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DEVELOPMENT OF MYCELIOPHTHORA THERMOPHILA 'C1' (A HIGHLY EXPRESSING FILAMENTOUS FUNGUS) INTO A NEXT-GENERATION THERAPEUTIC PROTEIN PRODUCTION SYSTEM

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Myceliophthora thermophila (named C1), Dyadic's proprietary gene-expression platform is a well-known industrial enzyme production host that has reached enzyme titers of over 120 g/l in a 6/7-day process. Enzymes produced by using the C1 technology have already obtained the FDA GRAS status for safety in food applications.

Since 2016, Dyadic's goal is to leverage C1's exceptional production potential to lower the cost of manufacturing proteins for animal and human health applications. To this end, we have further developed certain C1 cell lines to reach very high productivity levels in a variety of biologics. Further, we have also worked to reduce C1's protease activity as well as humanize C1's glycan structures for expressing glycoproteins such as monoclonal antibodies.

During this work we have characterized over fifty C1 proteases with transcriptomics, proteomics and biochemical methods. Deletion of the critical protease genes has led to low protease C1 strains that result in 50-fold lower total protease activity than wild type strains. With these upgraded C1 strains, we have expressed a variety of biologics with very high yield, including full-length mAbs at final titers of up to 24.5 g/l, Fc-fusion proteins at 13.2 g/l and Fab fragments at 14.5 g/l. The antigen binding properties of two mAbs produced from C1 were comparable to CHO-produced mAbs. We believe there are still potential and opportunities to generate C1 strains with even higher productivity.

We also generated data from difficult-to-express proteins rVaccines, VLPs and bi-specific antibodies, including a secreted 60-mer VLP protein at 2.24 g/l and another viral antigen at 1.8 g/l in 5-7 days fermentation.

The original C1 glycan structure consists mostly of high mannose glycans reaching up to Man9 which is a superior glycan structure for humanization than that of yeast. We are continuing our work to modify C1's glycan pattern towards complex human glycoforms by deleting native genes and expressing various heterologous genes.

The data generated from our third party and internal research programs supports the thesis that the C1 platform has the potential to now be developed into a safe and efficient expression system that will help speed up the development, lower production costs and improve the performance of biologic vaccines and drugs.