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Construction of mutant strains with inducible mating type genes to identify the A mating pathway in *Flammulina velutipes*

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Mating is the initial step of sexual development in higher fungi and involves the fusion of two compatible mycelia, which brings together complementary sets of mating type genes; HD and PR mating type genes. This pairing of complementary HD and PR genes results in activation of the respective A (HD) and B (PR) mating type pathways. Although HD and PR mating type genes have been identified in many fungi, their downstream pathways have remained largely unknown. The present study aims to develop a tetracycline inducible gene based system for basidiomycetous fungi, followed by the construction of mutants with inducible HD and PR mating type genes to study these pathways in detail. We successfully adapted the doxycycline (a tetracycline derivative) inducible promoter system (TET-On) using two expression units in a single vector; the reverse tetracycline transactivator (rtTA2S-M2) gene that encodes the doxycycline binding TetR protein, and the tetO7 (TetR binding) sequence fused with a minial *gpd* promoter to control expression of the gene of interest. The functionality of this system was confirmed in *F. velutipes* with two different reporter genes, one for a Green Fluorescent Protein (gfp) and one for a Red (dTomato). Next, we selected complementary HD and PR mating type genes for *F. velutipes* strain L11, that were subsequently cloned in the doxycycline inducible system. The first doxycycline inducible HD mating type mutants have recently been obtained and were confirmed by qPCR. These mutants are currently being analyzed, while transformation experiments for inducible PR and HD+PR *F. velutipes* mutants are underway.

Biography

Irum Mukhtar has expertise in fungal taxonomy, genetics and plant pathology. Her passion in fungal genetics and skill is improving information about mating in higher fungi. Her current experimental work is based on inducible system to in study detail mating genes and downstream pathways involve in A and B pathways in *Flammulina velotipes* in which initial results showed that this approach is responsive in determining pathways in *F. velotipes* and can be used in other higher fungi.

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