

4th International Conference on Clinical Microbiology and Microbial Genomics

October 05-07, 2015 Philadelphia, USA

Biochemical, molecular and ecological characterization of bacterial endophytes within the ancient crop, finger millet that have antifungal activity

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inger 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 strains 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.

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Cross-reactivity of synthetic corticosteroids on cortisol serum levels: A comparison between three different immunoassays

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Immunoassays are widely used in clinical laboratories as they have high specificity and are easy in use. A downside of these assays is cross-reactivity with structurally similar molecules to the analyte, which could lead to false clinical interpretation. Due to structural similarity between methylprednisolone and prednisolone and the analyte, falsely elevated cortisol levels have been reported with cortisol immunoassays. This study made a comparison between a manual radio immunoassay (RIA) and two automated cortisol assays on Cobas[®] 8000 (Cortisol I) and Modular[®] E170 (Cortisol II). Patient's serum samples were pooled to obtain two concentration levels: a lower cortisol concentration of 5µg/dl and a higher concentration in normal range of 15µg/dl. Serum samples were spiked with different concentrations of prednisolone and methylprednisolone (methylprednisolone), as found in literature. Serum was also spiked with a single concentration of dexamethasone, corresponding to a supra-therapeutic dosage of 800 mg per OS, which was expected to have no cross-reactivity with either assay. In one volunteer, serum cortisol levels were measured at different time intervals after the intake of 32 mg Medrol[®], the next day after a late-night intake of 2 mg dexamethasone. Differences in elevated cortisol levels by each assay could be indicative for the interference of in vivo metabolites of methylprednisolone.

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