

Review

Microbes – friends and foes of sugarcane

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Sugarcane is an important cash crop for many countries because it is a major source of several products including sugar and bioethanol. To obtain maximum yields there is a need to apply large quantities of chemical fertilizers. Worldwide yields are also severely affected by more than sixty diseases, mostly caused by fungi but viruses, phytoplasmas, nematodes and other pests can also damage this crop. For most of these diseases, chemical control is not available and breeders are struggling with the development of pest resistant varieties. Many members of the grass family Poaceae establish associations with beneficial microbes which promote their growth by direct and indirect mechanisms. They can be used as means to reduce the need for chemical fertilizer and to minimize the impacts of pathogen invasion. This review highlights the diversity of the microbes associated with sugarcane and the role of beneficial microbes for growth promotion and biocontrol. More extensive use of beneficial microbes will help the sugarcane grower not only to reduce the use of chemical fertilizers but also minimize the disease. In this paper, a brief description of both the non-pathogenic and pathogenic microbes associated with sugarcane is provided. Future prospects for the expanded use of beneficial microbes for sugarcane are also discussed and detailed herein.

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Introduction

Organisms that cannot be seen without a microscope are known as microbes. They are abundant on earth and are ubiquitous, living in soil, air, rocks, water, snow and hot springs. They are intimately associated with plants, animals, and humans. Microbes in the broad sense include bacteria, fungi, viruses, and protozoa. Depending on their interaction with their hosts, they can be either pathogenic or non-pathogenic. Non-pathogenic microbes live in or outside of their host and have a commensal or mutualistic relationship with their hosts whereas pathogenic microbes attack living cells and induce diseases in their hosts.

Sugarcane (genus *Saccharum*) belongs to family *Poaceae* (*Gramineae*) and is a tall, perennial. It is grown in over 110 countries, in tropical and sub-tropical regions. It thrives

in a range of climates from hot and dry to cool and moist. Although grown most for refining of sugar, it also produces numerous valuable by-products such as ethanol, bagasse, press mud, molasses, as well as essential items for industries producing chemicals, plastics, paints, synthetics, fiber, insecticides and detergents (www.pakissan.com). Similar to other grass crops, sugarcane gets its nutrient requirements fulfilled by adding costly chemical fertilizers. It is a general practice to apply 250 kg N ha⁻¹ y⁻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 percent of production in Brazil and India. In India alone, sugarcane is grown on over 4.2 million ha, producing about 250 million tons of canes annually. For cultivation of sugarcane, it is a general practice to apply 250 kg N ha⁻¹ y⁻¹, or most in most countries. In India, the nitrogen applications for sugarcane range between 250–350 kg ha⁻¹. Brazil, on the other hand, which is the largest sugarcane producer in the world, with the crop occupying more than 5 million hectares producing a yield of 495 M tons

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in 2007/2008 [129] and 16 million m³ of alcohol in 2006 [73, 85] uses only 50 kg N ha⁻¹. This contrasting difference between India and Brazil is 1) due to the use of sugarcane varieties that need less chemical fertilizer and 2) the utilization of beneficial microbes which convert the atmospheric nitrogen into ammonium and provide it to the plants thus minimizing their dependence on chemical fertilizers. Researchers in Brazil are intensively working on further reducing the use of N-fertilizer application by one half to 25 kg N ha⁻¹. This would be a saving of 125,000 tons N y⁻¹ saving producers an estimated US\$ 62.5 million y⁻¹. This approach could have a significant impact on reducing the cost of bio-energy worldwide.

Nonetheless, sugarcane is subject to several diseases that significantly lessen its yield. Of the sixty four known diseases, more than fifty are fungal induced whereas the remainder is caused by either bacteria, viruses, or phytoplasma. There are still some diseases for which the causal pathogen remains un-known. Other factors that contribute towards reducing sugarcane yield are parasitic nematodes and pests attacks. In this review, a brief description about the pathogenic and non-pathogenic microbes, isolated from and associated with, sugarcane are provided.

Non-pathogenic microbes

Many bacteria and fungi that associate with sugarcane are beneficial. Bacteria which enhance plant growth and development have been termed “plant growth promoting rhizobacteria” (PGPR) or “plant growth promoting bacteria” (PGPB). They live in free-living soil, the rhizosphere, rhizoplane, or phyllosphere or inside the plant as endophytes. Many bacteria provide the plants with nutrients that include nitrogen through nitrogen fixation and phosphorus by phosphate solubilization. They are also capable of producing phytohormones, siderophores for iron acquisition, and antibiotics that inhibit the growth of pathogenic bacteria and fungi.

The most beneficial fungi associated with the plants roots are known as mycorrhizae. They are naturally occurring soil fungi that are intimately associated with plant roots in a symbiotic relationship. These fungi derive their energy via carbohydrates from plants and in return by virtue of their large surface areas improve the water and phosphorus absorption of plants from the soil and protection against stress, drought, and pathogens. Both groups of microbes have been discussed in the following sections. The details of beneficial bacterial genera and their effects on plants have already been discussed by Mehnaz [69]. Therefore, this review focuses on mycorrhizal fungi and the pathogenic bacteria and

fungi. However, a brief account on beneficial bacteria is also presented and some new information has been updated on them in the text and in Table 1.

Bacteria

Members of alpha, beta and gamma proteobacteria have all been isolated from sugarcane. Species and strains of more than forty different genera of bacteria are reported. A complete list of genera and species that have been isolated to this point is presented in Table 1.

Azospirillum is a Gram negative, facultative endophyte known for its association with cereals and grass roots [45]. It has well-known growth promoting capabilities due to phytohormone production and nitrogen fixation. Recently, Barrassi *et al.* [12] described additional growth promotion features of *Azospirillum* namely, the production of bacteriocin, siderophores and antimicrobial compounds. This genus has more than ten species and three, *A. amazonense*, *A. brasilense* and *A. lipoferum*, have been isolated from the rhizosphere and from all parts of sugarcane growing in Brazil, Egypt, India, Pakistan, South Africa and Spain [24, 31, 38, 42, 49, 70, 97, 106, 128].

Azotobacter is a nitrogen-fixing, Gram negative, obligate aerobic bacterium that fixes at least 10 mg N per gram of carbohydrate [13]. Like *Azospirillum*, it is also known for its association with grass roots. Two of its species, *Azotobacter chroococum* and *A. vinelandii*, have been isolated from sugarcane roots and rhizosphere [42, 49, 108, 128].

Beijerinckia – The nitrogen-fixing species of this genus in association with sugarcane were reported for the first time from Brazil. *B. fluminensis* occurred predominantly in soils where sugarcane was cultivated [28]; 95% of sugarcane soil samples contained *Beijerinckia*. Dobereiner *et al.* [32] showed that nitrogenase activity was higher in sugarcane roots than in the rhizosphere and non-rhizospheric soil and found *Beijerinckia indica* to be one of the most abundant bacterial species in roots and soil samples. Subsequently, it was calculated that this bacterium contributes about 50 kg N ha⁻¹ y⁻¹ to the soil plant⁻¹ system (based on nitrogenase activity; [33]). The most recent report about isolation of *Beijerinckia* sp. was published by Vendruscolo [131].

The genus *Burkholderia* is a Gram-negative, obligate aerobic bacteria, that may be known as opportunistic animal, human and plant pathogens but the vast majority of *Burkholderia* species promote plant growth and bio-remediation. This genus has 30 properly described species, five of which are nitrogen fixers and three species are known to synthesize vitamins and phyto-hormones [122]. More than 10 species are reported

Table 1. List of the bacteria isolated from the sugarcane.

