

Supplementary Material 2

Genomic BLAST screening of sucrose-, glucose orthologs in *Metschnikowia pulcherrima* strain APC 1.2

S1. Methods

S1.1 Genomic data sources

Predicted proteomes were obtained for *Metschnikowia aff. pulcherrima* strain APC 1.2 (BioProject [PRJNA508581](#); genome assembly [GCA_004217705.1](#), ASM421770v1) and *Saccharomyces cerevisiae* S288C (RefSeq proteome, genome assembly [GCF_000146045.2](#), assembly name R64; BioProject [PRJNA128](#)).

S1.2 Candidate gene identification, extraction, and BLAST analysis

Candidate *S. cerevisiae* genes of interest were compiled from the primary literature (see Section S2, 'Rationale for Gene Selection'). The official gene symbol, full protein name, RefSeq protein accession number (NP_), NCBI Gene ID, corresponding NCBI record link and general protein information were manually retrieved for each candidate gene from the NCBI Gene database. This was necessary because several genes were only generically annotated in the *S. cerevisiae* source proteome FASTA header (e.g. 'alpha-glucoside permease', 'glucose sensor', 'hexokinase'), precluding reliable identification by keyword search alone. This information was compiled into a reference table. The protein sequences corresponding to each verified NP accession were then extracted directly from the *S. cerevisiae* RefSeq proteome using the SeqIO module of Biopython and written to a combined query FASTA file.

A local protein BLAST database was constructed from the *M. pulcherrima* APC 1.2 predicted proteome using NCBI BLAST+ v2.17.0 (makeblastdb -dbtype prot) and the combined query file was searched against this database using blastp (-evalue 1e-5 -outfmt 6 -max_target_seqs 3, including the query and subject length fields). Query and subject percent coverage were calculated from the raw alignment coordinates as follows: Query coverage (%) = $100 \times (\text{qend} - \text{qstart} + 1) / \text{qlen}$; subject coverage (%) = $100 \times (\text{send} - \text{sstart} + 1) / \text{slen}$.

To validate orthology, the *M. pulcherrima* proteins returned by the forward search were extracted using Biopython and searched against a second local database built from the *S. cerevisiae* RefSeq proteome using blastp (-evalue 1e-5, -outfmt 6 and -max_target_seqs 1).

S1.3 Software versions and custom scripts

- NCBI BLAST+ v2.17.0 (Windows)
- Biopython (SeqIO module) for sequence extraction
- Python 3

Custom Python scripts were used to extract query sequences by verified accession, identify and extract reciprocal-search target sequences from BLAST output, calculate alignment coverage, and construct the results tables described above. These tables were then adjusted manually for Tables S3.1.1–S3.2.3. These scripts are not independently published or peer-reviewed bioinformatics tools; they perform routine, non-algorithmic data handling steps using the Biopython library. The scripts, the raw BLAST output and the constructed results tables are provided in the accompanying Supplementary Data archive (Supplementary2_Data_MP_Dinic_2026.zip) deposited on Zenodo under DOI 10.5281/zenodo.22698070 and are organised as follows: 01_Scripts (custom Python scripts); 02_Raw_Results (raw BLAST and reciprocal BLAST output and constructed results tables); and 03_Supplementary_Documents (this document and related supplementary files).

S2. Rationale for Candidate Gene Selection

The candidate genes were selected based on their established roles in sucrose and disaccharide metabolism, hexose transport and glucose repression signalling in *S. cerevisiae*, as summarised below.

Sucrose/maltose/isomaltose pathway gene candidates

The genes SUC2, IMA1–IMA5, MAL12, MAL32, MAL11 (AGT1), MAL31, MPH2 and MPH3 were chosen based on their established roles in sucrose and alpha-glucoside metabolism. SUC2 was initially investigated as the primary sucrose-hydrolysis gene. Marques et al. (2017) revealed that IMA1/IMA5 upregulation sustains sucrose utilisation in SUC2/MAL-deficient strains, and that combined deletion of all five IMA genes eliminates this ability. The same study also showed that deleting MAL12, MAL22 and MAL32 alongside SUC2 was necessary to eliminate residual sucrose hydrolysis. This implicates this gene family in the SUC2-independent route. MPH2 and MPH3 (YDL247w and YJR160c) were added based on the work of Day et al. (2002), who characterised them as alpha-glucoside transporters via heterologous complementation.

