Chapter 7
Plant Growth-Promoting Bacteria Associated with Sugarcane

Samina Mehnaz

7.1 Introduction

Sugarcane is an important industrial and cash crop in many countries of the world. It is grown in over 110 countries, in tropical and sub-tropical regions, in a range of climates from hot dry environment near sea level to cool and moist environment at higher elevations. Besides sugar production, sugarcane produces numerous valuable by-products like ethanol, bagasse, press mud, molasses, and essential items for industries like chemicals, plastics, paints, synthetics, fiber, insecticides, and detergents (http://www.pakissan.com). This crop is perhaps the most economically competitive source of ethanol and can effectively contribute to a cleaner environment. Ways of improving its productivity are subject to investigation in several countries. Worldwide climate change due to the intense use of greenhouse gas-producing energy sources has resulted in the development of sustainable energy. Consequently, sustaining and enhancing the growth and yield of sugarcane have become a major focus of research.

Sugarcane and other grasses such as rice, wheat, maize, and sorghum, currently have much of their nitrogen (N) needs supplied by costly mineral fertilizers. It has been a general practice to apply 250 kg N ha^{-1} $year^{-1}$, or more in most of the sugarcane cultivating countries. In 2008, an estimated 1,743 million metric tons of sugarcane were produced worldwide, with about 50% of production occurring in Brazil and India. In India, sugarcane is grown over 4.2 million ha, producing about 250 million tons of canes annually and the nitrogen requirement of Indian sugarcane ranges from about 250 to 350 kg ha^{-1}. Brazil is the largest sugarcane producer in the world, with the crop occupying more than five million hectares with a yield of 495 M tons in 2007/2008 (UNICA 2009), 16 million m^3 of alcohol in 2006 (Mendes

S. Mehnaz
Department of Microbiology and Molecular Genetics, University of the Punjab, Quaid-i-Azam Campus, Lahore 54590, Pakistan
and
Institute of Pharmaceutical Biology, Bonn University, Bonn 53115, Germany
e-mail: saminamehnaz@gmail.com

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et al. 2007; Oliveira et al. 2006) and annual application of nitrogen fertilizer for sugarcane is around 50 kg N ha^{-1}, with a cost near US$ 500 t^{-1} (http://www.udop.com.br). Researchers in Brazil are intensively working on further reducing the use of N-fertilizer application by one half (<125,000 t N $year^{-1}$) due to the biological nitrogen fixation (BNF), so the producers could save estimated US$ 62.5 m $year^{-1}$ (Oliveira et al. 2006). This approach can significantly reduce the cost of bio-energy in the whole world.

7.2 Plant Growth-Promoting Rhizobacteria

The biological reaction that counterbalances the loss of nitrogen from soils or agro-ecosystems is the BNF, which is the enzymatic reduction of the atmospheric di-nitrogen (N_2) to ammonia, catalyzed by nitrogenase and this process is unique to Bacteria and Archaea. Bacteria which fix nitrogen can also be beneficial for the plants by using other mechanisms such as phytohormone production, phosphate solubilization, etc. They are grouped under the name plant growth-promoting rhizobacteria (PGPR) also known as plant growth-promoting bacteria (PGPB) defined as "free-living soil, rhizosphere, rhizoplane, endophytic, and phyllosphere bacteria that under certain conditions are beneficial for plants" (Bashan and de Bashan 2005). They are capable of promoting plant growth through different mechanisms, including BNF, phytohormone production, phosphate solubilization, siderophore production, and biological control. PGPR belong to diverse genera including *Azospirillum*, *Azotobacter*, *Herbaspirillum*, *Bacillus*, *Burkholderia*, *Pseudomonas*, *Rhizobium,* and *Gluconacetobacter*, among others. In this article, only those PGPRs will be discussed which have been isolated from sugarcane. A complete list of genera, species, source of isolation, country of origin and references are provided in Table 7.1.

7.2.1 Azospirillum

Azospirillum belong to the facultative endophytic group of bacteria which colonizes the surface and interior of the roots. Bacteria are micro-aerophilic, gram-negative rods and often associated with roots of cereals and grasses (Grifoni et al. 1995). They are very well known for nitrogen fixation and higher production of indole acetic acid. In the group of bacteria responsible for associative nitrogen fixation, *Azospirillum* is as important as *Rhizobium* in the bacterial group known for symbiotic nitrogen fixation. It is a very well-studied PGPR. Several reports have been published about its beneficial effect due to nitrogen fixation and growth hormone production. Due to its nonpathogenic behavior, it is the safest choice to use as a biofertilizer for any crop.

Three species of this genus namely, *Azospirillum amazonense*, *A. brasilense,* and *A. lipoferum* have been isolated from sugarcane. In 1976, Dobereiner and Day

Table 7.1 List of the bacterial genera and species isolated from the sugarcane

Bacteria	Source	Country	References
Acinetobacter baumanii	Apoplast	Cuba	Velázquez et al. (2008)
Agrobacterium tumefaciens	Stem	China	Xing et al. (2006)
Azospirillum sp.	Rhizosphere, roots	Egypt, South Africa, Brazil, India	Gangwar and Kaur (2009), Hegazi et al. (1979), Purchase (1980), Ruschel (1981)
A. brasilense	Rhizosphere, root, stem, leaves	Spain, Pakistan, Brazil	Graciolli et al. (1983), Mehnaz et al. (2010), Reis et al. (2000), Tejera et al. (2005)
A. lipoferum	Root, stem, leaves	Brazil	Dobereiner and Day (1976), Reinhardt et al. (2008), Reis et al. (2000), Tejera et al. (2005)
A. amazonense	Roots, stem	Brazil	Cavalcante and Dobereiner (1988), Reis et al. (2000)
Azotobacter chroococum	Roots	Spain	Tejera et al. (2005)
A. vinelandii	Rhizosphere, roots	Egypt, Brazil	Graciolli et al. (1983), Hegazi et al. (1979), Rennie (1980)
Bacillus spp.	Rhizosphere, roots, Stem	South Africa, India, Pakistan	Antwerpen et al. (2002), Gangwar and Kaur (2009), Hassan et al. (2010)
B. cereus	Apoplast	Cuba	Velázquez et al. (2008)
B. pumilus	Apoplast	Cuba	Velázquez et al. (2008)
B. subtilis	Apoplast, rhizosphere	Cuba, Pakistan	Hassan et al. (2010), Velázquez et al. (2008)
Beijerinckia sp.	Root	Brazil	Vendruscolo (1995)
B. fluminensis	Rhizosphere	Brazil	Dobereiner (1961), Dobereiner and Alvahydo (1959)
B. indica	Rhizosphere, roots	Brazil	Dobereiner et al. (1972)
Brevibacillus sp.	Stem, leaves	Brazil	Magnani et al. (2010)
Burkholderia spp.	Stem, leaves	South Africa, Brazil	Antwerpen et al. (2002), Perin et al. (2006b)
B. ambifaria	Rhizosphere, roots	South Africa	Omarjee et al. (2008)
B. cepacia	Rhizosphere, roots, stem	Brazil, South Africa	Luzivotto et al. (2010), Mendes et al. (2007), Omarjee et al. (2008)
B. cenocepacia	Roots, stem	Brazil	Mendes et al. (2007)
B. fungorum/ graminis	Rhizosphere, roots	South Africa	Omarjee et al. (2008)
B. gladioli	Rhizosphere, roots, stem	South Africa	Omarjee et al. (2004, 2008)
B. plantarii/glumae	Stem	Papua New Guinea	Omarjee et al. (2004)
B. sacchari	Rhizosphere	Brazil	Bramer et al. (2001)
B. silvatlantica	Rhizosphere, roots, leaves	Brazil	Omarjee et al. (2008), Perin et al. (2006a)
B. tropica	Rhizosphere, roots	South Africa, Mexico	Omarjee et al. (2008), Perin et al. (2006b), Reis et al. (2004)