Bacteria	References
<i>Achromobacter</i>	[127]
<i>Acinetobacter baumannii</i>	[130]
<i>Agrobacterium tumifaciens</i>	[140]
<i>Anaeromyxobacter</i> sp.	[94]
<i>Azospirillum</i> sp.	[38, 49, 97, 115]
<i>A. brasilense</i>	[42, 70, 94, 106, 128]
<i>A. lipoferum</i>	[31, 103, 106, 128]
<i>A. amazonense</i>	[24, 106]
<i>Azotobacter chroococum</i>	[128]
<i>A. vinelandii</i>	[42, 49, 108]
<i>Bacillus</i> spp.	[3, 38, 47, 94]
<i>B. cereus</i>	[130]
<i>B. pumilus</i>	[130]
<i>B. subtilis</i>	[47, 130]
<i>Belnapia</i> sp.	[94]
<i>Beijerinckia</i> sp.	[131]
<i>B. fluminensis</i>	[29, 30]
<i>B. indica</i>	[32]
<i>Bradyrhizobium japonicum</i>	[94]
<i>B. elkanii</i>	[94]
<i>Brevibacillus</i> sp.	[66]
<i>Burkholderia</i> spp.	[3, 91]
<i>B. ambifaria</i>	[86]
<i>B. caribensis</i>	[94]
<i>B. cepacia</i>	[65, 73, 86]
<i>B. cenocepacia</i>	[73]
<i>B. fungorum/ graminis</i>	[86]
<i>B. gladioli</i>	[86, 87]
<i>B. hospita</i>	[94]
<i>B. plantarii/glumae/</i>	[87]
<i>B. sacchari</i>	[22]
<i>B. silvatlantica</i>	[86, 90]
<i>B. tropica</i>	[86, 91, 107]
<i>B. unamae</i>	[23, 87, 91]
<i>B. vietnamiensis</i>	[40]
<i>Caulobacter crescentus</i>	[70]
<i>Citrobacter</i> sp.	[66]
<i>Comamonas testosterone</i>	[130]
<i>Cupriavidus</i> sp.	[94]
<i>Curtobacterium</i> sp.	[66]
<i>Delftia acidovorans</i>	[70]
<i>Derxia gummosa</i>	[109, 42]
<i>Enterobacter</i> sp.	[62, 66]
<i>E. aerogenes</i>	[70]
<i>E. cloacae</i>	[42, 70, 74, 108, 109]
<i>E. oryzae</i>	[70]
<i>Erwinia cyripedii</i>	[130]
<i>E. herbicola</i>	[42, 109]
<i>Gluconacetobacter diazotrophicus</i>	[7, 14, 24, 34, 37, 39, 61, 78, 96, 104, 130, 143]
<i>G. sacchari</i>	[35]
<i>Herbaspirillum seropedicae</i>	[7, 8, 83]
<i>H. rubrisubalbicans</i>	[7, 83, 92]
<i>Hyphomicrobium</i> sp.	[94]
<i>Klebsiella</i> spp.	[3, 66]
<i>K. oxytoca</i>	[70, 74]
<i>K. pneumoniae</i>	[40, 42, 62, 113]
<i>K. variicola</i>	[113]
<i>Kocuria kristinae</i>	[130]
<i>Labrys</i> sp.	[94]

(Continued)

Table 1. (Continued)

Bacteria	References
<i>Lactococcus lactis</i> subsp. <i>Lactis</i>	[25]
<i>Massilia</i> sp.	[94]
<i>Methylobacterium</i> sp.	[94]
<i>Methylobacterium</i> sp.	[94]
<i>Microbacterium oleivorans</i>	[130]
<i>M. testaceum</i>	[73]
<i>Micrococcus luteus</i>	[130]
<i>Novosphingobium</i> sp.	[94]
<i>Ochrobacterium intermedium</i>	[47]
<i>Paenibacillus</i> sp.	[94]
<i>P. azotofixans</i>	[24, 117]
<i>P. polymixa</i>	[42, 108, 109]
<i>Pannonibacter phragmitetus</i>	[70]
<i>Pantoea</i> sp.	[64, 66]
<i>P. ananatis</i>	[73]
<i>P. herbicola</i>	[43]
<i>P. stewartii</i>	[73]
<i>Pseudolabrys</i> sp.	[94]
<i>Pseudomonas</i> spp.	[3, 38, 61, 66]
<i>P. aeruginosa</i>	[98, 136]
<i>P. aurantiaca</i>	[71]
<i>P. fluorescens</i>	[135, 72, 73, 98]
<i>P. libaniensis</i>	[98]
<i>P. plecoglossicida</i>	[98]
<i>P. putida</i>	[72, 135]
<i>P. reactans</i>	[70]
<i>Rahnella</i> sp.	[127]
<i>R. aquatilis</i>	[70]
<i>Ralstonia</i> sp.	[94]
<i>Ramlibacter</i> sp.	[94]
<i>Rhizobium</i> sp.	[70, 94]
<i>R. rhizogenes</i>	[130]
<i>R. tropici</i>	[94]
<i>R. multihospitium</i>	[94]
<i>Rhodoplanes</i> sp.	[94]
<i>Saccharibacillus sacchari</i>	[111]
<i>Serratia</i> spp.	[3]
<i>Shinella</i> sp.	[127]
<i>Staphylococcus</i> sp.	[66]
<i>S. epidermidis</i>	[130]
<i>S. saprophyticus</i>	[130]
<i>Stenotrophomonas</i> sp.	[127]
<i>S. maltophilia</i>	[47, 70]
<i>S. pavanii</i>	[100]
<i>Steroidobacter</i> sp.	[94]
<i>Tumebacillus</i> sp.	[94]
<i>Variovorax</i> sp.	[94]
<i>Xanthomonas</i> spp.	[3, 70, 127]
<i>X. campestris</i>	[130]
<i>X. oryzae</i>	[130]
<i>Zymomonas</i> sp.	[3]

from all parts of the sugarcane plant and its rhizosphere. *B. tropica*, *B. unamae*, *B. cepacia* are commonly found in association with sugarcane. Table 1 contains the complete list of the species names. Although *B. cepacia*, *B. gladioli*, *B. graminis*, *B. glumae*, *B. plantarii* are non-pathogenic on sugarcane, they may be pathogenic

on other crops [122]. Recently, Pisa *et al.* [94] isolated *B. hospita* and *B. caribensis* from the rhizosphere of Brazilian sugarcane.

The **Enterobacteriaceae** is a large family of Gram-negative, facultative anaerobic bacteria; most of them are capable of nitrogen fixation. There are several reports about the isolation of nitrogen-fixing members of Enterobacteriaceae from sugarcane. The majority are *Enterobacter* or *Klebsiella*. The most commonly isolated species of these two genera are *E. cloacae* and *K. pneumoniae*. Recently, *E. oryzae* and *E. aerogenes* have been isolated from sugarcane [70]. Isolation of *K. pneumoniae* has been reported from Australia, Brazil and India [40, 42, 62]. *K. oxytoca* was isolated from sugarcane roots, stems and rhizosphere [70, 74]. Rosenblueth *et al.* [113] isolated *K. variicola* from different crops including sugarcane. Three species of *Pantoea*, *P. ananatis*, *P. herbicola*, and *P. stewartii*, have been isolated from roots, stems and leaves of sugarcane plants [43, 73]. Un-identified strains of *Pantoea* spp. have also been isolated [61, 63]. Other members of Enterobacteriaceae isolated from sugarcane are *Citrobacter* spp., *Erwinia herbicola*, *Erwinia cyripedii* and *Serratia* spp. [3, 42, 66, 109, 130].

Gluconacetobacter is a Gram-negative, aerobic, nitrogen-fixing, phytohormone- and acetic acid-producing bacterial genus that grows well below pH 5.0. The first nitrogen-fixing representative of *Gluconacetobacter* was isolated by Johana Dobereiner *et al.* and named as *Gluconacetobacter diazotrophicus* [24]. It has been isolated from all parts of sugarcane, including the apoplast, sugarcane residues, and from a mealy bug associated with sugarcane plants [89]. Its isolation has been reported from Brazil, Mexico, India, Cuba, Egypt, Argentina, the Philippines, and Australia (Table 1). A non-nitrogen fixing member of this genus, *G. sacchari* has been isolated from leaf sheaths of an Australian sugarcane crop [35].