Glucose transport and sensing

All 20 members of the HXT hexose transporter family (HXT1–17, GAL2, and the sensors SNF3/RGT2) (Özcan & Johnston, 1999) were tested to assess whether limited glucose transport capacity could explain the strain's preference for sucrose over glucose. Although Diderich et al. (1999) found that HXT1–7 exhibited the strongest activity and were the only members whose transcription correlated directly with extracellular glucose concentration (with GAL2, SNF3, RGT2 and HXT8–17 being expressed only weakly or under specific conditions), Wieczorke et al. (1999) showed that deleting only HXT1–7 was insufficient to block the uptake of glucose, mannose and fructose. In their CEN.PK2-1C-derived strain, complete abolition of hexose transport required the deletion of all HXT1–17, as well as MAL11, MPH2 and MPH3 (also known as AGT1, YDL247w and YJR160c, respectively). This confirms that the remaining transporters, which are individually minor, are collectively sufficient to sustain uptake. Therefore, all 20 family members were tested here rather than restricting the analysis to HXT1–7. HXT8–17 have received less attention, and many of their assigned functions are not directly related to sugar utilisation. However, expression of all except HXT11 and HXT12 is glucose-regulated (Rintala et al., 2008), and some, including HXT13, HXT15, HXT16 and HXT17, have been specifically linked to hexitol transport with distinct substrate specificities (Jordan et al., 2016). HXT12 and HXT14 were excluded from testing as they are absent/non-functional in the S288C reference strain.

Core glucose-repression signaling pathway

The candidate genes SNF1, SNF4, MIG1, HXK1, HXK2, GLK1, REG1, GLC7, TUP1, SNF3, and RGT2 were selected based on their established roles in the glucose repression pathway of *S. cerevisiae* based on reviews of Busti et al. (2010), and Kayikci & Nielsen, (2015). The core catalytic complex, which is formed by SNF1 and its regulatory subunit SNF4, is activated upon glucose limitation to phosphorylate and inactivate MIG1, relieving the repression of glucose-repressed genes. MIG1 mediates this repression in conjunction with the general co-repressor TUP1 (via its obligatory partner SSN6/CYC8). Through its nuclear association with MIG1 at target promoters, such as SUC2 GLK1 and HXK1 were kept as hexokinase-family homologues to act as parallel comparison points, despite not being directly involved in this signalling function. SNF1 is rendered inactive by the phosphatase complex formed by REG1 and GLC7, which serves as the pathway's 'off-switch'. SNF3 and RGT2 are upstream regulators and are included in the above group (glucose transport and sensing (HXT family, GAL2, SNF3/RGT2-type sensors)). Additional pathway components identified in both reviews include RGT1, MTH1, STD1, SAK1, ELM1, TOS3, MIG2, ADR1 and CAT8. These components were not included in the present analysis, but represent candidates for a broader analysis in the future.

S3. Results Tables

Tables S3.1. Top BLAST hit per query gene, with reciprocal BLAST validation

For each *S. cerevisiae* query gene, the single top-scoring hit against the *M. pulcherrima* proteome is reported, with percent identity, query- and subject-side coverage, and bit score. Each *M. pulcherrima* hit was also searched back against the *S. cerevisiae* proteome (reciprocal BLAST); the resulting top reciprocal hit and its percent identity are shown in the final two columns, alongside a verdict on orthology confirmation