(*continued*)

Table 7.1 (continued)

Bacteria	Source	Country	References
B. unamae	Stem	Papua New Guinea, Brazil, Mexico	Caballero-Mellado et al. (2004), Omarjee et al. (2004), Perin et al. (2006b)
B. vietnamiensis	Stem	India	Govindrajan et al. (2007)
Caulobacter crescentus	Roots	Pakistan	Mehnaz et al. (2010)
Citrobacter sp.	Rhizosphere	Brazil	Magnani et al. (2010)
Comamonas testosteroni	Apoplast	Cuba	Velázquez et al. (2008)
Curtobacterium sp.	Stem	Brazil	Magnani et al. (2010)
Delftia acidovorans	Stem, leaves	Pakistan	Mehnaz et al. (2010)
Derxia gummosa	Rhizosphere	Brazil	Graciolli et al. (1983), Rennie (1980)
Enterobacter sp.	Rhizosphere, roots	Brazil, Australia	Li and Macrae (1992), Magnani et al. (2010)
E. aerogenes	Stem	Pakistan	Mehnaz et al. (2010)
E. cloacae	Rhizosphere, roots, stem	Pakistan, Brazil	Graciolli et al. (1983), Mehnaz et al. (2010), Mirza et al. (2001), Rennie (1980), Rennie et al. (1982)
E. oryzae	Stem	Pakistan	Mehnaz et al. (2010)
Erwinia cypripedii	Apoplast	Cuba	Velázquez et al. (2008)
E. herbicola	Stem	Brazil	Graciolli et al. (1983), Rennie et al. (1982)
Gluconacetobacter diazotrophicus	Roots, stem, leaves, apoplast, bud, sugarcane juice	Brazil, Australia, India, Egypt, Cuba, Mexico, Philippines, Argentina	Asis et al. (2000), Bellone et al. (1997), Cavalcante and Dobereiner (1988), Dong et al. (1994), Fuentes-Ramirez et al. (1993), Gillis et al. (1989), Li and Macrae (1991), Muthukumarasamy et al. (1994), Prabudoss and Stella (2009), Reis et al. (1994), Velázquez et al. (2008), Youssef et al. (2004)
G. saccharii	Leaf sheath	Australia	Franke et al. (1999)
Herbaspirillum seorpedaceae	Stem, leaves	Brazil, Philippines	Asis et al. (2000), Baldani et al. (1992), Olivares et al. (1996)
H. rubrisubulbicans	Leaves	Brazil, Philippines	Asis et al. (2000), Olivares et al. (1996), Pimentel et al. (1991)
Klebsiella spp.	Stem	Brazil, South Africa	Antwerpen et al. (2002), Magnani et al. (2010)
K. oxytoca	Rhizosphere, roots, stem	Pakistan	Mehnaz et al. (2010), Mirza et al. (2001)
K. pneumoniae	Roots, stem	India, Brazil, Australia	Govindrajan et al. (2007), Graciolli et al. (1983), Li and Macrae (1992), Rennie et al. (1982)
K. variicola	Stem	Mexico	Rosenblueth et al. (2004)

(*continued*)

Table 7.1 (continued)

Bacteria	Source	Country	References
Kocuria kristinae	Apoplast	Cuba	Velázquez et al. (2008)
Lactococcus lactis subsp. *lactis*	Leaves	Colombia	Cock and de Stauvenel (2006)
Microbacterium oleivorans	Apoplast	Cuba	Velázquez et al. (2008)
M. testaceum	Stem	Brazil	Mendes et al. (2007)
Micrococcus luteus	Apoplast	Cuba	Velázquez et al. (2008)
Ochrobactrum intermedium	Rhizosphere	Pakistan	Hassan et al. (2010)
Paenibacillus azotofixans	Roots	Brazil, Hawaii	Cavalcante and Dobereiner (1988), Seldin and Penido (1986)
P. polymyxa	Roots, stem	Brazil	Graciolli et al. (1983), Rennie (1980), Rennie et al. (1982)
Pannonibacter phragmitetus	Root	Pakistan	Mehnaz et al. (2010)
Pantoea sp.	Stem, leaves	Cuba, Brazil	Loiret et al. (2004), Magnani et al. (2010)
P. ananatis	Stem	Brazil	Mendes et al. (2007)
P. herbicola	Roots, stem, leaves	Brazil	Graciolli et al. (1986)
P. stewartii	Stem	Brazil	Mendes et al. (2007)
Pseudomonas spp.	Rhizosphere, roots, stem, leaves	Brazil, South Africa, Australia, India	Antwerpen et al. (2002), Gangwar and Kaur (2009), Li and Macrae (1991), Magnani et al. (2010)
P. aeruginosa	Stem	India	Viswanathan et al. (2003)
P. aurantiaca	Stem	Pakistan	Mehnaz et al. (2009b)
P. fluorescence	Roots, stem	India, Pakistan, Brazil	Mehnaz et al. (2009a), Mendes et al. (2007), Viswanathana and Samiyappan (2002)
P. putida	Rhizosphere, roots, stem	India, Pakistan	Mehnaz et al. (2009a), Viswanathana and Samiyappan (2002)
P. reactans	Stem	Pakistan	Mehnaz et al. (2010)
Rahnella aquatilis	Roots	Pakistan	Mehnaz et al. (2010)
Rhizobium sp.	Roots	Pakistan	Mehnaz et al. (2010)
R. rhizogenes	Apoplast	Cuba	Velázquez et al. (2008)
Saccharibacillus sacchari	Apoplast	Spain	Rivas et al. (2008)
Serratia spp.	Stem	South Africa	Antwerpen et al. (2002)
Staphylococcus sp.	Stem, leaves	Brazil	Magnani et al. (2010)
S. epidermidis	Apoplast	Cuba	Velázquez et al. (2008)
S. saprophyticus	Apoplast	Cuba	Velázquez et al. (2008)
Stenotrophomonas maltophilia	Rhizosphere	Pakistan	Hassan et al. (2010), Mehnaz et al. (2010)
S. pavanii	Stem	Brazil	Ramos et al. (2010)
Xanthomonas spp.	Stem	South Africa, Pakistan	Antwerpen et al. (2002), Mehnaz et al. (2010)
X. campestris	Apoplast	Cuba	Velázquez et al. (2008)
X. oryzae	Apoplast	Cuba	Velázquez et al. (2008)
Zymomonas sp.	Stem	South Africa	Antwerpen et al. (2002)

