




RESEARCH NOTE

Identifying the *gluc-1* and *gluc-2* mutations in *Neurospora crassa* by genome resequencing

KEVIN McCLUSKEY¹ and SCOTT E. BAKER^{2,3*} 

¹*Bolt Threads, 5858 Horton, Emeryville, CA 94608, USA*

²*DOE Joint BioEnergy Institute, 5885 Hollis St, Emeryville, CA 94608, USA*

³*Functional and Systems Biology Group, Environmental Molecular Sciences Division, Pacific Northwest National Laboratory, 3335 Innovation Blvd, Richland, WA 99354, USA*

*For correspondence. E-mail: scott.baker@pnnl.gov.

Received 12 July 2022; revised 11 August 2022; accepted 16 August 2022

Abstract. Genome resequencing is an efficient strategy for associating mutant phenotypes with physical genomic loci (Baker 2009). A pilot study of this approach demonstrated that the *Neurospora crassa* genetic map was critical in narrowing the possible candidate mutations in a strain to a small number in a limited, defined region of the genome (McCluskey *et al.* 2011). In this study, we utilize a resequencing strategy to identify the mutations underlying the *gluc-1* and *gluc-2* genes in *N. crassa*.

Keywords. resequencing; genomics; beta-glucosidase; phenotype; genotype; *Neurospora crassa*.

Introduction

Fungal CAZymes are of special interest for biofuel production because they can deconstruct plant-derived biomass into fermentable sugars (Ottum *et al.* 2021). *Neurospora crassa* has been a good model to learn about the biochemistry and regulation of cellulose deconstructing enzymes for many decades (Eberhart *et al.* 1977; Yazdi *et al.* 1990a,b; Tian *et al.* 2009; Schmoll *et al.* 2012; Znameroski *et al.* 2012; Coradetti *et al.* 2013; Gabriel *et al.* 2021). In cellulose saccharification, beta glucosidases release glucose from cellobiose (Ketudat Cairns and Esen 2010). The *N. crassa*, beta-glucosidase system has been well characterized and exploited for advances in biotechnology related to sugar utilization (Eberhart *et al.* 1964; Mahadevan and Eberhart 1964a; Eberhart and Beck 1970, 1973; Galazka *et al.* 2010; Ha *et al.* 2011; Karkehabadi *et al.* 2018).

Numerous studies of biomass deconstruction continue to be facilitated by the generation and wide distribution of *N. crassa* gene knockout library (Colot *et al.* 2006; Znameroski and Glass 2013; Seibert *et al.* 2016). By screening gene knockout strains many of the regulatory circuits and enzymes that drive *N. crassa* biomass deconstruction have been characterized (Schmoll *et al.* 2012; Sun *et al.* 2012;

Coradetti *et al.* 2013; Reilly *et al.* 2015; Gabriel *et al.* 2021). While these advances have had significant impact, in many cases, connections to prior literature and classical or forward genetic mutant strains have not been made. Fractionation studies indicated *N. crassa* had at least two beta-glucosidases one with activity that was thermostable (Eberhart *et al.* 1964). The induction and enzymatic properties of beta-glucosidases were characterized, in part, utilizing two mutations, *gluc-1* and *gluc-2* which reduce measured thermostable beta glucosidase activity by ~90% and over 99% respectively. *gluc-1* and *gluc-2* map to linkage group III and were proposed to be allelic (Eberhart and Beck 1970, 1973; Mahadevan and Eberhart 1962, 1964b).

Results and discussion

To bridge the current beta-glucosidase studies with prior literature, we sequenced the genomes of two *N. crassa* strains, one with *gluc-1* (FGSC 1224) and the other with *gluc-1* and *gluc-2* (FGSC 1227). Combined with prior genetic mapping of the mutant to LG III, our sequencing analysis led us to discover that *gluc-1* and *gluc-2* are both encoded by mutations in NCU08755 (table 1). Sequence

Table 1. Strain resequencing information.

