Title A genetic approach to elucidate the pathway of patulin degradation by a biocontrol yeast

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Abstract

The basidiomycetous yeast *Rhodotorula glutinis* (teleomorph *Rhodosporidium* spp.) can be isolated from diverse environments. *R. glutinis* strain LS11 was isolated from apple, and was subsequently found to protect apples from postharvest rots caused by *Penicillium expansum* and *Botrytis cinerea*. It is also able to degrade and detoxify the mycotoxin patulin, which is formed in apples infected with *P. expansum*. Patulin is a very potent toxin and only minute concentrations are permitted in commercial apple juice. The extreme sensitivity of *Escherichia coli* to patulin and its insensitivity to the degradation products was utilized to develop an assay for screening of mutants defective in patulin degradation. In order to find the genes responsible for enzymatic degradation, we generated *Agrobacterium*-mediated insertion mutants and are in the process of constructing a genomic library. The genes of mutants that do not degrade patulin are being identified. The genome library is being constructed in *Cryptococcus neoformans* strain JEC43, a basidiomycetous yeast strain that is sensitive to patulin and is unable to degrade it. To have additional genetic tools for *R. glutinis* LS11 uracil auxotrophs were selected on minimal medium supplemented with 5-FoA (5-fluoro-orotic acid). This approach yielded mutants in genes for enzymes involved in uracil biosynthesis, *URA3* (orotidine-5'-phosphate decarboxylase) or *URA5* (orotate phosphoribosyltransferase). In order to identify the gene mutated, each auxotroph was complemented with the *URA3* and *URA5* genes of *C. neoformans*. Of eight auxotrophs analysed, two were auxotrophs for URA3 and six for URA5. The transformants were confirmed phenotypically and genotypically.