reported the isolation of *Spirillum lipoferum* (now known as *A. lipoferum*) from sugarcane roots. Dobereiner also isolated *A. amazonense* from sugarcane roots but data were not published. The information was communicated without any detail in 1988, when isolation of *Gluconacetobacter diazotrophicus* was reported (Cavalcante and Dobereiner 1988). *A. brasilense* was also isolated from roots of Brazilian cultivars by Graciolli et al. (1983). Reis et al. (2000) isolated *A. brasilense*, *A. lipoferum,* and *A. amazonense* from four genotypes of Brazilian sugarcane and found them in all parts of the plant except that *A. amazonense* was not found in leaves. Other than Brazil, isolation of *Azospirillum* spp. have been reported from Egypt, India, Pakistan, South Africa, and Spain (Gangwar and Kaur 2009; Hegazi et al. 1979; Mehnaz et al. 2010; Purchase 1980; Tejera et al. 2005).

7.2.2 *Azotobacter*

Azotobacter is a gram-negative, polymorphic, obligate aerobic bacterium, although it can grow under low pO_2. It is very well known for its nitrogen-fixing ability and can fix at least 10 mg N g^{-1} of carbohydrate (Becking 1992). *Azotobacter* is a poor competitor for nutrients in soil and mostly isolated from roots of the grasses. Two species, namely *Azotobacter chroococum* and *A. vinelandii*, have been isolated from sugarcane. Hegazi et al. (1979) and Rennie (1980) isolated the *A. vinelandii* from sugarcane rhizosphere. Graciolli et al. (1983) reported its isolation from roots. *A. chroococcum* is recently reported from roots of sugarcane cultivars growing in south of Spain (Tejera et al. 2005). The author could not find any other report about the isolation of this organism from sugarcane.

7.2.3 *Beijerinckia*

The occurrence of nitrogen-fixing bacteria in this genus was mentioned for the first time in Brazil. Dobereiner and Alvahydo (1959) and Dobereiner (1961) reported the first observations of selective stimulation of nitrogen-fixing bacteria in sugarcane in Brazil. Additional studies on the occurrence of this genus in soil of several Brazilian States (Rio de Janeiro, São Paulo, Pernambuco and Paraná) led to the description of a new species named *B. fluminensis* (Dobereiner and Ruschel 1958). Analysis of 158 samples collected in different regions of Brazil showed that this species occurred predominantly in soils where sugarcane was cultivated (Dobereiner 1959a), as 95% of sugarcane soil samples contained *Beijerinckia*. In the sugarcane rhizosphere and on the root surface there were 20–50 times more *Beijerinckia* and two to five times less other microorganisms than in control soil (Dobereiner 1961). Additional studies showed that roots, leaves, and stems had positive influence on *Beijerinckia* population. A direct influence of the plant on the development of bacteria was suggested (Döbereiner 1959b).

In 1970s, the introduction of acetylene reduction methodology stimulated further studies involving *Beijerinckia* and sugarcane. The nitrogenase activity in sugarcane roots was much higher than rhizosphere and nonrhizospheric soil (between the plant rows) and *Beijerinckia indica* was the most abundant bacterial species in roots and soil samples (Dobereiner et al. 1972). Quantitation of BNF in sugarcane based on the extrapolation of the nitrogenase activity data indicated a contribution of 50 kg N ha^{-1} $year^{-1}$ (Dobereiner et al. 1973). Vendruscolo (1995) also isolated *Beijerinckia* sp. from the roots of sugarcane. Before the discovery of *G. diazotrophicus*, *Beijerinckia* was considered as the most important genus, responsible for nitrogen fixation in sugarcane growing in Brazil. Isolation of this bacterium, from sugarcane, is not reported from any other country although it has been isolated from other crops.

7.2.4 Burkholderia

The genus refers to a group of ubiquitous Gram-negative, motile, obligate aerobic rod-shaped bacteria including animal, human, and plant pathogens as well as some environmentally-important species. Some of these organisms are useful for promoting plant growth and bio-remediation. However, the problem about the threat to human health remains open. Until very recently, the genus *Burkholderia* included 30 properly described species, but the number of novel *Burkholderia* species has continuously increased (Perin et al. 2006b). Five of them, *Burkholderia vietnamiensis*, *B. kururiensis*, *B. unamae*, *B. tropica*, and *B. xenovorans* can fix atmospheric nitrogen, three species namely *B. tuberum*, *B. phymatum*, and *B. caribensis* help the formation of nitrogen-fixing tubers of bean in tropical regions whereas *B. vietnamiensis*, *B. ambifaria*, and *B. phytofirmans* are known to synthesize vitamins and phytohormones that help crop growth and development (Stoyanova et al. 2007).

