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Supporting Information

for

A *myo*-inositol dehydrogenase involved in aminocyclitol biosynthesis of hygromycin A

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Table S1: Characterized enzymes from PF01408.

Gene name	Uniprot ID	Function	Biological role	Reference
Inositol metabolism				
IolG	P26935	*Myo*-inositol 2-dehydrogenase	*Myo*-inositol catabolism	[1]
	Q9WYP5	*Myo*-inositol 2-dehydrogenase	*Myo*-inositol catabolism	[2]
	O68965	*Myo*-inositol 2-dehydrogenase	*Myo*-inositol catabolism	[3]
	Q8NTY7	*Myo*-inositol 2-dehydrogenase	*Myo*-inositol catabolism	[4]
	P40332	*Scyllo*-inositol 2-dehydrogenase	*Scyllo*-inositol catabolism	[5]
	Q8NNS7	*Scyllo*-inositol 2-dehydrogenase	*Scyllo*-inositol catabolism	[4]
IolU	O05265	*Scyllo*-inositol 2-dehydrogenase	*Scyllo*-inositol catabolism	[6]
IolW	O32223	*Scyllo*-inositol 2-dehydrogenase	*Scyllo*-inositol catabolism	[6]
	Q8NTY4	*Scyllo*-inositol 2-dehydrogenase	*Scyllo*-inositol catabolism	[4]
Natural product biosynthesis				
KijD10	B3TMR8	dTDP-3,4-didehydro-2,6-dideoxy-alpha-D-glucose 3-reductase	L-Digitoxose biosynthesis	[7]
SpnN	Q9ALN5	dTDP-3,4-didehydro-2,6-dideoxy-alpha-D-glucose 3-reductase	Forosamine biosynthesis	[8]
OleW	Q9RR32	dTDP-3,4-didehydro-2,6-dideoxy-alpha-D-glucose 3-reductase	L-Oleandrose biosynthesis	[9]
IgdH	F0M433	Levoglucosan dehydrogenase	Levoglucosan biosynthesis	[10]
NtdC	O07564	Glucose-6-phosphate 3-dehydrogenase	Kanosamine biosynthesis	[11]
KanD2	Q6L737	Glucose-6-phosphate 3-dehydrogenase	Kanosamine biosynthesis	[12]
RifL	Q7BUE1	UDP-glucose dehydrogenase	Kanosamine biosynthesis	[13]
Irp3	W9BA38	Thiazolinyl imine reductase	Yersiniabactin biosynthesis	[14]
Carbohydrate metabolism				
NagA	A4Q8F7	α-N-Acetylgalactosaminidase	Blood group antigen degradation	[1-5][15]
	A4Q8G1	α-N-Acetylgalactosaminidase	Blood group antigen degradation	[15]
	Q8ECL7	α-N-Acetylgalactosaminidase	Blood group antigen degradation	[15]
AraA	Q53TZ2	L-arabinose 1-dehydrogenase	L-Arabinose degradation	[16]
Gal	P11886	Galactose 1-dehydrogenase	Galactose metabolism	[17]

ApsD	B1G894	Apiose dehydrogenase	D-Apiose catabolism	[18]
	B9JK80	Apiose dehydrogenase	D-Apiose catabolism	[18]
Xdh	Q8GAK6	D-Xylose 1-dehydrogenase	D-Xylose degradation	[19]
	Q5UY95	D-Xylose 1-dehydrogenase	D-Xylose catabolism	[24–26][20]
PLH35	T2KNC8	Oxidoreductase P35	Ulvan degradation	[21]
Gfo	A0A143PP41	Aldose-Aldose Oxidoreductase	Unknown	[22]
YjhC	P39353	2,7-anhydro-Neu5Ac dehydrogenase	Sialic acid metabolism	[23]
YcjS	P77503	Glucoside 3-dehydrogenase	Unknown carbohydrate catabolism	[24]
LPS biosynthesis				
WbpB	G3XD23	UDP-N-acetyl-2-amino-2-deoxy-D-glucuronate dehydrogenase	LPS biosynthesis.	[25–27]
WlbA	I6UWH1	UDP-N-acetyl-2-amino-2-deoxy-D-glucuronate dehydrogenase	LPS biosynthesis	[28]
GnnA	A0A543Q2K9	UDP-N-acetylglucosamine 3-dehydrogenase	LPS biosynthesis	[29]
Other functions				
PhtC	Q05184	Phthalate cis-4,5-dihydrodiol dehydrogenase	Cis-3,4-dihydrodiol phthalate catabolism	[30]
DgpA	A0A3Q9WWX8	C and O glycoside isomerase	Puerarin catabolism	[31]
LigC	Q9KWL3	4-carboxy-2-hydroxymuconate-6-semialdehyde dehydrogenase	Protocatechuate catabolism	[32]
Ddh	Q5L9Q6	*m*-Diaminopimelate dehydrogenase	L-Lysine biosynthesis	[33]

Table S2: Comparison of the hygromycin A biosynthetic cluster [34] and unknown hygromycin A-like cluster.

