Genome Inspired Discovery of an Ancient Co-Evolution Between an African Host Crop, A Beneficial Bacterial Endophyte and A Pathogenic Fungus

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Finger millet is an ancient African cereal crop, domesticated 7000 years ago in Ethiopia, reaching India at 3000 BC. Unlike other cereals, finger millet is resistant to the toxigenic fungal pathogen *Fusarium graminearum*. As this fungus is also ancient to Africa, we hypothesized that the crop may host beneficial endophytes (plant-colonizing microbes) that co-evolved to combat *Fusarium*. Here we describe the first ever report of endophytes from finger millet. We describe a novel *Enterobacter* species (strain M6). *In vitro* experiments demonstrate that strain M6 can inhibit the growth of *Fusarium*. When M6 was allowed to colonize the genetically related cereal crops, corn and wheat, which are susceptible to *Fusarium*, they acquired resistance to this pathogenic fungus, as demonstrated by replicated greenhouse trials. Confocal microscopy using GFP-tagged M6 showed that M6 colonizes the internal tissues of corn, wheat and millet and thus confirms that M6 behaves as an endophyte. To help understand the anti-*Fusarium* mechanism of action, M6 was co-cultured with *Fusarium*; microscopy using vitality stains demonstrated that M6 causes cleavage of fungal hypha at septa *in vitro*. To discover the anti-*Fusarium* genes, Tn5 mutagenesis followed by whole genome sequencing were conducted. Screening of 4800 Tn5 insertion events led to the discovery of 10 candidate genes/operons from M6. Expression of the candidate genes was studied by real time PCR, which showed that most of the genes are inducible by *Fusarium*. The importance of the Tn5 knockouts was confirmed in replicated greenhouse trials. The candidate anti-*Fusarium* genes from M6 include operons that encode phenazine (a potent anti-fungal metabolite), butanediol (an elicitor of host plant defences), and a fusaric acid resistance protein (FARP). Fusaric acid is a metabolite produced by *Fusarium* pathogen to inhibit the biosynthesis of bacterial phenazine; FARP biosynthesized by M6 bacteria is an apparent efflux transporter for fungal fusaric acid. Since both *Fusarium* and finger millet appear to be ancient to Africa, the phenazine-butanediol-fusaric acid-FARP interaction network may represent a fascinating example of three-way co-evolution between an endophyte, a pathogen and a host. It is hoped that modern agriculture will benefit from this ancient selection pressure in Africa.

Biography

Walaa Mousa completed her BSc in Pharmaceutical Sciences from Mansoura University Egypt in 2005. Walaa completed her MSc in Biochemistry in 2010. In 2011, Walaa moved to Canada to study her PhD in Raizada lab at University of Guelph. Her current research involves: the development of new bacterial biocontrol agents to help control fungal pathogens of plants and elucidate the underlying mechanisms of antifungal activity.

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