More than ten species of this genus are reported to be associated with sugarcane plants. These were found in all parts of the plant and rhizosphere as well. Most of the reports are from South Africa and Brazil. The association of *B. tropica*, *B. unamae*, and *B. cepacia* has been reported more frequently as compared to the rest of the species. *B. vietnamiensis* is reported only from Indian sugarcane cultivars (Govindrajan et al. 2007). Complete list of the species name and their references are given in Table 7.1. Although the species isolated from sugarcane are nonpathogenic to this host at least five of them namely, *B. cepacia*, *B. gladioli*, *B. graminis*, *B. glumae*, and *B. plantarii* are reported pathogens for other crops (Stoyanova et al. 2007). Of all *Burkholderia* species, *B. cepacia* is of greatest importance. It is an extremely versatile and flexible microorganism, which can be considered like friend or foe of humans. Although *B. cepacia* is known as a plant pathogen, today it is accepted as one of the most important agents for plant protection and plant growth promotion but its use as a biofertilizer/biocontrol agent in fields at "commercial level" still seems to be very difficult.

7.2.5 *Enterobacter, Klebsiella, and Pantoea*

These three genera belong to the Enterobacteriaceae, a large family of bacteria. Members of Enterobacteriaceae are rod-shaped, gram-negative, facultative anaerobes, motile or non-motile and most of them reduce nitrate to nitrite. Isolation of members of Enterobacteriaceae has been reported from several crops including rice, wheat, sorghum, sugarcane, grasses, and dicotyledonous plants (Li and Macrae 1992). Most of these isolates are capable of nitrogen fixation.

Since 1980, there are several reports about isolation of nitrogen-fixing members of Enterobacteriaceae from sugarcane. Rennie et al. (1982) described the members of Enterobacteriacea as the dominating nitrogen-fixing bacteria isolated from sugarcane roots as most of them belonged to the genera *Enterobacter* and *Klebsiella*. The most commonly isolated species of these two genera are *Enterobacter cloacae* and *Klebsiella pneumoniae*. *E. cloaceae* has been reported from Brazil and Pakistan (Graciolli et al. 1983; Mehnaz et al. 2010; Mirza et al. 2001; Rennie 1980; Rennie et al. 1982). Li and Macrae (1992) also reported the isolation of *Enterobacter* but species were not identified. *E. oryzae* and *E. aerogenes* have been reported from Pakistan (Mehnaz et al. 2010). Isolation of *K. pneumoniae* has been reported from Australia, Brazil, and India (Govindrajan et al. 2007; Graciolli et al. 1983; Li and Macrae 1992). *K. oxytoca* has been reported from Pakistan, and bacteria were isolated from root, stem and rhizosphere (Mehnaz et al. 2010; Mirza et al. 2001). Magnani et al. (2010) and Antwerpen et al. (2002) also reported the association of *Klebsiella* spp. with sugarcane. Rosenblueth et al. (2004) isolated a new nitrogen-fixing species of *Klebsiella* from different crops including sugarcane and named it as *K. variicola*.

Pantoea, another nitrogen-fixing member of Enterobacteriacea, has been isolated from all parts of the sugarcane plants. Three species of this genus, *Pantoea ananatis*, *P. herbicola*, and *P. stewartii* have been isolated from roots, stem, and leaves of Brazilian sugarcane plants (Graciolli et al. 1986; Mendes et al. 2007). The unidentified strains of *Pantoea* sp. have been reported by Loiret et al. (2004) and Magnani et al. (2010) from Cuba and Brazil, respectively. Other members of Enterobacteriacea, isolated from sugarcane are *Citrobacter* sp., *Erwinia herbicola*, *E. cypripedii,* and *Serratia* sp. (Antwerpen et al. 2002; Graciolli et al. 1983; Magnani et al. 2010; Rennie et al. 1982; Velázquez et al. 2008). These were isolated from roots and stems of Brazilian and South African sugarcane plants.

7.2.6 *Gluconacetobacter*

This genus belongs to the family Acetobacteraceae. Members of this family are known to produce acetic acid, which are usually acid-tolerant and grow well below pH 5.0. They are gram-negative, aerobic, and rod-shaped bacteria. *Gluconacetobacter* is a nitrogen-fixing and acetic acid-producing bacterium. The first nitrogen-fixing *Gluconacetobacter* was isolated and described in Brazil by Cavalcante and

Dobereiner (1988). Initially a new genus and species was suggested for this organism and it was named as *Saccharobacter nitrocaptans*. When paper was in press, based on the results of DNA/RNA Tm and DNA/DNA binding values, it was named as new species of *Acetobacter*, i.e., *Acetobacter nitrocaptans* (Cavalcante and Dobereiner 1988). Later on, it was changed to *A. diazotrophicus* (Gillis et al. 1989) and then renamed as *G. diazotrophicus* (Yamada et al. 1997). It has been isolated from all parts of sugarcane, including apoplast, in trash of sugarcane and also from a mealy bug associated with sugarcane plants (Pedraza 2008). In addition to Brazil, it has been reported from Mexico, India, Cuba, Egypt, Argentina, Philippines, and Australia (Table 7.1). Isolation of this organism is not easy as it is slow growing and affected by several factors including the presence of high nitrogen fertilizer which decreases its population.

This organism has lack of nitrate reductase and only partial inhibition of nitrogenase activity by ammonium ion, enables it to fix nitrogen in the presence of soil nitrogen. The minimum use of nitrogen fertilizer for sugarcane crop in Brazil is believed to be due to natural occurrence of this organism in their soils. In addition to nitrogen fixation, *G. diazotrophicus* is also a phytohormone producer. There is so much research done on this organism in Brazil and other countries and its effect on sugarcane growth in labs and field has been studied extensively. The whole genome of this organism is sequenced (http://www.biomedcentral.com/1471-2164/10/450). Several scientific papers and reviews have been written about the isolation and significance of this organism (Boddey et al. 2003; James and Olivares 1997; Pedraza 2008). A recent review by Pedraza (2008) provides detailed information about all the work done up till now on this organism.

Recently, another species of *Gluconacetobacter*, i.e., *G. sacchari* has been isolated from leaf sheath of Australian sugarcane crop (Franke et al. 1999). Unfortunately, this bacterium does not fix nitrogen. There are no reports about isolation of any other species of *Gluconacetobacter* from sugarcane.

7.2.7 *Herbaspirillum*

The genus comprises several diazotrophic species, some of which exhibit the potential of endophytic and systemic colonization of a number of plants. Two of them, namely *Herbaspirillum seropedicae* and *H. rubrisubulbicans* are repeatedly isolated and reported from sugarcane. *H. seropedicae* could be detected on root surface and as endophyte in intercellular spaces, as well as within intact root cells (Olivares et al. 1997). *H. rubrisubalbicans* was described as a diazotrophic endophyte with slight pathogenicity in some sugarcane varieties (Baldani et al. 1996; Olivares et al. 1997). The bacteria are gram-negative, curved rods with polar flagella and grow best on dicarboxylic acids, gluconate, glucose, and mannitol, fix N_2 at a pH range of 5.3–8, and very high sucrose concentrations (up to 10%), even though they cannot metabolize this substrate (James and Olivares 1997).

