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Next generation biotherapeutic production system: The filamentous fungus *Trichoderma reesei*

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The filamentous fungus *Trichoderma reesei* is an important production organism used by industrial enzyme companies worldwide. It is a low cost production system that secretes its native enzymes at levels exceeding 100 g/L of culture medium. Several *T. reesei* produced enzymes have obtained the generally recognized safe status by the Food and Drug Administration. *T. reesei* has tremendous prospects to be a cost efficient and high yield system for producing therapeutic proteins. We have adapted the fungus to become more suitable for bio-therapeutic production by reducing secreted protease activity and altering glycosylation pathways needed for adding mammalian glycoforms. Expression strains for monoclonal antibodies, Fab antibody fragments, interferon alpha-2b, insulin-like growth factor 1, and fibroblast growth factor 21 were constructed, cultivated in bioreactors, and expression levels were measured from the culture medium. After deleting 13 of the most critical protease genes, the general secreted protease activity was reduced over 30-fold. Monoclonal antibodies could be produced up to 7.6 g/L, Fab antibody fragments up to 8.2 g/L, interferon alpha-2b at 7.9 g/L, and insulin-like growth factor fusion protein at 8 g/L. With protease inhibitor treatment, interferon alpha-2b could be produced at over 10 g/L, insulin-like growth factor fusion protein at 19 g/L, and full length fibroblast growth factor 21 at 200 mg/L in addition to a shorter form at 3.5 g/L. Human glycoforms such as G0 and FG0 were produced on monoclonal antibodies. Expression levels and product quality improved dramatically after multiple protease deletions and optimization of culture conditions. While the production levels achieved are already relatively high, the strains could be developed further to reach the 100 g/L potential of the organism. This study demonstrates the excellent prospects of *T. reesei* as a host for therapeutic protein production.

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