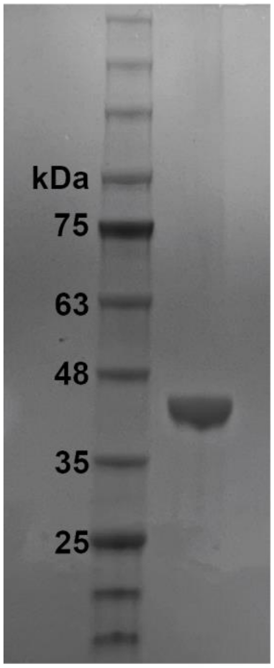
Protein	Annotated function	HygA biosynthetic role	% ID to unknown cluster
Hyg1	AfsR regulatory protein	Regulation	---
Hyg2	*p*-Hydroxybenzoate hydroxylase	Cinnamic acid	---
Hyg3	Regulatory protein	Regulation	---
Hyg4	Chorismate lyase	Cinnamic acid	---
Hyg5	Mannose dehydratase	Furanose	---
Hyg6	Methyltransferase	Aminocyclitol	56
Hyg7	Unknown	Aminocyclitol	69
Hyg8	Aminotransferase	Aminocyclitol	65
Hyg9	ACP	Cinnamic acid	31
Hyg10	b-ketoacyl synthase I	Cinnamic acid	50
Hyg11	Unknown	Unknown	---
Hyg12	CoA-ligase	Cinnamic acid	37
Hyg13	ACP	Cinnamic acid	
Hyg14	3-Hydroxyacyl ACP dehydratase	Cinnamic acid	34
Hyg15	3-Hydroxyacyl ACP reductase	Cinnamic acid	44
Hyg16	Glycosyltransferase	Furanose attachment	45
Hyg17	*Myo*-inositol dehydrogenase	Aminocyclitol	50
Hyg18	*Myo*-inositol-1-phosphate synthase	Aminocyclitol	---
Hyg19	Transmembrane protein	Unknown	---
Hyg20	Transglucosylase	Furanose	57
Hyg21	Phosphotransferase	Resistance	37
Hyg22	Acyltransferase	Cinnamic acid	---
Hyg23	Fucose synthase	Furanose	32
Hyg24	Unknown	Unknown	
Hyg25	*Myo*-inositol-1-phosphatase	Aminocyclitol	---
Hyg26	Short chain dehydrogenase	Furanose	---
Hyg27	DAHPS synthase	Cinnamic acid	---
Hyg28	ABC transporter	Unknown	---
Hyg29	Methyltransferase	Unknown	---

Table S3: Genomic neighborhood analysis of PF01408 sequences.

PFAM	Function	# Sequences with PFAM neighbors	# SSN clusters that contain PFAM
	NP biosynthetic enzymes		
PF00109	Ketoacyl synthase	584	45
PF00550	Acyl carrier protein (ACP) domain	340	71
PF00975	Thioesterase (TE) domain*	1,193	22
	Aminotransferases		
PF01041	DegT/DnrJ/EryCq/StrS aminotransferase	15,139	340
PF00202	Aminotransferase – class III	1,236	149
PF00155	Aminotransferase – class I and II	1,318	136
	Putative resistance proteins		
PF04655	Aminoglycoside antibiotic resistance kinase	22	4
PF02537	CrcB-like protein, Camphor Resistance	81	5
PF00903/ PF13660	Glyoxalase/Bleomycin resistance protein/Dioxygenase superfamily	1354	160
PF13536	Putative multidrug resistance efflux protein	2	2
PF05099	Tellurite resistance protein TerB	8	5

*TE domains only on their own. Some other TE domains are also found with ACP domains.

Figure S1: SDS-PAGE gel of purified Hyg17.



Expected MW
36 kDa

Figure S2: pH Activity profile for reactions with 10 mM *myo*-inositol, 10 mM NAD^+, 1 μM Hyg17, 100 mM HEPEs, 100 mM Tris, 100 mM CHES, and 100 mM CAPS, 50 mM NaCl, pH 9.5–11.

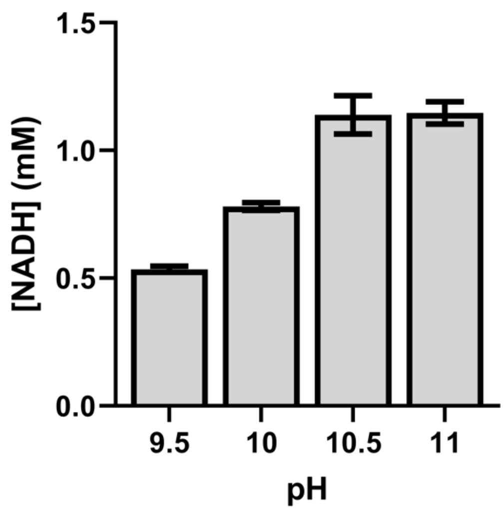
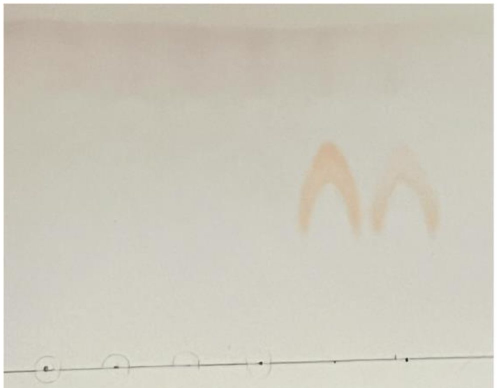


Figure S3: TLC analysis for Hyg17 and BsIDH reactions with *myo*-inositol and NAD$^+$. *p*-Anisaldehyde was used to stain ketones light pink.



-	+	-	+	+	+	NAD$^+$
-	-	+	+	+	+	*myo*-inositol
-	-	-	-	+	-	Hyg17
-	-	-	-	-	+	BsIDH

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