H. seropedicae was originally thought to be a new *Azospirillum* species because of similar growth characteristics in the semi-solid, N-free, malate NFb medium devised for the isolation of *Azospirillum* spp. (Tarrand et al. 1978). However, further analyses showed that it was a completely new genus, i.e., *Herbaspirillum* (Baldani et al. 1986). The similarity of *Herbaspirillum* and *Azospirillum* made further isolation of *Herbaspirillum* difficult, and therefore, Baldani et al. (1992) devised a new semi-solid malate medium (JNFb medium) to more easily distinguish *Herbaspirillum* from *Azospirillum* spp.

Gillis et al. (1990) reported that *H. seropedicae* was very closely related by phenotypical and genotypical characteristics to a mild pathogen of sugarcane and sorghum called "*Pseudomonas*" *rubrisubalbicans*, which also fixes N_2. After further analyses, "*Pseudomonas*" *rubrisubalbicans* was renamed as *Herbaspirillum rubrisubalbicans*. It was proven to be able to incorporate ^{15}N from labeled N_2 gas (Baldani et al. 1992) and is only the second confirmed diazotrophic plant pathogen, the first being *Agrobacterium tumefaciens* (Kanvinde and Sastry 1990). Most of the reports about these two organisms are from Brazil. James and Olivares (1997) published a very comprehensive review, describing the details of these two organisms and *G. diazotrophicus*, with special emphasis on their association with sugarcane.

7.2.8 *Pseudomonas*

The genus belongs to the family Pseudomonadaceae and itself contains large number of species which are distributed into subgroups. *Pseudomonas* is known for different beneficial and pathogenic characteristics. It is a very well known PGPR due to its ability to produce phytohormones, siderophores, antibiotics, phosphate solubilization, and production of antifungal compounds. Some species also fix nitrogen in addition to above-mentioned characteristics. *Pseudomonas fluorescens* and *Pseudomonas putida* are very well known and well-studied species of this genus. These species have been isolated very frequently from different crops and also used in several studies as inoculum to promote plant growth. *Pseudomonas aurantiaca* and *Pseudomonas chlororaphis* are known to be used as biocontrol agents, due to the production of antifungal phenazine compounds.

Pseudomonas spp. have been isolated from stem, root, leaves, and rhizosphere of sugarcane growing in Australia, Brazil, India, Pakistan, and South Africa (Table 7.1). *P. fluorescence* and *P. putida* have been frequently isolated and reported from India (Gangwar and Kaur 2009; Kumar et al. 2002; Viswanathana and Samiyappan 2002). Viswanathan et al. (2003) isolated *P. aeruginosa*, in addition to *P. fluorescence* and *P. putida* from sugarcane stalk. In 2009, these species have also been reported from Pakistani sugarcane cultivars (Mehnaz et al. 2009b). *P. aurantiaca* and *P. reactants* have been recently isolated from stem of sugarcane plants (Mehnaz et al. 2009a, 2010). Magnani et al. (2010) reported the *Pseudomonas* spp. as dominant bacterial community in leaves of Brazilian

sugarcane cultivars. Although there are several reports for the isolation of *Pseudomonas* spp. from sugarcane, reports for identified *Pseudomonas* species are not many.

7.2.9 Other Bacteria

Although the genera described above are those which are very frequently reported and represent dominating bacterial community associated with sugarcane, however there are several other bacteria which have been isolated from sugarcane. These include the diazotrophic and nondiazotrophic organisms. *Bacillus* spp., *Bacillus subtilis*, *B. cereus,* and *B. pumilus* have been isolated from rhizosphere, root, stem, and apoplast of sugarcane (Antwerpen et al. 2002; Gangwar and Kaur 2009; Hassan et al. 2010; Velázquez et al. 2008). *Brevibacillus* sp. was isolated from stem and leaves of Brazilian cultivars (Magnani et al. 2010). *Caulobacter crescentus* and *Delftia acidovorans* from rhizosphere, *Curtobacterium* sp. from stem and leaves and *Derxia gummosa* from root and rhizosphere have been isolated (Graciolli et al. 1983; Mehnaz et al. 2010; Rennie 1980). Cock and de Stauvenel (2006) isolated a lactic acid-producing bacteria, *Lactococcus lactis* subsp. *lactis*, from leaves of sugarcane. Recently, this bacterium has started getting attention due to its probiotic nature. *Paenibacillus azotofixans* and *P. polymyxa* have been isolated from root and stem (Graciolli et al. 1983; Seldin and Penido 1986). Rivas et al. (2008) isolated a bacterium from apoplastic fluid of sugarcane and identified it as *Saccharibacillus sacchari*, a new genus and species of Paenibacillaceae. The closely related genus to the *Saccharibacillus* is *Paenibacillus*. Two nitrogen-fixing bacteria *Rahnella aquatilis*, *Rhizobium* sp., and *R. rhizogenes* have been isolated from roots and apoplast (Mehnaz et al. 2010; Velázquez et al. 2008). Recently, nitrogen-fixing species of *Stenotrophomonas* have been isolated from stem and apoplast (Mehnaz et al. 2010; Ramos et al. 2010). Xing et al. (2006) isolated *Agrobacterium tumefaciens* from sugarcane stalk. Strains of *Acinetobacter*, *Comamonas*, *Mycobacterium*, *Micrococcus*, *Staphylococcus*, *Xanthomonas*, *Zymomonas*, *Ochrobactrum, Kocuria,* and *Pannonibacter* have also been reported from sugarcane (Antwerpen et al. 2002; Hassan et al. 2010; Magnani et al. 2010; Mehnaz et al. 2010; Mendes et al. 2007).

7.3 Role of PGPR in Sugarcane Growth

Beneficial effects savored by the host plant in a PGPR–plant interaction have been speculated to be the result of BNF by the colonizing bacteria, plant growth-promoting substances produced by the rhizobacteria, antifungal, and antibacterial compounds or biocontrol agent. In some cases, a cumulative participation of all of the above mechanisms was observed.

