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RESEARCH NOTE

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

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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 $\sim$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	*gluc-1*	PRJNA249728	L425P	gluc-1, supercontig_3:5256440, A->G
1227	*gluc-1*	PRJNA249732	L425P	gluc-1, supercontig_3:5256440, A->G
	gluc-2		W46*	gluc-2, 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.

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