FGSC strain	Gene	NCBI BioProject accession	Mutation	Mutation location
1224	<i>gluc-1</i>	PRJNA249728	L425P	<i>gluc-1</i> , supercontig_3:5256440, A->G
1227	<i>gluc-1</i>	PRJNA249732	L425P	<i>gluc-1</i> , supercontig_3:5256440, A->G
	<i>gluc-2</i>		W46*	<i>gluc-2</i> , supercontig_3:5257648, C->T

homology demonstrates that NCU08755 is a member of the glycoside hydrolase family 3 (GH3) beta-glucosidase. The *gluc-1* mutation encodes a leucine to proline change at amino acid residue 425 while *gluc-2* encodes a tryptophan to STOP mutation at amino acid residue 46, presumably resulting in a truncated and nonfunctional peptide.

In summary, we have identified mutations in the gene NCU08755 that underly the classical mutant alleles, *gluc-1* and *gluc-2*, connecting a robust biochemistry and genetics literature with current systems biology studies enabled by the *N. crassa* gene knockout strain library as well as by the diverse tools available for research into fundamental and applied areas of fungal biology and biotechnology.

Acknowledgements

Genome sequence analysis was performed as part of the DOE Joint BioEnergy Institute (<https://www.jbei.org/>) and genome sequencing was performed by the DOE Joint Genome Institute (<https://jgi.doe.gov/>), both of which are supported by the US Department of Energy (DOE), Office of Science, Office of Biological and Environmental Research, through Contract DE-AC02-05CH11231 between Lawrence Berkeley National Laboratory and the US DOE.