7.3.1 Biological Nitrogen Fixation and Phytohormones

Systematic study by various workers in Brazil over the years led to the observation that some sugarcane varieties grown for decades or even a century do not show any decline in the soil nitrogen reserve or yield despite the supply deficit of nitrogen (Boddey et al. 1995). In some varieties of sugarcane, grown in well-irrigated and fertilized tank (with proper supply of K and P) without nitrogen, yield increase was in the range of 170–230 t ha^{-1} in the first year. In sugarcane varieties CB45-3, SP70-1143 and Krakatau, the trend of yield increase continued for three subsequent years. Researchers were convinced that in these varieties, 60–80% of the nitrogen accumulated was a result of BNF (Boddey et al. 1995). Studies have shown that BNF by the endophytic bacteria has contributed significantly to the nitrogen nutrition of some sugarcane cultivars in Brazil (Boddey et al. 1991) and Australia (Li and MacRae 1991). Nitrogen balance and ^{15}N-aided dilution studies have confirmed nitrogen nutrition benefits by sugarcane; however, there have been cultivar differences in amounts of fixed nitrogen ranging from 4 to over 70% Ndfa (nitrogen derived from atmosphere) of the total nitrogen from the atmosphere (Lima et al. 1987; Urquiaga et al. 1992; Yoneyama et al. 1997; Asis et al. 2002).

In the last decade, numerous studies were undertaken to optimize conditions and reap maximum benefit from various bacteria–non-legume interactions. However, most of the experiments to test the performance of bacteria were conducted under controlled conditions. For sugarcane, these studies include the tissue culture, pot and field experiments but only few PGPR have been used in these experiments. Most of these experiments are conducted with inoculums of *G. diazotrophicus*, *H. ruribulbicans,* and *H. seropedicae*. *G. diazotrophicus* increased 26% plant dry weight of micropropagated sugarcane plants in green house (Muñoz-Rojas and Caballero-Mellado 2003), plant biomass almost 19–50% in a pot trial (Suman et al. 2005, 2007) and 13–16% yield increase in field trials (Govindarajan et al. 2006). Oliveira et al. (2006) used *H. rubrisubalbicans* and *H. seropedicae* in a green house experiment and observed 35% increase in dry matter whereas Govindarajan et al. (2006) used *H. seropedicae* in a field experiment and reported 5–12% increase in yield. *B. vietnamiensis* increased 19.5% yield and *Klebsiellea* sp. GR9 enhanced the plant biomass 13–19.5% in field trials (Govindrajan et al. 2006). *Burkholderia* MG43 inoculation in sugarcane resulted in an effect greater than increasing the fertilizer from half to the full recommended rate, saving the cost of ~140 kg ha^{-1} N fertilizer (Govindarajan et al. 2006). *Enterobacter,* inoculated to roots of micropropagated sugarcane, assimilated 29% of nitrogen by atmospheric fixation (Mirza et al. 2001).

PGPR can be used discretely or as a mixture for inoculating plants in pots or fields. A mixture of bacterial isolates used as an inoculum gave a synergistic result in terms of plant growth and development (Govindarajan et al. 2008). A mixture of *G. diazotrophicus* LMG7603, *A. amazonense,* and *Burkholderia s*p. when applied to sugarcane gave a comparatively lower yield than individual inoculation of *B. vietnamiensis* MG43 and *G. diazotrophicus* LMG7603 (Govindarajan et al. 2006;

Oliveira et al. 2002). Oliveira et al. (2002) inoculated micropropagated sugarcane with five different strains of nitrogen-fixing bacteria (*G. diazotrophicus, H. seropedicae*, *H. rubrisubalbicans*, *A. amazonense* and *Burkholderia* sp.) originally isolated from sugarcane. These strains were used together in various combinations. Plantlets were transferred to pots containing N^{15} for assessment of nitrogen fixation by the N^{15} isotope dilution technique. The bacterial inoculation documented a maximum rise of 39% in total biomass over the uninoculated control and assimilated 30% nitrogen by BNF (Oliveira et al. 2002). These studies emphasize the importance of strain selection in a mixed inoculum for obtaining higher performance in the plant.

Some of the factors that may affect the performance of PGPR are nitrogen content of the soil, soil type, host plant age, and variety. Soil provided with high amount of nitrogen fertilizer (ammonia) reduced the colonization of sugarcane by both *G. diazotrophicus* and *H. seropedicae* (Fuentes-Ramírez et al. 1999; Reis et al. 2000; Muthukumarasamy et al. 1999, 2002). Muñoz-Rojas and Caballero-Mellado (2003) observed a drastic decrease in the *G. diazotrophicus* population with the age of the plant and the genotype. In some sugarcane varieties, apparently, the persistence of the endophyte was for a longer period and in higher numbers. Lima et al. (1987) compared four varieties and found that IAC 52-150 yielded only a small positive N balance that was only one-eighth of that shown by CB 47-89.

Environmental factors like the soil hydric stress and seasonal changes also contribute to the observed variation in diazotrophic bacteria number (Reis et al. 2000). Oliveira et al. (2006) observed the influence of the soil type, inoculation mixture, and nitrogen fertilization level in the yield response and BNF contribution of two sugarcane varieties. Inoculation promoted increases as well as decreases in the productivity of the sugarcane, with regard to the interaction of the soil classes, sugarcane varieties, and nitrogen rates. The inoculants showed better growth-promoting effects in the soils with lower and medium fertility, and without nitrogen fertilizer. More field trials are therefore required to optimize these parameters including time and way of application of the PGPR and environmental factors. Moutia et al. (2010) described the influence of genotype and drought stress on plant growth promotion by *Azospirillum* sp. Two agronomically contrasting sugarcane cultivars R570 and M1176/77 adapted to different agro-climatic zones were inoculated with *Azospirillum* sp. with and without stress. After 103 days of planting, cultivar M1176/77 responded positively with 15% improved growth in shoot height and 75% more root dry mass when subjected to drought stress whereas R570 responded negatively particularly in the absence of drought stress.

