

## Molecular phylogeny of *Bipolaris* and *Curvularia* species associated with browntop millet

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Browntop millet (*Urochloa ramosa*, Poaceae) is a warm season grass commonly used as a cover crop in pasture management systems. This species is inexpensive compared to other forage crops and its ability to easily reseed and to remain viable in the soil for years makes browntop millet an excellent regenerating food plot for wildlife. The genera *Bipolaris* and *Curvularia* (Pleosporaceae) are closely related and comprise many pathogenic species associated with poaceous hosts. Of these, only *B. setariae* and *C. hawaiiensis* have previously been reported from *U. ramosa*. The taxa belonging in these sister genera are often difficult to identify based on morphology alone, especially when co-occurring on a single host. In May 2015, infected seeds of *U. ramosa* grown in Tifton, Georgia, USA were observed and collected. Conidia and conidiophores were observed using compound and dissecting light microscopy. Single spore isolation techniques were used to obtain pure cultures, which were also observed scanning electron microscopy. Genomic DNA was extracted from isolates of *Bipolaris* and *Curvularia* using the DNeasy Plant Mini Kit (Qiagen). The ITS, GPDH, TEF and RPB gene regions were amplified and the resulting amplicons were sequenced using the BigDye Terminator v. 3.1 on an Applied Biosystems 3130xl Genetic Analyzer. Phylogenetic analyses were carried out using maximum parsimony and maximum likelihood methods. Five *Curvularia* and nine *Bipolaris* cultures were isolated, and a total of 42 new DNA sequences were generated in this study. GPDH gene region resolved most species in both genera. Maximum parsimony analysis of combined ITS, GPDH and TEF loci for *Bipolaris* included 58 isolates and 81 isolates for *Curvularia*. Based on morphological and phylogenetic data, the isolates found on the seeds of *U. ramosa* are identified as *Bipolaris yamadai*, *Curvularia geniculata* and a previously undescribed species of *Curvularia*. To our knowledge, these are the first records of these fungal species in association with *U. ramosa*. Surface ornamentation and texture of *Curvularia* conidia were clearly observed under SEM than under light microscope. Although other conidial cells are smooth, basal cell of the conidium can be either smooth or verrucose and this was used as a morphological character to delimit some members of the "geniculata" group. *Curvularia geniculata*, *C. urochloae* and *B. yamadai* had conidia with smooth basal cell under SEM. Therefore SEM imaging will be useful to observe micromorphological characters that are difficult to distinguish using conventional light microscopy. The discovery of these potentially pathogenic fungi on an economically important host provides new insights for disease surveillance to

plant pathologists, quarantine officials and others with interests in plant disease management.

**Keywords:** Hyphomycetes, Molecular systematics, Pathogens

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**Biological Sciences**

**Screening of beach sand for bacterial treasures  
- A preliminary approach**

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Marine microorganisms are of great interest as novel and rich sources of biologically active products. The micro-organisms living in both sea water and sand must be able to survive and grow under harsh conditions. Due to these adaptations, the metabolites produced by marine micro-organisms possess unique features and they serve as promising choices for many different biotechnological applications. The aforesaid micro-organisms are referred to as ‘microbial treasures’ at present.

In this study, preliminary screening for different culturable bacteria present in shoreline sand was carried out and tested for different biological activities. Morphologically different bacterial isolates were identified using the standard morphological and biochemical characterization procedures in microbiology. The results indicated that the majority of the isolates belong to the genus *Bacillus* and the others were the species of *Micrococcus*, *Aerococcus*, *Pseudomonas*, and *Staphylococcus*. The degradation of two dyes, crystal violet and saffranin were tested and the degradation was only observed for crystal violet with two *Bacillus* isolates i.e. SSMA-15 and SSPC-16. Some isolated bacteria accumulated the dyes in their cells. The highest indole acetic acid (IAA) production was observed for the *Bacillus*-SSPC-11. Antagonistic effect against all three selected bacteria, i.e. *S. aureus*, *B. subtilis* and *E. coli* was only shown by the *Pseudomonas*-SSMA-7. Along with the antagonistic effects, it also produced the protease and cellulase enzymes. The bacterial isolate-SSMA-9 belonging to the family *Enterobacteriaceae* exhibited the highest number of positive activities during this study. It included the production of cellulase, laccase, amylase and protease enzymes and the accumulation of the saffranin and crystal violet dyes. The *Bacillus*-SSPC-3 showed positive production of three enzymes; amylase, protease and cellulase. *Staphylococcus*-SSPC-12 has the ability to accumulate the two dyes in addition to the production of the two enzymes amylase and protease. Based on the positive activities and produced components, bacterial isolates SSMA-9, SSMA-7 and SSPC-12 were identified as possible bacterial treasures.

**Keywords:** Bacterial treasures, Cellulase, Indole acetic acid, Sea sand