Herbaspirillum is a Gram negative, obligate or facultative endophyte. Two species of this genus, *H. seropediaceae* and *H. rubrisubalbicans*, are repeatedly isolated and reported from sugarcane. *H. rubrisubalbicans* is described as a diazotrophic endophyte with slight pathogenicity [9, 82]. These bacteria can fix N₂ at a pH range of 5.3 to 8, and in the presence of 10% sucrose [48]. *H. seropedicae* has been detected on root surfaces, in intercellular spaces, and within intact root cells [82]. Detailed information about *G. diazotrophicus* and *H. seropediaceae* and their association with sugarcane, is available in a review by James and Olivares [50].

The genus *Pseudomonas* is very well known for phosphate solubilization and the production of phytohormones, siderophores, antibiotics, and anti-fungal

compounds. Some species fix nitrogen. *Pseudomonas* spp. have been isolated from stems, roots, leaves, and rhizospheres of sugarcane (Table 1). Magnani *et al.* [66] reported that *Pseudomonas* spp. are the dominant bacterial community in leaves of Brazilian sugarcane cultivars. Two species, *P. fluorescens* and *P. putida* have been isolated very frequently from Indian sugarcane cultivars [38, 57, 135]. Viswanathan *et al.* [136] isolated *P. aeruginosa*, in addition to *P. fluorescens* and *P. putida* from sugarcane stalks. *P. aurantiaca* and *P. reactans* have been recently isolated from stems of sugarcane plants [70, 71].

Other bacteria: The dominating bacterial community associated with sugarcane mostly contains the above mentioned genera but there are several other diazotrophic and non-diazotrophic bacteria that have been isolated from sugarcane. These genera and species have also been included in Table 1.

Fungi

Beneficial fungal species isolated from sugarcane up until now mostly belong to the division Glomeromycota, which contains the arbuscular mycorrhizal (AM) or vesicular arbuscular mycorrhizae (VAM) fungi. After spore germination, fungal hyphae enter into the plant cells by invaginating the whole cell membrane and then proliferate extending the hyphal network inside the root. Within certain root cells, either balloon-like structures (vesicles, for storage) or branched hyphae (arbuscules, for phosphate transport) develop. The structure of the arbuscules greatly increases the contact surface area between the fungal cell and the host cell cytoplasm to facilitate the transfer of nutrients between them. AM or VAM fungi are obligate plant symbionts and improve the host's ability to absorb phosphorus and take up mineral forms of nitrate, potassium and other nutrients. This symbiotic association also enhances the host resistance to drought, heavy metals and soil salts, and protect the plants from soil pathogens and foliar-feeding insects [60]. The hyphae of these fungi produce an insoluble glue-like substance, 'glomalin', a major component of the organic matter in the soil which contributes to soil aggregate formation, critical for soil structure and stability against erosion [139]. AM associations are present in over 80% of plant species [1]. The Poaceae, the family that includes sugarcane, is considered as one of the best hosts of AM fungi.

The benefit of the mycorrhizal symbiosis to the plant in part depends on the AM species or species community involved in the symbiosis. AM fungal species vary in their ability of nutrient uptake from soil and its transport to the host [119]. According to reports from

Australia, Brazil, India, Iran and Pakistan and according to information available in these reports, six genera and forty-three species of AM fungi have been isolated from sugarcane. A complete list of genera and species isolated from sugarcane has been provided in Table 2. These genera are discussed briefly. Most of the information is taken from the websites developed by Dr. Janusz Blaszkowski. (<http://www.agro.ar.szczecin.pl/~jblaszkowski.html>; <http://www.zor.zut.edu.pl/Glomeromycota>).

Table 2. List of the non-pathogenic fungi isolated from sugarcane.

Fungi	Reference
<i>Acaulospora</i> spp.	[105]
<i>A. colombiana</i>	[113]
<i>A. laevis</i>	[95]
<i>A. rugosa</i>	[52]
<i>A. scrobiculata</i>	[105, 121]
<i>Glomus aggregatum</i>	[80, 112, 121]
<i>G. ambisporum</i>	[52]
<i>G. caledonium</i>	[112]
<i>G. claroidium</i>	[52, 80]
<i>G. clarum</i>	[52, 105, 112]
<i>G. coremioides</i>	[52]
<i>G. convolutum</i>	[80]
<i>G. coronatum</i>	[112]
<i>G. deserticola</i>	[121]
<i>G. diaphanum</i>	[105, 112]
<i>G. eburneum</i>	[112]
<i>G. etunicatum</i>	[105, 52]
<i>G. fasciculatum</i>	[27, 52, 80, 98, 121]
<i>G. geosporum</i>	[52, 121]
<i>G. halonatum</i>	[80]
<i>G. intraradices</i>	[27, 52]
<i>G. lamellosum</i>	[52]
<i>G. liquidambaris</i>	[52]
<i>G. luteum</i>	[52]
<i>G. macrocarpum</i>	[52, 80]
<i>G. microcarpum</i>	[80, 112]
<i>G. monosporum</i>	[80]
<i>G. mosseae</i>	[27, 80, 98, 121]
<i>G. occultum</i>	[105]
<i>G. pachycaulis</i>	[121]
<i>G. pakistanica</i>	[121]
<i>G. rubiformus</i>	[52]
<i>G. sinosum</i>	[52, 121]
<i>G. versiforme</i>	[27, 52, 98]
<i>G. viscosum</i>	[52]
<i>Gigaspora aurigbola</i>	[80]
<i>Gi. erythropha</i>	[80]
<i>Gi. gigantea</i>	[105]
<i>Gi. gregaria</i>	[105]
<i>Gi. heterogama</i>	[80]
<i>Gi. margarita</i>	[80, 98, 105, 121]
<i>Gi. minuta</i>	[80]
<i>Gi. nigra</i>	[80]
<i>Paraglomus occultum</i>	[112]
<i>Pacispora scintillans</i>	[112]
<i>Scutellospora heterogama</i>	[105, 121]

Acaulospora: This genus is member of the family *Acaulosporaceae*, a genus with thirty three species. Recently another species *A. colombiana*, which was isolated from sugarcane plants growing in Iran, was added to this genus [112]. Two species, *A. rugosa* and *A. scrobiculata* and some unidentified species have been isolated from sugarcane cultivars of Brazil, India and Iran [52, 105, 121]. Spores are produced “laterally” from the neck of the sporiferous saccule, a characteristic that differentiates this group from *Entrophospora* where spores are produced within the neck of fully expanded saccule. Resting spores are mostly smooth but sometimes have ornamentation on outer walls. The mycorrhiza produces arbuscules, knobby or irregular vesicles in the roots and straight or coiled intra-radical hyphae. The coils are mostly concentrated at entry points.

Gigaspora: Spores are produced from bulbous sporogenous cells formed at the end of a fertile hypha connected with mycorrhizal roots. The outermost wall of the spore is smooth. *Gigaspora* species also produce clusters of auxiliary cells, which have spines. The mycorrhizae formed by *Gigaspora* species consist of arbuscules only, which form fine branches directly from basal hyphae: vesicles are not produced. Members of this genus are known to harbor intracellular bacteria [16]. Eight *Gigaspora* species have been isolated from sugarcane. Five are reported from Pakistan, two from Brazil and *G. margarita* has been isolated from Pakistani, Brazilian, and Indian sugarcane cultivars [80, 95, 105, 121].

Glomus (Sclerocystis): This is the only member of family *Glomeraceae* and largest genus of the AM fungi; 85 species for this genus has been described. Spores of *Glomus* spp. mostly develop at the end of a sporogenous hypha, and rarely form intercalarily. In most species, the sporogenous hypha develops from extraradical hyphae of mycorrhizal roots. Spores of some species occur inside roots whereas some species additionally produce inner spores from a sporogenous hypha arising from the subtending hypha of the parent spore. Mycorrhizae consist of arbuscules with cylindrical or slightly flared trunks with branches tapering towards tips, and thin-walled, ellipsoid vesicles, although vesicles are not always produced. *Glomus* spp. also produce, intra- and extra-radical hyphae. Intraradical hyphae usually spread along roots and frequently form Y-shaped branches, H-shaped connections, and coils at entry points.