Some workers observed that the overall growth promotion and nitrogen assimilation in a plant inoculated with PGPR is not solely due to BNF. Sevilla et al. (2001) suggested the participation of other growth-promoting factors in addition to nitrogen fixation as both wild and *nif*H^- mutants of *G. diazotrophicus* promoted growth of sugarcane in the presence of nitrogen. Similarly, *P. fluorescence*, *P. putida*, indole acetic acid-producing strains, increased the plant biomass of micro-propagated sugarcane, from 2- to 5-folds as compared to un-inoculated plants in *in vitro*, experiments (Mehnaz et al. 2009b; Fig. 7.1). Moutia et al. (2010) reported the 75%

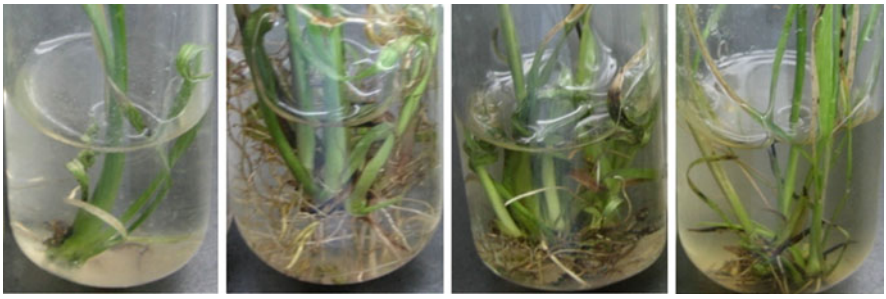

Fig. 7.1 Effect of sugarcane isolates, *Pseudomonas fluorescence* (PB-St1) and *Pseudomonas putida* (OK-St and QR2) on root growth of sugarcane plantlets under gnotobiotic conditions

increase in root dry weight of sugarcane plants due to auxin production by *Azospirillum* sp. rather than nitrogen fixation. Most PGPRs are producers of phytohormones: indoleacetic acid, gibberellins, and cytokinins (Biswas et al. 2000a, b; Verma et al. 2001; Yanni et al. 2001), iron-sequestering siderophores (Verma et al. 2001; Yanni et al. 2001), phosphate-solubilizing enzymes, (Verma et al. 2001) and 1-aminocyclopropane-1-carboxylate (ACC) deaminase (Khalid et al. 2005). Growth hormones produced by the bacteria enhance the development of lateral roots and improve the plant's nutrient uptake from the rhizosphere.

7.3.2 Biocontrol Agent

PGPR induce resistance in plants against fungal, bacterial and viral diseases, insects and nematodes. Induced resistance (IR) is defined as an enhancement of the plant's defensive capacity against a broad spectrum of pathogens and pests that is acquired after appropriate stimulation. PGPR bring about IR through fortifying the physical and mechanical strength of the cell wall as well as changing the physiological and biochemical reaction of the host leading to the synthesis of defense chemicals against the challenge pathogen. PGPR provide different mechanisms for suppressing plant pathogens. They include competition for nutrients and space, antibiosis by producing antibiotics, viz., pyrrolnitrin, pyocyanin, 2,4-diacetyl phloroglucinol and production of siderophores, viz., pseudobactin which limits the availability of iron necessary for the growth of pathogens. Other important mechanisms include production of lytic enzymes such as chitinases and β-1,3-glucanases which degrade chitin and glucan, respectively, present in the cell wall of fungi, present in the cell wall of fungi, HCN production and degradation of toxin produced by pathogen.

Environmental and health concerns about the extended use of pesticides in agriculture necessitate the finding of alternative control approaches for eliminating or controlling pathogens from crops. Several authors have reported on the use of bacteria or fungi as a biocontrol agent. Strains that inhibit the growth of pathogens

and also have nitrogen-fixing properties could be inoculated into sugarcane varieties and thereby enhance the growth of the crop in the field. The well-known diseases of sugarcane are smut (*Ustilago scitaminea*), stem rot (*Fusarium* spp.), red rot (*Colletotrichum falcatum*), and Nematodes (*Melaidogyn* sp.). Red rot disease caused by the fungus *C. falcatum* is one of the major production constraints. It is responsible for the deterioration of sugarcane cultivars and continues to be problem in other countries such as USA, Australia, Taiwan, Thailand, India, and Bangladesh. Plant protection chemicals are currently not recommended for this disease. One approach adopted by the farmer is the use of disease-free seed canes for planting. Such measures are impractical due to the difficulty in diagnosing the dormant fungal infection in seed canes. One key to overcome this situation is the use of biocontrol agent to contain this disease.

Researchers are already working on this aspect and several published reports are available. Antwerpen et al. (2002) checked the antifungal activity of *Burkholderia* isolates from the sugarcane rhizosphere, against *U. scitaminea* (sugarcane smut) and *Fusarium* spp. (stalk rot). Forty-seven strains inhibited the growth of *Ustilago* while 72 strains inhibited the growth of *Fusarium* in vitro. Twenty-one of these bacterial strains inhibited the growth of both *Fusarium* and *Ustilago* (Fig. 7.2a and b). Kumar et al. (2002) isolated *P. fluorescens* strains from sugarcane and reported antifungal activity against *Fusarium oxysporum* and *Rhizoctonia bataticola*. Hassan et al. (2010) reported the antifungal activity of sugarcane isolates, *Ochrobactrum intermedium*, *P. putida*, *B. subtilis*, *Bacillus* sp., and *Stenotrophomonas maltophilia* against local strains of *Colletotrichum falcatum*. Malathi et al. (2002) conducted a study on the possible detoxification of phytotoxin produced by the sugarcane red-rot pathogen *C. falcatum* Went by antagonistic fungal and bacterial strains. Eleven *P. fluorescens* strains and two *Trichoderma harzianum* strains, isolated from sugarcane rhizosphere, were grown on a medium containing the partially purified toxin from the *C. falcatum* pathotype Cf 671. Results of this study confirm the efficacy of some strains of biocontrol agents in detoxifying the pathogen toxin. Viswanathan et al. (2003) isolated and checked the antifungal activity of *P. putida*, *P. fluorescens,* and *P. aeruginosa* isolates against *C. falcatum* and observed that seven isolates of these three species were strong inhibitors. *P. aurantiaca*, isolated from sugarcane stalk, also

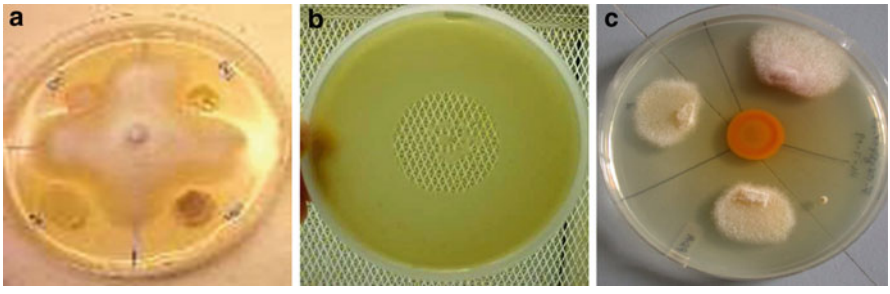

Fig. 7.2 Antifungal activity of sugarcane isolates against fungal pathogens of sugarcane. *Fusarium* sp. (**a**), *Ustilago scitaminea* (sporidia) (**b**) and *C. falcatum* (**c**)

showed antifungal activity against four local isolates of *C. falcatum* (Mehnaz et al. 2009a; Fig. 7.2c).