References

- Baker S. 2009 Selection to sequence: opportunities in fungal genomics. *Environ. Microbiol.* **11**, 2955–2958.
- Colot H. V., Park G., Turner G. E., Ringelberg C., Crew C. M., Litvinkova L. *et al.* 2006 A high-throughput gene knockout procedure for *Neurospora* reveals functions for multiple transcription factors. *Proc. Natl. Acad. Sci. USA* **103**, 10352–10357.
- Coradetti S. T., Xiong Y. and Glass N. L. 2013 Analysis of a conserved cellulase transcriptional regulator reveals inducer-independent production of cellulolytic enzymes in *Neurospora crassa*. *Microbiologyopen* **2**, 595–609.
- Eberhart B., Cross D. F. and Chase L. R. 1964 β -glucosidase system of *Neurospora crassa* I. β -Glucosidase and Cellulase Activities of Mutant and Wild-Type Strains. *J. Bacteriol.* **87**, 761–770.
- Eberhart B. M. and Beck R. S. 1970 Localization of the beta-glucosidases in *Neurospora crassa*. *J. Bacteriol.* **101**, 408–417.
- Eberhart B. M. and Beck R. S. 1973 Induction of beta-glucosidases in *Neurospora crassa*. *J. Bacteriol.* **116**, 295–303.
- Eberhart B. M., Beck R. S. and Goolsby K. M. 1977 Cellulase of *Neurospora crassa*. *J. Bacteriol.* **130**, 181–186.
- Gabriel R., Thieme N., Liu Q., Li F., Meyer L. T., Harth S. *et al.* 2021 The F-box protein gene *exo-1* is a target for reverse engineering enzyme hypersecretion in filamentous fungi. *Proc Natl Acad Sci U S A* **118**, e2025689118.
- Galazka J. M., Tian C., Beeson W. T., Martinez B., Glass N. L. and Cate J. H. 2010 Cellodextrin transport in yeast for improved biofuel production. *Science* **330**, 84–86.
- Ha S.-J., Galazka J. M., Rin Kim S., Choi J.-H., Yang X., Seo J.-H. *et al.* 2011 Engineered *Saccharomyces cerevisiae* capable of simultaneous cellobiose and xylose fermentation. *Proc. Natl. Acad. Sci. USA* **108**, 504–509.
- Karkehabadi S., Hansson H., Mikkelsen N. E., Kim S., Kaper T., Sandgren M. *et al.* 2018 Structural studies of a glycoside hydrolase family 3 β -glucosidase from the model fungus *Neurospora crassa*. *Acta Crystallogr. f. Struct. Biol. Commun.* **74**, 787–796.
- Ketudat Cairns J. R. and Esen A. 2010 β -Glucosidases. *Cell Mol. Life Sci.* **67**, 3389–3405.
- Mahadevan P. and Eberhart B. M. 1964a The β -glucosidase system of *Neurospora crassa*: II. Purification and characterization of aryl β -glucosidase. *Arch Biochem. Biophys.* **108**, 22–29.
- Mahadevan P. and Eberhart B. M. 1964b The β -glucosidase system of *Neurospora crassa*: III. Further studies on an aryl β -glucosidase mutant. *Arch Biochem. Biophys.* **108**, 30–35.
- Mahadevan P. and Eberhart B. M. 1962 A dominant regulatory gene for aryl-beta-glucosidase in *Neurospora crassa*. *J. Cell Comp. Physiol.* **60**, 281–283.
- McCluskey K., Wiest A. E., Grigoriev I. V., Lipzen A., Martin J., Schackwitz W. *et al.* 2011 Rediscovery by whole genome sequencing: classical mutations and genome polymorphisms in *Neurospora crassa*. *G3 (Bethesda)* **1**, 303–316.
- Ottum E., Baker S. E. and Bredeweg E. L. 2021 Production of biofuels from biomass by fungi. In *Encyclopedia of mycology* (ed. O. Zaragoza and A. Casadevall) pp. 555–576. Elsevier. Amsterdam.
- Reilly M. C., Qin L., Craig J. P., Starr T. L. and Glass N. L. 2015 Deletion of homologs of the SREBP pathway results in hyperproduction of cellulases in *Neurospora crassa* and *Trichoderma reesei*. *Biotechnol. Biofuels* **8**, 1–18.
- Schmoll M., Tian C., Sun J., Tisch D. and Glass N. L. 2012 Unravelling the molecular basis for light modulated cellulase gene expression—the role of photoreceptors in *Neurospora crassa*. *BMC Genomics* **13**, 1–19.
- Seibert T., Thieme N. and Benz J. P. 2016 The renaissance of *Neurospora crassa*: how a classical model system is used for applied research. In *Gene expression systems in fungi: advancements and applications* (eds. M. Schmoll and C. Dattenböck), Springer, Cham, pp. 59–96.
- Sun J., Tian C., Diamond S. and Glass N. L. 2012 Deciphering transcriptional regulatory mechanisms associated with hemicellulose degradation in *Neurospora crassa*. *Eukaryot Cell* **11**, 482–493.
- Tian C., Beeson W. T., Iavarone A. T., Sun J., Marletta M. A., Cate J. H. *et al.* 2009 Systems analysis of plant cell wall degradation by the model filamentous fungus *Neurospora crassa*. *Proc. Natl. Acad. Sci. USA* **106**, 22157–22162.
- Yazdi M., Radford A., Keen J. and Woodward J. 1990a Cellulase production by *Neurospora crassa*: purification and characterization of cellulolytic enzymes. *Enzyme Microb. Technol.* **12**, 120–123.

- Yazdi M., Woodward J. and Radford A. 1990b The cellulase complex of *Neurospora crassa*: activity, stability and release. *Microbiology* **136**, 1313–1319.
- Znameroski E. A., Coradetti S. T., Roche C. M., Tsai J. C., Iavarone A. T., Cate J. H. *et al.* 2012 Induction of lignocellulose-degrading enzymes in *Neurospora crassa* by cellobextrins. *Proc. Natl. Acad. Sci. USA* **109**, 6012–6017.
- Znameroski E. A. and Glass N. L. 2013 Using a model filamentous fungus to unravel mechanisms of lignocellulose deconstruction. *Biotechnol. Biofuels* **6**, 1–8.

Corresponding editor: DURGADAS P. KASBEKAR