Table S3.1.1. Sucrose/maltose/isomaltose pathway gene candidates

Gene	SC accession	MP top hit	MP annotation	Identity (%)	Query cov (%)	Subject cov (%)	Bit score	Reciprocal SC hit	Reciprocal identity (%)
SUC2	NP_012104.1	NO HIT	n/a	-	-	-	-	n/a	-
IMA1	NP_011803.3	QBM87903.1	alpha-glucosidase	47.5	97.5	94.4	546	IMA1	47.5
IMA2	NP_014485.1	QBM87903.1	alpha-glucosidase	47.8	97.5	94.4	558	IMA1	47.5
IMA3	NP_012096.1	QBM87903.1	alpha-glucosidase	47.7	97.5	94.4	553	IMA1	47.5
IMA4	NP_012314.1	QBM87903.1	alpha-glucosidase	47.7	97.5	94.4	553	IMA1	47.5
IMA5	NP_012319.1	QBM87903.1	alpha-glucosidase	45.2	98.5	94.6	528	IMA1	47.5
MAL12	NP_011808.3	QBM87903.1	alpha-glucosidase	47.6	97.8	94.6	532	IMA1	47.5
MAL32	NP_009858.3	QBM87903.1	alpha-glucosidase	47.7	97.8	94.6	533	IMA1	47.5
MAL11 (= AGT1)	NP_011805.3	QBM90166.1	MFS transporter, SP family, general alpha glucoside:H+ symporter	44.6	89.1	95	503	MPH3	46.0
MAL31	NP_009857.1	QBM90166.1	MFS transporter, SP family, general alpha glucoside:H+ symporter	43.8	91.5	97.7	514	MPH3	46.0
MPH2	NP_010034.1	QBM90166.1	MFS transporter, SP family, general alpha glucoside:H+ symporter	47.3	88	92.9	518	MPH3	46.0
MPH3	NP_012694.1	QBM90166.1	MFS transporter, SP family, general alpha glucoside:H+ symporter	46	92.7	96.7	520	MPH3	46.0

Table S3.1.2. Glucose transport and sensing

Gene	SC accession	MP top hit	MP annotation	Identity (%)	Query cov (%)	Subject cov (%)	Bit score	Reciprocal SC hit	Reciprocal identity (%)
HXT1	NP_011962.1	QBM86508.1	MFS transporter, SP family, sugar:H+ symporter	57.4	93.3	98.7	639	HXT6	58.9
HXT2	NP_013724.1	QBM87556.1	MFS transporter, SP family, sugar:H+ symporter	62.4	91.9	91.2	643	HXT6	60.5
HXT3	NP_010632.1	QBM86508.1	MFS transporter, SP family, sugar:H+ symporter	57.7	92.6	96.9	639	HXT6	58.9
HXT4	NP_011960.2	QBM86508.1	MFS transporter, SP family, sugar:H+ symporter	58.9	94.8	99.8	656	HXT6	58.9
HXT5	NP_011964.1	QBM86508.1	MFS transporter, SP family, sugar:H+ symporter	60.1	88.5	96.9	657	HXT6	58.9
HXT6	NP_010630.1	QBM86508.1	MFS transporter, SP family, sugar:H+ symporter	58.9	95.8	99.1	673	HXT6	58.9
HXT7	NP_010629.3	QBM86508.1	MFS transporter, SP family, sugar:H+ symporter	58.7	95.8	99.1	671	HXT6	58.9
HXT8	NP_012321.1	QBM87556.1	MFS transporter, SP family, sugar:H+ symporter	59.3	90.5	94.2	627	HXT6	60.5
HXT9	NP_012316.1	QBM86508.1	MFS transporter, SP family, sugar:H+ symporter	58.4	95.6	98.9	655	HXT6	58.9
HXT10	NP_116644.1	QBM87556.1	MFS transporter, SP family, sugar:H+ symporter	57.4	97.1	96.4	616	HXT6	60.5
HXT11	NP_014486.1	QBM86508.1	MFS transporter, SP family, sugar:H+ symporter	59.3	93.8	97.1	651	HXT6	58.9
HXT13	NP_010845.1	QBM86508.1	MFS transporter, SP family, sugar:H+ symporter	49.4	94.9	98.7	554	HXT6	58.9
HXT15	NP_010036.1	QBM86508.1	MFS transporter, SP family, sugar:H+ symporter	51.1	92.2	96.5	553	HXT6	58.9
HXT16	NP_012692.3	QBM86508.1	MFS transporter, SP family, sugar:H+ symporter	50.9	92.2	96.5	550	HXT6	58.9
HXT17	NP_014470.1	QBM86508.1	MFS transporter, SP family, sugar:H+ symporter	49.3	94.9	98.7	551	HXT6	58.9
GAL2	NP_013182.1	QBM86508.1	MFS transporter, SP family, sugar:H+ symporter	56.6	94.6	99.1	632	HXT6	58.9
SNF3	NP_010087.1	QBM86342.1	MFS transporter, SP family, sugar:H+ symporter	55.7	56.1	67	583	SNF3	55.7
RGT2	NP_010143.1	QBM86342.1	MFS transporter, SP family, sugar:H+ symporter	56.6	62.5	65.1	573	SNF3	55.7