Most of the species are thought to be obligate symbionts. These cannot be cultured in lab without their hosts as they are dependent on them to complete their life cycles. They are found in all terrestrial habitats including grasslands, tropical forests, deserts, and tundras. Twenty-nine species of this genus have

been isolated from sugarcane growing in Australia, Brazil, India, Iran and Pakistan. Ten of these species, *G. aggregatum*, *G. claroideum*, *G. clarum*, *G. etunicatum*, *G. fasciculatum*, *G. geosporum*, *G. macrocarpum*, *G. microcarpum*, *G. mosseae* and *G. sinuosum* are common compared to the rest of the species because they have been reported to have been isolated from more than one country.

The genus *Sclerocystis* was established in 1873, as a member of Glomeraceae [15]. In 2000, Redecker *et al.* [102] did a molecular analysis of this genus and transferred it to *Glomus*. However, some researchers are still using the older name. Srikumar *et al.* [121] reported three species of *Glomus* under the genus name *Sclerocystis*, i.e. *Sclerocystis pachycaulis*, *S. pakistanica*, and *S. sinuosa* from sugarcane.

Pacispora: Genus was introduced in 2004, within the family Glomeraceae [81]. Later, Walker and Schussler [137] introduced a new family Pacisporaceae, which includes this genus. *Pacispora scintillans*, previously known as *Glomus scintillans*, is the type species. Spore formation is similar to *Glomus*. The mycorrhizae consist of arbuscules, vesicles, intra- and extraradical hyphae, and auxillary cells. The arbuscules, vesicles, and hyphae morphologically resemble *Glomus* spp. The auxiliary cells are knobby and occur outside and inside of roots. This genus has seven species and the formation of VA mycorrhiza is confirmed only in two species including *P. scintillans*. Species of this genus have been isolated from Mediterranean, tropical and temperate regions indicating its ubiquitous occurrence. Isolation of *P. scintillans* from sugarcane is reported by Rokni *et al.* [116] from Iran.

Paraglomus is a member of family Paraglomeraceae, order Paraglomerales. Spores of this genus produce at the tip of mycorrhizal extraradical hyphae, like *Glomus* spp. The morphological difference between the two genera is in the properties of the mycorrhizae. The arbuscules of *Paraglomus* spp. are cylindrical or have slightly flared trunks with branches progressively tapering in width toward the tips. Vesicles are absent and intraradical hyphae are frequently coiled within and between cortical cells. This genus has three species and *P. occultum* is the type species. From sugarcane, only one species *P. occultum* has been isolated [112].

Scutellospora: Spores of this genus develop from a bulbous sporogenous cell formed at the end of a fertile hypha connected to the mycorrhizal roots. The mycorrhizae of this genus are like *Gigaspora*, i.e., without vesicles; only arbuscules are produced, which develop from swollen basal hyphae. Intra-radicle hyphae are straight or coiled and auxillary cells are smooth or knobbed. The isolation of one species of this genus, *S. heterogama* has been reported from sugarcane plants in Brazil and India [105, 121].

Pathogenic microbes

Sugarcane suffers from a number of diseases particularly from pathogenic fungi. However, bacteria, viruses, and phytoplasma also contribute to reducing crop yield by causing disease. In addition, nematodes, termites, and insects also damage plants. In total, more than 60 diseases have been reported by American phytopathological society all over the world. A complete list of pathogenic microbes of sugarcane has been provided in Table 3. Detailed Information about sugarcane diseases is taken from Rott *et al.* [114] and names of the diseases have been collected from following websites. http://www.isppweb.org/names_sugarcane_common.asp http://edis.ifas.ufl.edu/topic_sugarcane_diseases

Bacteria

Six bacterial genera, *Acidovorax*, *Herbaspirillum*, *Leifsonia*, *Pectobacterium*, *Pseudomonas*, and *Xanthomonas* are known to be pathogenic to sugarcane. These bacteria cause leaf scald, ratoon stunting, red stripe, mottled stripe, spindle rot and gumming disease. Two of these, ratoon stunting and leaf scald, are considered as major diseases whereas the rest of them as minor.

Leifsonia xyli subsp. *xyli*, is a member of Actinomycetales, an order that contains other plant pathogens including *Clavibacter*, *Curtobacterium*, and *Streptomyces*. It is a Gram positive, aerobic, rod shaped bacterium and responsible for 'ratoon stunting' (growth-hindering) disease of sugarcane. It is considered the most important cause of sugarcane varietal degeneration and also reduces germination and yield. Progressive yield decline takes place and the ratoon crop suffers more than parent crop. Primary spread is through infected setts, but may also spread through harvesting equipments contaminated with juice of infected plants. Characteristic symptoms include orange dots on internal tissue in the nodal regions. Other symptoms include stunted growth, thin stalks with short internodes, pale yellow foliage, and rapid upward tapering of the stem. This disease causes greater economic loss to the sugarcane industry worldwide. The amount of loss depends on susceptibility of the variety in question and disease incidence.

Xanthomonas is a Gram-negative rod-shaped bacterium. It has 27 plant-associated species, and most of them are pathogenic. Individual species have multiple pathogenic variants (pathovars, pv.). Members of the genus are known to cause disease on monocot and dicot species, including fruit and nut trees, cereals and plants of the families Solanaceae and Brassicaceae. Disease symptoms include necrosis, cankers, spots, and blight-affecting leaves, stems, and fruits. 'Leaf scald' is a vascular disease of sugarcane and common in many countries.

Table 3. List of pathogenic microbes and diseases of sugarcane.

Pathogen	Disease
Bacteria	
<i>Acidovorax avenae</i>	Red stripe, top& spindle rot
<i>Herbaspirillum rubrisubalbicans</i>	Mottled stripe
<i>Leifsonia xyli</i> subsp. <i>xyli</i>	Ratoon stunt
<i>Pectobacterium chrysanthemi</i>	Bacterial mottle
<i>Pseudomonas syringae</i> pv. <i>Syringae</i>	Red streak
<i>Xanthomonas</i> sp.	False red stripe
<i>X. albilineans</i>	Leaf scald
<i>X. axonopodis</i> pv. <i>Vasculorum</i>	Gumming
Fungi	
<i>Acremonium furcatum</i>	Wilt complex
<i>Ac. implicatum</i>	Wilt complex
<i>Alternaria alternate</i>	Seedling foliage blight
<i>Bipolaris sacchari</i>	Eye spot; seedling foliage blight
<i>Capnodium</i> sp.	Sooty mould
<i>Ceratocystis adipose</i>	Black rot
<i>Ce. paradoxa</i>	Pineapple sett rot
<i>Cercospora longipes</i>	Brown spot
<i>Claviceps</i> sp.	False floral smut
<i>Cl. purpurea</i>	Ergot
<i>Cl. pusilla</i>	Ergot
<i>Clypeoportha iliau</i>	Leaf sheath binding
<i>Cochliobolus stenospilus</i>	Brown stripe
<i>Co. hawaiiensis</i>	Seedling foliage blight
<i>Co. lunatus</i>	Seedling foliage blight
<i>Corticium rolfsii</i>	Sclerotium sheath rot
<i>Curvularia sengalensis</i>	Seedling foliage blight
<i>Cytospora sacchari</i>	Sheath rot
<i>Deightonella papuana</i>	Veneer blotch
<i>Dimeriella sacchari</i>	Red leaf spot
<i>Didymosphaeria taiwanensis</i>	Leaf blast
<i>Elsinoe sacchari</i>	White rash
<i>Fumago sacchari</i>	Sooty mould
<i>Fusarium moniliforme</i>	Stem rot; Pokkah boeng
<i>F. subglutinans</i>	Pokkah boeng
<i>F. sacchari</i>	Wilt complex
<i>F. oxysporium</i>	Wilt complex
<i>Gloeocercospora sorghi</i>	Zonate leaf spot
<i>Glomerella tucumanensis</i>	Red rot
<i>Helminthosporium</i> sp.	Target blotch
<i>Hendersonia sacchari</i>	Collar rot
<i>Leptosphaeria bicolor</i>	Leaf scorch
<i>L. sacchari</i>	Ring spot
<i>L. taiwanensis</i>	Leaf blight
<i>Ligniera vasculorum</i>	Dry top rot
<i>Marasmius sacchari</i>	Basal stem, root and sheath rot
<i>M. stenospilus</i>	Basal stem, root and sheath rot
<i>Mycosphaerella striatiformans</i>	Leaf splitting
<i>Mycovellosiella koepkei</i>	Yellow spot
<i>My. vaginae</i>	Red spot of leaf sheath
<i>Myriogenospora atramentosa</i>	Leaf binding
<i>Pachymetra chaunorhiza</i>	Root rot
<i>Peronosclerospora miscanthi</i>	Leaf splitting
	Downy mildew

