing of *Jatropha* seeds coated with bacterial inoculum in earthen pots with regularly watered sandy loamy soil, the *Enterobacter* was able to enhance various parts of *J. curcas* such as root length by 124.14%, fresh root mass by 81%, fresh shoot mass by 120.02%, dry root mass by 124%, dry shoot mass by 105.54%, leaf number by 30.72%, chlorophyll content by 50.41% and biomass by 87.20% as compared to control under experimental conditions (Jha et al., 2012). Another study carried out to investigate the remediation of heavy metals such as As, Cr, Cu and Mg by the rhizospheric microflora of *J. multifida* (Nanda and Abraham, 2011). Soils spiked with heavy metals were amended with wastewater biosludge and biofertilizer increased the available nutrients in soil and PGPR like *Pseudomonas*, *Azotobacter* and *Rhizobium* isolated from the soil showed tolerance against heavy metals (Table 1). Plant growth promoting rhizobacteria (PGPR) *Enterobacter* sp has been isolated from soil and rhizosphere of plants, for example, rice (Mehnaz et al., 2001), mustard (Ahmad and Khan, 2010), raspberry (Orhan et al., 2006), leguminous plants (Yoon et al., 1996), and showing its natural association to this environment. *Enterobacter* enhance root length (Glick et al., 1995; Li et al., 2000). Similarly *Pseudomonas* stimulates plant growth and it is hypothesized that colonization of this microbes suppress deleterious rhizosphere microflora, especially deleterious endorhizosphere bacteria (Van Peer and Schippers, 1989). *Azotobacter*, *Rhizospbium* found in soil of *Jatropha* are well characterized from the rhizospheric of many plants (Ahmad et al., 2005; Jaleel et al., 2007; Joseph et al., 2007). *Azotobacter* are well recognized for their potential in N₂ fixation and PGPR activity. Systematic studies on N₂ fixers association with plant and N₂ fixing rate in soil or rhizosphere of *J. curacs* are lacking.

PGPR promotes plant growth by through ACC (1-Aminocyclopropane-1-Carboxylate) deaminase activity. This enzyme catalyzes the conversion of ACC, the immediate precursor of ethylene synthesis in plants, to ammonia and α -ketobutyrate as ethylene inhibits plant

growth. ACC exuded from seeds or plant roots are metabolized by PGPR microbes expressing ACC deaminase activity, which stimulates plant ACC efflux under stress, decreasing the root ACC concentration and root ethylene evolution and increasing root growth (Glick et al., 1995). Thus PGPR with ACC deaminase activity promote plant growth under a variety of stressful conditions, such as flooding (Grichko and Glick, 2001), saline conditions (Mayak et al., 2004a), and drought (Mayak et al., 2004b). Secondly PGPRs exert plant growth-promoting response by depriving native microflora of iron by producing extracellular siderophores (microbial iron transport agents) which efficiently complex environmental iron, making it less available to certain native microflora (Kloepper et al., 1980). Recently the functional diversity of rhizospheric microorganisms investigated in select *Jatropha* field soils located in Gujarat, India. With combinatorial effect of species richness and diversity complex microbial diversity identified. PGPR isolates like phosphate solubilisation, siderophore production, indole acetic acid production, ACC deaminase production, HCN production, EPS production and ammonia production were enumerated from the rhizosphere of *Jatropha curcas* (Jha et al., 2011). This review also identifies presence of actinomycetes specific PLFA in the rhizosphere of *J. curcas* (Table 1). With cultivation independent approach increasing numbers of endophytic bacteria are being isolated and identified revealing a rich vein of microbial interaction within a variety of crop plants (Franco et al., 2007). These microbial groups are well characterized for their potential as (a) source of agroactive compounds, (b) plant growth promoting organisms, and (c) biocontrol tools of plant diseases (Doubou et al., 2001). PGPR stimulate systematic plant resistance against pathogens (Jetiyanon et al., 2003; Zehnder et al., 2001). Some of these rhizobacteria may also be used in integrated pest management programmes. Thus there is greater application of PGPR in *Jatropha* for biocontrol of plant pathogens and biofertilization.

CONCLUDING REMARKS AND FUTURE PERSPECTIVES OF RESEARCH HARNESSING RHIZOSPHERIC MICROORGANISMS FOR SUSTAINABLE *JATROPHA CURCAS* BIOENERGY CROP

It is tempting to say that rhizosphere of bioenergy crop *J. curcas* is dominated by microbial groups that are important for nutrient availability and mobilization. Particularly AMF, actinomycetes are more prevalent than bacteria in the rhizosphere. However most of the literatures explore structure of the AMF/fungal communities based on PLFA, microscopic characterization and genomic studies. Phospholipid fatty acids (PLFAs) are widely accepted as biomarkers that

indicate viable microbial biomass and provide a microbial community 'fingerprint'. But this approach is appropriate for quantitative than qualitative analysis of microbial community. The combination of cultivation dependent and independent techniques in the study is recommended to characterize whole microbial community. Cultivation-based techniques will enable us to recover and test potential isolates, whereas cultivation-independent techniques enable the screening for variations in the total rhizospheric microbial communities (Van Overbeek and van Elsas, 2008). Also there is need of understanding on the linkage between plant type and its rhizospheric microbial community. Because plant factors drive the bacterial communities in terms of the richness and evenness, and microbial strain associated during different stages of colonization of the roots of plants. In addition, there is need of research to define the endosymbiotic microbial community. Endosymbionts might play important role because of habitat of *J. curcas* that is, growing in degraded or marginal land or under nutrient limited conditions. Plants grown under such conditions undergoes stress and produces compounds, such as terpenoids, benzoxazinone and particularly flavonoids and isoflavonoids during stress, causing its root interior and even exterior parts a forbidden territories for a vast majority of beneficial soil beneficial bacteria (Bais et al., 2006). Endophytic population acts more efficiently as sink to such products and help in plant growth. Identifying the endophytic microbes (efficient ACC deaminase producers and/or toxins that is, curcumin degraders) associated with *Jatropha* will potentially improve plant growth. Research on endophytic diversity, colonization mechanism (that is, plant selective process) and microbial-plant interaction are required for sustainable biofuel crops. The review identifies information gaps relating to the microbial gene expression relevant to nutrient cycling in rhizosphere of *J. curcas*. The nutrient cycle involves complex interaction between biological, chemical and plant, some of which are not yet fully understood. Nutrient cycling genes expression profile in different varieties, various stages of crop and management are relevant to understand microbial plant interaction. To better understand activity regulation within biological systems in the rhizosphere (ecto-endo), gene expression profiling of N_2 fixers, P solubilizers, and other PGPRs are essential. Apart from rhizosphere, there is need of extensive research on the diversity and response of epiphytes microbes particularly prevalent in the phyllosphere of *J. curcas*. Phyllosphere bacteria can promote plant growth and both suppress and stimulate the colonization and infection of tissues by plant pathogens (Lindow and Brandl, 2003; Rasche et al., 2006). Bacterial mediated nitrogen fixation in the phyllosphere in many tropical plants occurs around 60 kg $N\ ha^{-1}$ (Freiberg, 1998). Such epiphytic N_2 fixing activity also recorded in many agronomic crops (Murty, 1984; Miyamoto et al., 2004). Identifying and characterizing the

epiphytic microbial community in *J. curcas* would provide mechanistic processes of plant microbial interaction for minimal agricultural input and enhanced biofuel production.

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