Table S3.1.3. Core glucose-repression signaling pathway

Gene	SC accession	MP top hit	MP annotation	Identity (%)	Query cov (%)	Subject cov (%)	Bit score	Reciprocal SC hit	Reciprocal identity (%)
SNF1	NP_010765.3	QBM87938.1	carbon catabolite-derepressing protein kinase	64.6	95.1	94.9	776	SNF1	66.0
SNF4	NP_011400.1	QBM89960.1	CBS domain-containing protein	66.6	98.1	93.8	441	SNF4	66.6
MIG1	NP_011480.1	QBM89872.1	zinc-finger protein CreA/MIG	55.7	18.5	20.8	122	MIG1	55.7
HXK1	NP_116711.3	QBM90772.1	hexokinase	68.9	99.4	99.4	706	HXK1	67.5
HXK2	NP_011261.1	QBM90772.1	hexokinase	68.2	99.4	99.4	700	HXK1	67.5
GLK1	NP_009890.1	QBM88337.1	hexokinase	48.6	97	97.7	445	GLK1	48.6
REG1	NP_010311.1	QBM86900.1	protein of unknown function DUF1752	36.4	33.9	40.7	194	REG1	36.4
GLC7	NP_011059.3	QBM90616.1	serine/threonine-protein phosphatase PP1 catalytic subunit	91.8	97.4	92.7	583	GLC7	91.8
TUP1	NP_010007.1	QBM90872.1	glucose repression regulatory protein TUP1	53.6	73.8	75.7	516	TUP1	58.2
CYC8	NP_009670.3	QBM87506.1	glucose repression mediator protein	68	38.3	39.8	567	CYC8	61.1

Tables S3.2. Secondary and tertiary BLAST hits per query gene (supplementary to Tables S1-S3)

This table lists the second- and third-ranked BLAST hits for each query gene, if available, as well as the top hit already reported in Tables S3.1. Only genes with more than one significant hit are shown beyond the first columns. Cells filled with n/a indicate that fewer than three significant hits were returned for that gene. As with Table S3.1, the results are presented in three subtables

Table S3.2.1. Sucrose/maltose/isomaltose pathway secondary and tertiary BLAST hits

Gene	MP hit 2	MP annotation 2	Identity 2 (%)	Query cov 2 (%)	Subject cov 2 (%)	Bit score 2	MP hit 3	MP annotation 3	Identity 3 (%)	Query cov 3 (%)	Subject cov 3 (%)	Bit score 3
SUC2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
IMA1	QBM90856.1	Alpha amylase, catalytic domain	41	54.8	98.1	246	n/a	n/a	n/a	n/a	n/a	n/a
IMA2	QBM90856.1	Alpha amylase, catalytic domain	41.4	54.8	98.1	243	n/a	n/a	n/a	n/a	n/a	n/a
IMA3	QBM90856.1	Alpha amylase, catalytic domain	41.4	54.8	98.1	243	n/a	n/a	n/a	n/a	n/a	n/a
IMA4	QBM90856.1	Alpha amylase, catalytic domain	41.4	54.8	98.1	243	n/a	n/a	n/a	n/a	n/a	n/a
IMA5	QBM90856.1	Alpha amylase, catalytic domain	39.3	55.9	99.7	227	QBM88055.1	1,4-alpha-glucan branching enzyme	24.2	31.7	21.5	47
MAL12	QBM90856.1	Alpha amylase, catalytic domain	41	55.5	99.7	233	n/a	n/a	n/a	n/a	n/a	n/a
MAL32	QBM90856.1	Alpha amylase, catalytic domain	41.3	55.5	99.7	234	n/a	n/a	n/a	n/a	n/a	n/a
MAL11 (= AGT1)	QBM85577.1	MFS transporter, sugar porter (SP) family	25.5	76.1	88.7	110	QBM88660.1	MFS transporter, sugar porter (SP) family	26.6	66.9	77.7	101
MAL31	QBM86508.1	MFS transporter, SP family, sugar:H ⁺ symporter	23.7	82.4	93.4	99	QBM85602.1	MFS transporter, sugar porter (SP) family	24.2	74.3	85	99
MPH2	QBM86508.1	MFS transporter, SP family, sugar:H ⁺ symporter	25.8	68.6	78.9	98	QBM85602.1	MFS transporter, sugar porter (SP) family	24.1	75.5	85.6	97
MPH3	QBM86508.1	MFS transporter, SP family, sugar:H ⁺ symporter	25.8	69.4	78.9	98	QBM85602.1	MFS transporter, sugar porter (SP) family	24.1	76.4	85.6	98