(Continued)

Table 3. (Continued)

Pathogen	Disease
<i>P. northii</i>	Downy mildew
<i>P. philippinensis</i>	Rind disease and sour rot
<i>P. sacchari</i>	
<i>Phaeocystroma sacchari</i>	
Pathogen	
<i>Phyllachora sacchari</i>	Tar spot
<i>Phyllosticta</i> sp.	Leaf spot
<i>Ph. hawaiiensis</i>	Dry rot
<i>Physalospora rhodina</i>	Leaf spot
<i>Phytophthora</i> spp.	Sett rot
<i>Phy. megasperma</i>	Black stripe
<i>Pseudocercospora atrofiliiformis</i>	Sett rot
<i>Puccinia kuehmii</i>	Orange rust
<i>Pu. melanocephala</i>	Brown rust
<i>Pythium</i> spp.	Root rot
<i>Pyt. myriotylum</i>	Root rot
<i>Pyt. arrhenomanes</i>	Root rot
<i>Sclerophthora macrospore</i>	Stunt
<i>Setosphaeria rostrata</i>	Seedling foliage blight
<i>Sphacelotheca erianthi</i>	Floral smut
<i>S. macrospore</i>	Covered smut
<i>Sporisorium cruentum</i>	Floral smut
<i>Sp. scitamineum</i>	Smut
<i>Stagonospora sacchari</i>	Leaf scorch
<i>Thanatephorus cucumeris</i>	Banded sclerotial disease
<i>T. sasakii</i>	Banded sclerotial disease
<i>Xylaria arbuscula</i>	Root and basal stem rot
<i>X. cf. warburgii</i>	Root and basal stem rot
Phytoplasmas	
	Grassy shoot, green grassy shoot, leaf yellows, white leaf
Viruses	
	Yellow leaf, red leaf mottle, leaf fleck, Fiji leaf gall, Fiji disease, mild mosaic, striate mosaic, streak mosaic

Xanthomonas albilineans is the causal organism. The disease is exacerbated by wet seasons, water stress, water logging, and low temperatures. The pathogen is confined mainly to the leaf and vascular bundles which are occluded with a gum-like substance. The initial symptom is a 1–2 mm white streak on leaf that later on, will follow the main vein. Eventually, this symptom disappears and the mature stalks suddenly wilt and die. The bacteria are transmitted by infected cuttings, harvesting equipments, soil, and water [19]. The disease causes yield loss at a large scale and can destroy a whole plantation of susceptible varieties in months. *Xanthomonas axonopodis* pv. *vasculorum* causes ‘gumming disease’. This disease was reported in more than twenty countries during first half of the 20th century although there are not many reports now. It is a vascular disease, and infection occurs through lesions on leaves. External symptoms include chlorosis of new leaves of mature plant and internal

symptoms include red discoloration at nodes, and gum pockets at growing points, nodal, and internodal tissues. In a highly infected plant, gum exudation can be observed if the stalk is cut transversely. Another disease 'false red stripe' is caused by *Xanthomonas* spp. and is characterized by a narrow stripe parallel to the leaf midrib. It was at one time confused with a disease caused by *Acidovorans avenae*. Leaf lesions appear at leaf tips first and then progress towards the base. This disease affects relatively more mature plants.

Acidovorax is a Gram-negative, straight or curved rod with a single polar flagellum. One of the pathogenic species of this genus, *A. avenae*, originally consisted of three sub-species, but recently Schaad *et al.* [116] emended their description and gave them species status. Therefore, *A. avenae* subsp. *avenae*, is now *A. avenae* and causes diseases in economically important crops including corn, rice, watermelon, orchids and sugarcane. In sugarcane, it causes 'spindle rot' which spreads by aerial means but not through stem cuttings. Symptoms appear on leaf spindle as light to dark brown elongated areas. At an advanced stage of disease, dark brown soft rotting tissue becomes prominent. It is not a truly vascular disease, does not spread to leaf sheath, apical meristem or stalk and mostly infects 4–5 month old plants and rarely the mature ones. It is not reported to cause significant economic loss.

Herbaspirillum rubrisubulbicans causes 'mottled stripe' of sugarcane and it is reported from 30 countries. Sugarcane is known as main host for this pathogen. The disease is spread through wind and rain. The disease symptoms are similar to red stripe and hence 'mottled stripe' is often confused with that disease. Fine narrow stripes run on both sides of midrib and are abundant at leaf bases. The color of the stripe varies from white cream to red, with heavily infected leaves showing a reddish appearance.

Pectobacterium chrysanthemi causes bacterial 'mottle' and often occurs in flooded fields. The pathogen enters the plant through the wounds caused by flood water. Disease occurrence is high after a heavy flood season. It affects small number of plants and does not spread rapidly therefore yield loss is not significant.

Pseudomonas syringae pv. *syringae*, causes 'red stripe' disease, and is reported mostly in Iran and Japan. Initial symptoms are yellowish red lines parallel to leaf veins. In severe infections, the lesions coalesce forming large, discolored, necrotic blotches resulting in the death of leaf portions. Application of bacteriocides and the use of healthy cuttings are used to manage the disease.

Fungi

Worldwide, forty- five fungal genera are known to cause diseases on sugarcane plants. A complete list of genera,

species and disease names are given in Table 3. Only fungal pathogens and diseases which are of importance are included as they significantly reduce crop yield.

Ceratocystis paradoxa (Pine apple disease). This genus is the member of the family Ceratobasidiaceae of the phylum Basidiomycota. *Ceratocystis* is an important genus of plant pathogens and causes major diseases of trees. *C. paradoxa* causes pineapple disease of sugarcane, and diseases of pineapple, banana, cacao, coconut and oil palm. Pineapple disease is economically important and occurs in almost all sugarcane-growing countries. It is a disease of setts and induces seed piece decay following planting. The rotting seed pieces smell like ripe pineapple due to production of ethyl acetate produced by fungus. The pathogen enters through the cut ends of the setts, and destroys parenchymatous tissues of internode. It retards bud germination, and shoot development thereby affecting early shoot vigor. Infected setts turn from red to brownish black due to the production of fungal spores. These spores are released into the soil upon seed piece decay and serve as a source of inoculum for the next crop. Sometimes the disease occurs in a standing crop because pathogen enters through the injured stalks caused by prior pest attack. The disease is essentially soilborne, and is transmitted by the fungal spores present mainly in the top 25 cm of the soil. Deep planting, wet or dry soil conditions, low temperatures, short or long hot-water treatments (used to control other diseases) increase susceptibility. The use of resistant cultivars is often the easiest, and most economical method for controlling this plant disease. In general, rapid germination decreases the impact of the disease. Therefore, if possible planting should take place when conditions favour rapid germination. The chemical fungicides, carbendazime and propiconazole, are also used to control this disease.

