Transformation of mutant Saccharomyces cerevisiae strains and establishing a disease model for ALG9-CDG

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Figure 3. s cerevisiae strains YGS414 & YGS415: Pre- and post-transformation.

Figure 2. Model of mannosyltransferase 7-9 (ALG).

Table 2. s cerevisiae strain and plasmid combinations utilized.

Table 1. Gene defects leading to deficient assembly of dolichol-linked oligosaccharides in CDG type I.

METHODS

Yeast Strains: Mutant strains of s cerevisiae derived from the temperature-sensitive, uracil-auxotrophic S322 strain (Mata ade2-101 ura-3-52 his3200 lys2-801) were obtained. Mutant strains included YGS414 and YGS415. YGS414 (SS322 w/ Dgal51 + KanMX) lacks the ALG9 gene, which encodes for mannosyltransferase 7-9, the deficient enzyme associated with ALG9-CDG (Figure 2). YGS415 (SS322 w/ wpl-1 Dgal51 + KanMX) lacks the ALG9 gene and also has additional down-regulation of the oligosaccharant transferase protein.

Plasmids: Mutant yeast strains were transformed with four different plasmids in separate experiments with the yeast/plasmid combinations shown in Table 2. All plasmids used in this study contain uracil synthetic pathways. pALG9 is a derivative of YEp352 and contains a 2.5 kb BglII-KpnI fragment of yeast genomic DNA encompassing the ALG9 ORF plus 0.5 kb upstream sequence in the Barri-KpnI plasmids. Plasmid HLA09 contains human genomic DNA incorporating the human ALG9 gene, while HLA09 sf53 and HLA09 Y286C plasmids contain the human ALG9 gene with single nucleotide polymorphism mutations known to correlate with reported clinical ALG9 cases.

Figure 1. Pathway of dolichol-linked oligosaccharide biosynthesis.

Figure 3. s cerevisiae strains YGS414 & YGS415: Pre- and post-transformation.

RESULTS

Uracil auxotrophic strains YGS414 and YGS415 were observed to grow on YPD media, however, did not grow on uracil-deficient media. Following the transformation procedure utilizing these strains, at 48 hours incubation, a slight haze of growth was observed on uracil-deficient YPD media. At 72 hours, heavy growth of budding yeast were identified as shown in Figure 3. Successful growth in the absence of uracil provides phenotypic evidence of successful transformation with uptake of plasmids and subsequent uracil prototrophy.

DISCUSSION

In addition to phenotypic analysis post-transformation, molecular sequencing will be employed to verify transformation and documentation of relevant ALG9 sequence including relevant mutations desired for disease modeling. Subsequently, further research will strive to characterize the role of ALG9 in organism development and function through comparison of mutant, transformed strains and wild type strains. Transcriptome analysis employing RNA microarray is currently underway for the purpose of gene expression changes that accompany the phenotypic change. The future goal of identifying potential therapeutic options. This current research describes the seminal work in Atlantic Canada where efforts are underway to establish disease models for ALG9-CDG using Saccharomyces cerevisiae and Danio rerio. This presentation describes the progress up to date with regards to the

REFERENCES