Table S3.2.2. Glucose transport and sensing secondary and tertiary BLAST hits

Gene	MP hit 2	MP annotation 2	Identity 2 (%)	Query cov 2 (%)	Subject cov 2 (%)	Bit score 2	MP hit 3	MP annotation 3	Identity 3 (%)	Query cov 3 (%)	Subject cov 3 (%)	Bit score 3
HXT1	QBM87556.1	MFS transporter, SP family, sugar:H+ symporter	56.1	94.7	97.1	624	QBM85602.1	MFS transporter, sugar porter (SP) family	37.9	87.4	91.6	321
HXT2	QBM86508.1	MFS transporter, SP family, sugar:H+ symporter	60.5	95.7	95.8	641	QBM85602.1	MFS transporter, sugar porter (SP) family	37	88.4	89	319
HXT3	QBM87556.1	MFS transporter, SP family, sugar:H+ symporter	59.8	88.9	92	635	QBM85602.1	MFS transporter, sugar porter (SP) family	38.6	86.4	91.4	328
HXT4	QBM87556.1	MFS transporter, SP family, sugar:H+ symporter	60.3	89.6	92.9	642	QBM85602.1	MFS transporter, sugar porter (SP) family	37	89.8	96.6	324
HXT5	QBM87556.1	MFS transporter, SP family, sugar:H+ symporter	59.1	84.6	92	634	QBM85602.1	MFS transporter, sugar porter (SP) family	36.2	85.1	93.9	317
HXT6	QBM87556.1	MFS transporter, SP family, sugar:H+ symporter	60.5	92.3	95.3	669	QBM85602.1	MFS transporter, sugar porter (SP) family	38.2	86.1	91.4	312
HXT7	QBM87556.1	MFS transporter, SP family, sugar:H+ symporter	60.3	92.3	95.3	667	QBM85602.1	MFS transporter, sugar porter (SP) family	37.8	87.9	93.5	311
HXT8	QBM86508.1	MFS transporter, SP family, sugar:H+ symporter	58.5	90.7	95.2	616	QBM85602.1	MFS transporter, sugar porter (SP) family	35.8	91.2	95.8	302