Fusarium moniliforme (Pokkah boeng). *Fusarium* belongs to the family Hypocreaceae of the phylum Ascomycota. It is a filamentous fungus that is widely distributed in plants and soil worldwide. Rice, bean, soybean, and other crops are hosts for *Fusarium* which is a common plant pathogen and can be an opportunistic human pathogen. Currently it includes over 20 species. *F. moniliforme* and/or *Fusarium subglutinans* are the causal agents of Pokkah boeng disease of sugarcane, which is found in most of the sugarcane growing countries. The fungus is viable for at least 12 months on decaying plant debris in the field. It can infect a wide range of grasses and also causes seedling blight, scorch, stalk rot, root rot, and stunting in different crops. Pokkah boeng which means in the local language "malformed-top", was reported for the first time reported in Java, and is considered as a major leaf disease of sugarcane. It has three phases based

on severity: chlorotic, top rot and knife cut. This is an airborne disease. Spores spread mainly through air currents, but infected seeds, irrigation water, rain, and infested soil can also play a role. Disease incidence is favored by dry climatic conditions followed by a wet season. Three to seven month-old plants appear to be most susceptible. Although the satisfactory control for pokkah boeng is the use of resistant varieties, a highly heritable, but in India spraying of fungicides such as 0.1% Bavistin, or 0.2% Blitox-50 or copper oxychloride or 0.3% Dithane M-45 have also been reported to be effective fungicides for reducing this disease. Two to three sprays at interval of 15 days reduced the multiplication of the pathogen and concomitantly losses in yield and cane quality. (www.vsisugar.com/india/agriculture_divisions/plantpathology).

Glomerella tucumanensis (Red rot). This genus is the member of phylum Ascomycota and family *Glomerellaceae*. The common name of the disease caused by this organism is "red rot". It is the oldest known disease of sugarcane and its presence has been reported from more than 70 countries. It is still a threat in several sub-tropical countries. The diagnostic symptom of the disease is a longitudinal split along the stalk and a red discoloration of internodal tissues interspaced by white patches. The pathogen can infect stem cuttings, stalks, leaf sheath, lamina and midrib but is mainly considered as stalk and seed disease. It infects stalk through the nodal region, leaf scar, growth ring, root primordia and buds. The main factor responsible for the spread of this disease is planting of infected seeds and debris of infected crop left in the field. Disease transmission is by wind, rain, heavy dews and irrigation water. Temperature and humidity play an important role in disease establishment. Rains during July to September, in tropical and sub-tropical regions make the disease most destructive. Symptoms are not very prominent at the early stage but eventually the disease leads to a breakdown of the cane stalk. Damage by this disease can include complete crop loss when it causes death of stalk and complete reduction of sugar content. One species of sugarcane *Saccharum officinarum* has already disappeared as a result of this disease while two others, *S. robustum* and *S. sinensis*, are also susceptible. The disease is well controlled only by planting resistant varieties.

Puccinia melanocephala (Rust). This genus belongs to the phylum Basidiomycota and family *Pucciniaceae*. *P. melanocephala*, the causal pathogen for rust, is an obligate parasite. This disease is found in almost every sugarcane growing country. It is a leaf disease with highest levels of infection occurring at the leaf tip and the lowest at the base. Disease spread takes place by wind.

Leaf wetness, soil moisture and atmospheric temperature are responsible for spore development. The highest infection rate is reported for soils with low pH and high levels of potassium and phosphorus nutrients. Yield loss due to this disease ranges from 20–25%. The best control is the use of resistant varieties but resistance is not stable due to rapid development of races that overcome resistance. Farmers are advised to use diverse varieties to minimize the chances of disease. Although some chemicals are available for sugarcane foliar diseases but they are not very effective for controlling this disease.

Ustilago scitaminea (Smut). *Ustilago* is the member of *Ustilaginaceae* family of phylum Basidiomycota. At least two hundred species of this genus are known to be pathogenic to grasses [56]. *U. scitaminea* causes sugarcane smut. It affects the stem of sugarcane and causes significant losses. The diagnostic symptom of this disease is the appearance of a curved smut 'whip' at the top of the affected plant which emerges from the terminal bud or lateral shoot of infected stalk. The whip is composed of host plant and the fungal tissue and serves as a source of spores. The spores can be spread by wind currents. Cane plants become infected in the buds and many infected buds remain dormant until the cane is cut for seed and planted. The use of infected seed cane thus is also responsible for disease spread. Insects may also play a role in spore dissemination as several insects have been associated with sugarcane and smut whips and spores have been found on their bodies. Irrigation was shown to be a factor in spreading the disease as water is necessary for spore germination. Therefore, special precautions have to be taken as to timing of irrigation to prevent enhancing disease spread. Plants grown under stress conditions are more susceptible. Dry and hot spring weather favors the disease. Losses due to smut have been reported to range from 30–40% in plant crops, up to 70% in ratoons and reductions of 3–7% in sucrose content are common. The use of resistant varieties is the best approach for smut control. Resistant varieties have been readily available and used to control outbreaks of smut in several countries. Using disease-free seed cane is also very important for disease control.

Phytoplasmas and viruses

Phytoplasmas are microbes without a cell wall and are obligate, intracellular parasites. They are pathogenic to important crops and are most prevalent in tropical and sub-tropical regions of the world. Four phytoplasma diseases are known in sugarcane. Two of them, grassy shoot and white leaf diseases are considered the most devastating to this crop. **Grassy shoot** is caused by *Candidatus phytoplasma*. Disease is spread by infected

seeds and phloem feeding insects. The pathogen lives in the phloem tissue. Infected plants do not produce chlorophyll, and thus leaves turn white. As photosynthesis does not take place, the cane gets a grassy appearance. The ratoon crop is more susceptible. Yield losses of 5–20% are common although 100% loss in yield and sugar production have been recorded in some parts of Southeast Asia and India. To prevent the diseases, use of healthy, disease free seeds is recommended.

More than ten viruses and viral diseases are reported for sugarcane. Most common is known as **sugarcane mosaic virus disease (SCMV)**. The disease is commonly referred to as 'mosaic' but it is not a single disease. These are four different diseases each caused by distinct viral pathogens, sugarcane mosaic, sorghum mosaic, maize dwarf mosaic and Johnson grass mosaic. All have been found in sugarcane-growing countries. Yield loss, due to mosaic diseases depends on the stage of the crop growth and sugarcane-growing area. Mosaic is identified by leaf symptoms. The intensity of the symptoms depends on crop variety, growing conditions, and viral strain. The most distinctive symptom is islands of normal green on a background of paler green or yellowish chlorotic areas on the leaf blade. Occasionally infection is accompanied with leaf reddening or necrosis. Chlorotic areas are common at the base of the leaf, sometimes on the leaf sheath but rarely on the stalk. Young, rapidly growing plants are more susceptible than mature, slower growing plants. Aphid and infected seed cane are responsible for spread of this disease. Application of insecticides to target vector aphids is not very helpful in reducing disease spread. Heat treatment of cuttings however, is also partially effective. Use of resistant varieties is the most effective method to control virus diseases and planting mosaic-free seed cane is also helpful.

Other diseases

Sugarcane can also be attacked by insects. Important pests include *Chilo infescatellus* (early shoot borer), *C. saccharifagus indicus* (internode borer), *Scirpophaga excerptalis* (top borer), *Melanaspis glomerata*, *Pyrilla purpusilla*, white fly and termites. Crop attacked by borer cause 20–30% yield loss and juice quality also deteriorates. Loss due to infestation by *M. glomerata* can be from negligible to total crop failure. It affects yield and quality of the cane. *Pyrilla* is most damaging leaf sucking pest of sugarcane and yield losses due to its infestations are about 30%. Termites attack stem of the plant and the affected plant dies. Termites become problematic more in long drought conditions and crops grown on light-textured soils. White fly can cause upto 20% yield loss and 3% loss in sucrose as it retards the cane

growth and reduces sugar content. Nematodes are also responsible for damaging the sugarcane crop. Species of *Meloidogyne* (root knot) and *Pratylenchus* (lesion) can cause 20–40% loss in productivity and also affects the survival of the ratoon crop. Other pathogenic genera include *Belonolimus* (sting), *Trichodorus* and *Paratrichodorus* (stubby root), *Criconemoides* (ring), *Tylenchorhynchus* and *Quinisulcius* (stunt) and *Hoplolimus* (lance). As most of these nematode species like sandy soils therefore sugarcane growing in sandy soil suffers more than crops grown in other types of soil. Nematocides are available but they are toxic to humans as well being environmentally damaging. Their impact is not very durable in most fields. There are some other sugarcane diseases, apex rot, chlorotic streak, dwarf, leaf gall and ramu streak, for which causal organisms remain unknown.