Ramamoorthy et al. (2001) published a review about the ability of PGPR to induce systemic resistance in plants against diseases and pests. The authors also mentioned that use of an endophytic PGPR strain for inducing systemic resistance is more beneficial for vegetatively-propagated crops like banana, sugarcane, and tapioca. There are several bacterial determinants involved in the induction of systemic resistance by PGPR, the most important being lipopolysaccharides present in the outer membrane of bacterial cells, siderophore, and salicylic acid production (Van Loon et al. 1998). PGPR bring about ISR through fortifying the physical and mechanical strength of the cell wall as well as changing the physiological and biochemical reaction of the host leading to the synthesis of defense chemicals against the challenge pathogen (Viswanathan and Samiyappan 1999a, b). Up till now, most of the reports about induced systemic resistance in sugarcane are from India. Viswanathan and his group worked on PGPR-mediated induced systemic resistance in sugarcane against *C. falcatum* and published several papers.

Viswanathan (1999) and Viswanathan and Samiyappan (1999a) revealed the utility of endophytic *P. fluorescens* strain EP1 isolated from stalk tissues of sugarcane in inducing systemic resistance against red rot (*C. falcatum*). In sugarcane, due to PGPR-mediated ISR against *C. falcatum*, enhanced levels of chitinase and peroxidase were noticed and specific induction of two new chitinase isoforms were found when inoculated with *C. falcatum* (Viswanathan and Samiyappan 1999a, b). Viswanathana and Samiyappan (2002) also reported that application of PGPR, as sett-treatment, induced systemic resistance against *C. falcatum* in addition to enhanced sett germination, tillering, and growth of the cane both under controlled conditions as well as field conditions. The *Pseudomonas*-mediated ISR was significantly higher in the disease susceptible cultivars than in the moderately resistant and moderately susceptible cultivars. Less pathogen-induced invertase enzyme activity was recorded in cane tissues from bacteria-treated stalks, and higher juice characters viz. sucrose percent and sugar yield as compared to the untreated stalk tissues, after pathogen inoculation. These studies clearly show that PGPR-mediated ISR and plant growth promotion can operate under field conditions.

Arencibia et al. (2006) described a new role for *G. diazotrophicus*. According to their report *G. diazotrophicus* induce systemic resistance against *Xanthomonas albilineans*-cause leaf scald disease of sugarcane. *G. diazotrophicus* passes and/or produce elicitor molecules which activate the sugarcane defense response resulting in plant resistance to *X. albilineans*, controlling the pathogen transmission to emerging agamic shoots. The disease was not observed in the presence of *G. diazotrophicus*. Their results point toward a form of induced systemic resistance which protects the plant against *X. albilineans* attack.

Defense mechanisms induced against insect pests in plants are different from that against pathogens. PGPR do not kill insects, but application of PGPR brings about some physiological changes in the host plant that prevents the insects from feeding. In nematode control, PGPR induce resistance by altering root exudates or inducing the host to produce repellents that affect nematode attraction or recognition

of the host (Oostendorp and Sikora 1990) and altering the syncytial development or sex ratio in the root tissue (Wyss 1989). Guyon et al. (2003) isolated *B. cepacia* complex, *B. graminis*, *B. gladioli*, *B. caribensis*, *B. fungorum* and *B. tropicalis* from sugarcane and observed that all isolates show anti-nematode activity against *Meloidogynae* strains of nematodes. Unfortunately, other than *B. tropicalis*, all strains are also human pathogen. Strain of *B. tropicalis* that was able to paralyze nematodes also fix atmospheric nitrogen and was isolated from the rhizosphere of sugarcane. This isolate can serve as an alternative of chemical control for nematodes.

An interesting strategy to use PGPR as a biocontrol was suggested by Omarjee et al. (2008). The authors studied the relationship between *Burkholderia* populations and plant parasitic nematodes in sugarcane and observed that more pathogenic nematode, *Xiphinema elongatum* was associated with *B. graminis*, *B. silvatlantica*, *B. gladioli*, and *B. fungorum* whereas the less pathogenic species, *Helicotylenchus dihystera* and *Pratylenchus zeae* were associated with *B. tropica*. On the basis of their results, the authors suggested that the *B. tropica* might be used to reduce nematode damage in sugarcane by promoting certain nematode species to create a less pathogenic nematode community.

7.4 Conclusion

Large number of PGPR have been isolated from sugarcane. Most of them are nitrogen-fixing bacteria and several species can infect the internal tissues of sugarcane. The endophytic nature of these PGPR makes them suitable for the use in vegetatively propagated crops such as sugarcane because of their capability to colonize and persist in the intercellular space of epidermal cells, also reducing the need for further application if the same vegetative parts are used as propagation material. The beneficial effects of PGPR include direct plant growth promotion through BNF and phytohormone production, biological control, and inducing systemic resistance in host plants. Complex interactions between plant genotype, specific environment for N_2 fixation and highly efficient diazotrophs are necessary to stimulate BNF in sugarcane but these are not clearly defined yet. It is also observed that instead of using single strain, it would be more effective to apply a mixture of strains to get good growth and broad spectrum activity against multiple pathogens and pests. Considering all the studies carried out for sugarcane, might be researchers are close to developing a biofertilizer for this crop but there is still need to carry out more studies, as most of the scientific literature available on sugarcane is dominated by Brazilian researchers and they are more focused on *G. diazotrophicus* and *H. seropedicae*. Brazilian soils are rich in these organisms and they are doing well there but several countries do not have the same climate as Brazil nor same bacterial community. Therefore, scientific community should focus on other PGPR like *Azospirillum*, *Pseudomonas*, *Klebsiella*, etc., and their performance should be evaluated in the field experiments. These organisms have greater potential to be used as biofertilizer due to their abilities of nitrogen fixation,

phytohormone production, and acting as biocontrol agent. These organisms are easy to isolate, well known for their ubiquitous distribution and association with grasses as compared to *G. diazotrophicus*.

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