HXT9	QBM87556.1	MFS transporter, SP family, sugar:H+ symporter	59.3	94.2	95.8	645	QBM85602.1	MFS transporter, sugar porter (SP) family	36.5	85.9	90.9	296
HXT10	QBM86508.1	MFS transporter, SP family, sugar:H+ symporter	59.5	89.9	90.8	607	QBM85602.1	MFS transporter, sugar porter (SP) family	37.2	86.6	88	314
HXT11	QBM87556.1	MFS transporter, SP family, sugar:H+ symporter	58	97.5	99.8	649	QBM85602.1	MFS transporter, sugar porter (SP) family	36.5	86.2	91.3	297
HXT13	QBM87556.1	MFS transporter, SP family, sugar:H+ symporter	52.6	88.3	92.2	551	QBM85602.1	MFS transporter, sugar porter (SP) family	35.8	86.5	90.7	290
HXT15	QBM87556.1	MFS transporter, SP family, sugar:H+ symporter	50.4	95.4	99.8	552	QBM85602.1	MFS transporter, sugar porter (SP) family	37.1	83.1	87.5	288
HXT16	QBM87556.1	MFS transporter, SP family, sugar:H+ symporter	50.2	95.4	99.8	548	QBM85602.1	MFS transporter, sugar porter (SP) family	37.1	83.1	87.5	286
HXT17	QBM87556.1	MFS transporter, SP family, sugar:H+ symporter	53.7	86	88.9	548	QBM85602.1	MFS transporter, sugar porter (SP) family	36.2	86.5	89.4	289
GAL2	QBM87556.1	MFS transporter, SP family, sugar:H+ symporter	60.5	87.3	90.7	630	QBM85602.1	MFS transporter, sugar porter (SP) family	37.9	84.8	90.9	305
SNF3	QBM85602.1	MFS transporter, sugar porter (SP) family	48.6	54.4	90.9	474	QBM86508.1	MFS transporter, SP family, sugar:H+ symporter	32.4	52.6	87.7	252
RGT2	QBM85602.1	MFS transporter, sugar porter (SP) family	48	61.7	88.2	475	QBM86508.1	MFS transporter, SP family, sugar:H+ symporter	33.3	61.3	87.1	272

Table S3.2.3. Core glucose-repression signaling pathway secondary and tertiary BLAST hits

Gene	MP hit 2	MP annotation 2	Identity 2 (%)	Query cov 2 (%)	Subject cov 2 (%)	Bit score 2	MP hit 3	MP annotation 3	Identity 3 (%)	Query cov 3 (%)	Subject cov 3 (%)	Bit score 3
SNF1	QBM88370.1	serine/threonine-protein kinase GIN4	46.2	43.3	21.8	265	QBM88815.1	serine/threonine-protein kinase HSL1, negative regulator of Swe1 kinase	45.3	42	19.6	250
SNF4	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
MIG1	QBM86280.1	zinc-finger protein CreA/MIG	64.9	14.7	12.7	113	QBM89661.1	hypothetical protein METSCH_D07410	42.4	19.6	46.9	81
HXK1	QBM88337.1	hexokinase	39.2	93.4	99.2	322	QBM87854.1	hexokinase	24	79	77.5	92
HXK2	QBM88337.1	hexokinase	40.4	91.6	97.3	320	QBM87854.1	hexokinase	28.2	64.6	62.1	105
GLK1	QBM90772.1	hexokinase	37.2	97.2	92.1	264	QBM87854.1	hexokinase	25.5	89	83.1	114
REG1	QBM90416.1	hypothetical protein METSCH_E06650	53.8	3.8	10.8	53	n/a	n/a	n/a	n/a	n/a	n/a
GLC7	QBM87224.1	serine/threonine-protein phosphatase PP1 catalytic subunit	61.4	99.4	62.8	389	QBM89050.1	serine/threonine-protein phosphatase PP1 catalytic subunit	59.5	93.3	55.6	388
TUP1	QBM90775.1	transcription initiation factor TFIID subunit 5	31	26.1	21.9	110	QBM90775.1	transcription initiation factor TFIID subunit 5	24.9	38.8	34.6	102
CYC8	QBM87276.1	Tetratricopeptide (TPR) repeat	29.7	17.3	26.1	84	QBM90366.1	anaphase-promoting complex subunit 8	27.6	16.1	26.6	51

S4. References

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Declaration of Generative AI and AI-assisted technologies in the writing process of the Supplementary

Material 2

During preparation of the Supplementary Materials for this work, the authors used Claude (Anthropic) to assist with improving English language, grammar, and clarity in the supplementary text, and to draft Python code used for generating results tables from BLAST output. Python code used for sequence extraction was independently written by the authors, with AI assistance limited to minor streamlining (e.g., variable naming, formatting, bug fixes). Selection of candidate genes and verification of all information/results, including reciprocal BLAST confirmation and resolution of ambiguous gene annotations, was performed manually by the authors without the help of AI. All code, results, and text were reviewed and verified by the author(s), who take full responsibility for the accuracy and content of the Supplementary Material.