How microbes promote the sugarcane growth?

Bacteria

Few decades ago, biological nitrogen fixation (BNF) was considered as the only mechanism used by the bacteria to promote the plant growth. Later on when it was reported that more than 80% soil bacteria produce phytohormones, then involvement of this mechanism in plant growth promotion was considered as important as BNF. Currently, researchers are more focused on indirect mechanisms of growth promotion which is dominated by the use of bacteria as biological control. PGPR community associated with sugarcane has been discussed earlier. Mechanisms used by these bacteria in growth promotion of sugarcane have been briefly discussed in this section.

In sugarcane growing countries, researchers conducted the experiments under controlled and natural conditions by using PGPR to get the maximum benefit. Boddey *et al.* [20, 21] reported 170 to 230 t ha⁻¹ yield increase for sugarcane varieties CB45-3, SP70-1143 and Krakatau, due to BNF as crop was grown without nitrogen (with potassium and phosphorus). Asis *et al.* [6] observed 14–37% nitrogen derived from atmosphere (%Ndfa) for sugarcane cultivar Nif-8 due to natural presence of nitrogen fixing endophytes. Later on, Momose *et al.* [75] reported the increase in root and shoot dry weight of sugarcane cultivar Nif-8 due to BNF, supported by the endophytic presence of *G. diazotrophicus* and %Ndfa. Taule *et al.* [127] reported 35–59% Ndfa due to BNF, for three sugarcane cultivars CP, TUC1, and LCP, growing in Uruguay. Mirza *et al.* [74] observed assimilation of 29% nitrogen by atmospheric fixation when plants were inoculated "in vitro" with *Enterobacter* sp.

Oliveira *et al.* [84] observed 39% increase in biomass and 30% nitrogen contributed through BNF when micro-propagated sugarcane plants were inoculated with five different strains of nitrogen fixing bacteria (*G. diazotrophicus*, *H. seropedicae*, *H. rubrisubalbicans*, *A. amazonense* and *Burkholderia sp.*). *B. vietnamiensis* and *Klebsiella sp.* increased 19% yield and 13–20% plant biomass in field trials, respectively. However, *Burkholderia sp.* inoculation in sugarcane field saved the cost of $\sim 140 \text{ kg ha}^{-1}$ N fertilizer [41]. Three groups reported 26% increase in plant dry weight of micropropagated sugarcane plants in green house, 19 to 50% increase in plant biomass in pot experiment and 13–16% yield increase in field experiment when *G. diazotrophicus* was used as inoculum [41, 77, 123, 124]. In a green house experiment, 35% increase in dry weight was observed when plants were inoculated with *H. rubrisubalbicans* and *H. seropedaceae* and with *H. seropedaceae* inoculum 5–12% yield increase was recorded in a field experiment [41, 85].

Growth hormones produced by the bacteria enhance the development of lateral roots and improve the plant's nutrient uptake from the rhizosphere. PGPRs are known for the production of phytohormones: indolacetic acid, gibberellins and cytokinins, iron-sequestering siderophores, phosphate-solubilising enzymes and 1-aminocyclopropane-1-carboxylate (ACC) deaminase [17, 18, 55, 132, 142]. Indole acetic acid producing and non-nitrogen fixing isolates of *P. fluorescence* and *P. putida*, increased the plant biomass of micro-propagated sugarcane, from 2–5 folds [72]. Moutia *et al.* [76] reported 75% increase in root dry weight of sugarcane plants due to auxin production by *Azospirillum sp.* Sevilla *et al.* [118] suggested the involvement of "other factors" when wild and *nifH*⁻ mutants of *G. diazotrophicus* promoted the sugarcane growth in the presence of nitrogen.

Increase in cane yield by application of phosphate solubilizing bacteria and farmyard manure is reported by Kumaraswamy *et al.* [58] and Kathiresan *et al.* [53]. Sundara *et al.* [125] conducted the field experiments to study the influence of *Bacillus megatherium* var. *phosphaticum* on sugarcane growth, when applied with and without phosphorus fertilizer. Inoculated plants showed increase in tillering, stalk weight, cane yield (12.6%) and improved juice quality and sugar yield. In combination with phosphorus fertilizer, these bacteria reduced the required phosphorus dosage by 25%. Yadav and Singh [141] observed increase in germination, tillering, cane yield and phosphorus uptake due to inoculation of *B. megatherium* with different doses of fertilizers.

As the awareness about environmental pollution is improving, pressure on the researchers to find a safe alternative for chemical pesticides and fungicides is

increasing. Therefore, use of microbes as a biocontrol agent is becoming a main focus of several researchers. Bacteria suppress the plant pathogens by different mechanisms including competition for nutrients and space, production of antibiotics, siderophores, lytic enzymes, HCN and degradation of toxins produced by pathogen.

There are several reports about anti-pathogenic activity of sugarcane isolates suggesting their use as a biocontrol agent. Pinon *et al.* [93] and Blanco *et al.* [19] reported the antagonistic behavior of *G. diazotrophicus* against *Xanthomonas albilineans* due to the production of a lysozyme-like bacteriocin. Malathi *et al.* [68] confirmed the detoxification of phytotoxin produced by the sugarcane red-rot pathogen *C. falcatum* Went by antagonistic *P. fluorescence*. Antwerpen *et al.* [3] observed the antifungal activity of *Burkholderia sp.* against *U. scitaminea* (sugarcane smut) and *Fusarium spp.* (stalk rot). Kumar *et al.* [57] reported antifungal activity of *P. fluorescence* against *F. oxysporium* and *Rhizoctonia bataticola*. Hassan *et al.* [47] reported the antifungal activity of *Ochrobacterium intermedium*, *Pseudomonas putida*, *Bacillus subtilis*, *Bacillus sp.* and *Stenotrophomonas maltophilia* against local strains of *Colletotrichum falcatum*, by using plate assay. Later on, Hassan *et al.* [48] used some of these isolates to study their antifungal effect "in vivo" and observed 44–60% reduction in disease severity by *Pseudomonas putida* NH50 in different field trials. *P. putida*, *P. fluorescence*, *P. aeruginosa* and *P. aurantiaca* also showed antifungal activity against *C. falcatum* [71, 136]. Muthukumarasamy *et al.* [79] demonstrated the antifungal activity of *G. diazotrophicus* against *Colletotrichum falcatum*. Recently, Logeshwaran *et al.* [63] reported the antifungal activity of *G. diazotrophicus* against *C. falcatum*, *Fusarium oxysporium*, *F. solani* and *Ceratocystis fimbriata* due to production of an antifungal compound, pyoluteorin.

It is known that bacteria induce resistance in plants against diseases, insects and nematodes. Induced resistance (IR) is the enhancement of the plant's defensive capacity against pathogens and pests that is acquired after appropriate stimulation. Resistance is induced by stimulating the physical and mechanical strength of cell wall, change in physiological and biochemical reaction of the host and synthesis of defense chemicals against pathogen. Arencibia *et al.* [5] observed the antibacterial activity of *G. diazotrophicus* against *X. albilineans*. *G. diazotrophicus* produces elicitor molecules which activate plant's defense response, controlling transmission of pathogen to emerging shoots. Viswanathan and Samiyappan [133, 134] described the role of *P. fluorescence* in induced resistance of sugarcane against *C. falcatum*. They observed enhanced levels of

chitinase and peroxidase and induction of two new isoforms of chitinase as a result of PGPR-mediated IR. Anti-nematode activity of PGPR had also been observed. Guyon *et al.* [46] reported the anti-nematode activity against *Meloidogyne* strains by *B. cepacia* complex, *B. graminis*, *B. gladioli*, *B. caribensis*, *B. fungorum* and *B. tropicalis*. Omarjee *et al.* [86] studied the interaction among *Burkholderia* and plant parasitic nematodes in sugarcane and suggested that the *Burkholderia tropica* can be used to reduce nematode damage by promoting certain nematode species to create a less pathogenic nematode community as their results showed that more pathogenic nematode, *Xiphinema elongatum* was associated with *B. graminis*, *B. silvatlantica*, *B. gladioli*, and *B. fungorum* whereas the less pathogenic species, *Helicotylenchus dihystrera* and *Pratylenchus zaeae* were associated with *B. tropica*.

Fungi

Mycorrhizas are known to provide plants with nutrients including phosphorus, molybdenum, copper and iron. They produce auxins and cytokinins and provide the plants resistance against drought, salinity, heat, heavy metal and root diseases [4].

Magarey *et al.* [67] studied the role of *Glomus clarum*, in sugarcane yield, when phosphorous fertilizer was applied at different level. Maximum yield was achieved with lower levels of P in the soil suggesting that fungus supported P uptake in low P soils but colonization of sugarcane roots by AM was decreased at high soil P levels. Prabudoss [95] observed significantly enhanced plant height, cane girth, root colonization of sugarcane and increase in spore number of *Glomus fasciculatum* when applied with recommended dose of farmyard manure.

Root colonization by arbuscular mycorrhizal fungi has been frequently reported to reduce root infection by various root borne pathogens. The mechanism involved in this biocontrol are not clear, but localized and systemic induced resistance [26] as well as increase in plant phosphorous status in response to mycorrhiza formation [44] appear to be involved. Rani *et al.* [101] observed the effect of different timings of application of mycorrhizae along different levels of phosphorous on yield and quality of sugarcane. Highest germination percentage, maximum sugar yield, cane yield, and higher nutrient uptake was observed when 75% of recommended dose of phosphorous (100 kg P₂O₅ ha⁻¹) with 12.5 kg ha⁻¹ mycorrhizae was applied at the time of planting. Nasim *et al.* [80] studied the correlation between red rot disease and colonization of sugarcane roots with AM fungi and observed that diseased plants have more than 50% reduction in colonization by AM fungi as compared to healthy sugarcane plants. Authors concluded

that presence of mycorrhiza in roots led to significantly lower infection levels of *C. falcatum* than observed in non-mycorrhizal plants and suggested that screening of AM flora should be done to select the best and most efficient AM endophyte suited for sugarcane crop as well as different aspects of interaction of AM fungi and pathogen should be known. Rao and Srinavas [100] observed higher colonization of AM in red rot resistant varieties of sugarcane as compared to susceptible varieties under inoculated (fungal pathogen) and uninoculated conditions.

There are several reports on increased disease tolerance of plants to soil-borne pathogens when pre-inoculated with mycorrhizal fungi. Ozgonen *et al.* [88] reported the reduction in disease incidence of pepper plant caused by *Phytophthora capsici* when inoculated with *Glomus mosseae*, *G. etunicatum*, *G. fasciculatus* and *Gigaspora margarita*. Authors noticed increase in the activity of phenolic compounds, enzymes and pathogenesis-related proteins and suggested that it could be involved in disease resistance. Kapoor [51] reported the effectiveness of mycorrhizal fungi, *Glomus macrocarpum* and *Glomus fasciculatum*, to control vascular wilt disease of tomato, caused by *Fusarium oxysporium*. This effectiveness can be due to increase in jasmonic acid concentration, resulted in higher trichome density, higher protein concentration, phenols accumulation and induction of phenylalanine ammonia lyase (PAL) activity.

Use of *Trichoderma spp.* has been recently reported to control sugarcane diseases and promote the plant growth. Singh *et al.* [120] tested the antagonistic activity of *T. harzianum* and *T. viride* strains against red rot pathogen (*C. falcatum* Went) on highly red rot susceptible sugarcane variety CoLk 7701 in field. *T. harzianum* strains were found to be more efficient than *T. viride* in dual culture and field as well. Red rot was controlled in 47–48% cane by *T. harzianum* and 24–28% by *T. viride*. This biocontrol effect was may be due to enzymatic action of metabolites released by bioagent. In addition to biocontrol, germination, number of tillers, millable canes and yield were also improved. Malathi *et al.* [68] confirmed the detoxification of phytotoxin produced by the sugarcane red-rot pathogen *C. falcatum* Went by antagonistic *T. harzianum* strains. Lal *et al.* [59] reported the inhibition of mycelia growth and teliospore germination of *Sporisorium scitamineum* when sugarcane setts were treated with culture filtrate of *T. viride* and leaf extract of *Solanum nigrum* and *Calendula officinalis* before transplantation. Sett treatments with culture filtrate of *T. viride* improved germination, millable canes (27%) and cane yield (38%) in plant crop and sprouting of clumps, millable canes (51%) and yield (49%) in ratoon crop.

Talukdar *et al.* [126] found the *T. harzianum* as effective to control pine apple disease of sugarcane caused by *Ceratocystis paradoxa* as fungicide (Bavistin 50WP – Carbendazim 50WP). *T. harzianum* treated setts also showed 20% increase in germination and 41% in cane yield over control.

Mycorrhiza-associated bacteria together with the fungal symbiont protect the plants against root pathogens. Recently, a review written by Frey-Klett *et al.* [36] discussed the role of bacteria in the establishment of mycorrhizal fungus. How they help each other and how they help the plant to get the nutrients and fight against pathogens was also discussed. Ardakani *et al.* [4] used *Glomus intraradices* in combination with *Azospirillum* and reported increase in wheat crop yield and improvement in iron, manganese, zinc and copper content of plant. Reis *et al.* [105] reported the natural occurrence of *Glomus*, *Gigaspora*, *Acaulospora* and *Scutellospora* spp., in fourteen varieties of sugarcane in association with *Gluconacetobacter diazotrophicus*, in almost all samples. Barea and Azcon-Aguilar [10] reported the production of auxin, gibberellic acid and four compounds with the cytokinin activity by *Glomus mosseae*. Barkur and Tagu [11] discussed the role of auxins and cytokinins in mycorrhizal symbiosis, in detail. Anjos *et al.* [2] reported the anti-nematodal activity of AM fungus *Scutellospora heterogama* against root knot nematode *Meloidogyne incognita*. Use of AM fungi can be an alternative of nematocides and nematode management.

Conclusion

Sugarcane is a host for a big diverse PGPR community which are associated with all plant parts, inside and outside, as well as rhizosphere. Similarly mycorrhizal fungal strains have also been isolated from sugarcane although reports are limited. Both groups of microbes are known for their beneficial effects on plants through different mechanisms including BNF, phytohormone production, biological control and induction of systemic resistance in host plants, compensating nutrient deficiency thus supporting the survival under harsh conditions. If we look at the experimental studies in which bacterial inoculums were used for plant growth promotion, these are more focused on *G. diazotrophicus* and *H. seropedaceae* as most of the reports are from Brazil and their soils are rich in these organisms. If we consider the literature reporting about use of bacteria as a biocontrol agent, most of the reports are from India and resistance induced by *P. fluorescence* or *P. putida* had been studied. Several PGPRs have already been isolated

from sugarcane and list is growing rapidly. Researchers should now start evaluating the potential of these organisms in the field. Preference should be given to those bacteria which can be used both as a biofertilizer and biocontrol agents. Mixture of nitrogen fixers e.g., *Azospirillum*, *Azotobacter*, *Klebsiella*, etc., and known biocontrol agent *Pseudomonas* spp. which are also phytohormone producer and phosphate solubilizer should be used as inoculum.

Similarly, AM fungi although known for several years for their beneficial effects on plants have not been studied in detail. Therefore, the information about AM fungal community associated with sugarcane and its contribution in later's growth promotion is very little. Researchers should get more know how about sugarcane associated AM fungal strains and their effect on sugarcane crop should also be evaluated. Although, there are some reports indicating increased disease tolerance in some economically important crops due to mycorrhizal inoculation, such an association has, so far, not been studied in sugarcane. Application of more than one bacterial or fungal strain, as well as their mixtures should also be tested in order to assess growth promotion in plants and broad spectrum activity of the tested strains against multiple pathogens and pests of sugarcane. It is also very important to get better understanding of the above mentioned mechanisms at molecular level, specifically in relation to growth promotion and bio-control. Researchers should also work on these lines to improve/increase the impact of these factors on sugarcane.

Conflict of Interest

There is no conflict of interest.

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