Identification of Purified Glyoxysome Proteins by Mass Spectrometry

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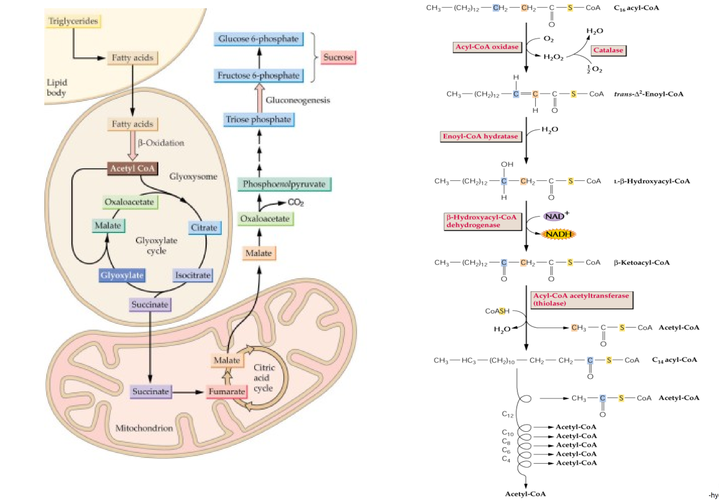
Abstract

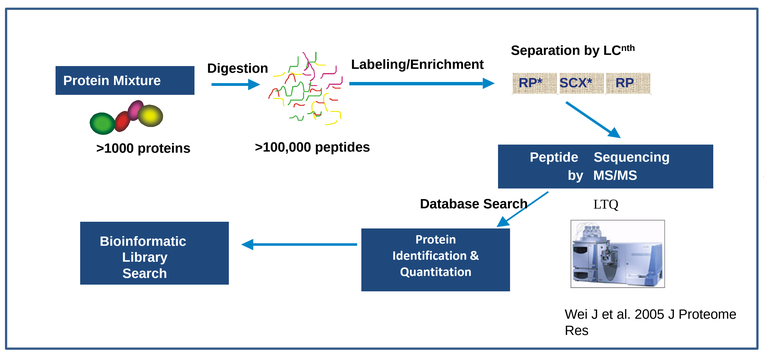
The purpose of this study was to construct a detailed and complete maize protein atlas. In maize cells, the glyoxysome, a specialized organelle, has the vital function of converting lipid storage into carbohydrates, which are needed for the growth of young plants. Our study constructed a protein atlas of 12,702 glyoxysome proteins. To our knowledge, this is the deepest proteome of any plant organelle. By comparing the abundance of proteins found in the glyoxysome and in the control tissue, enrichment values for each protein were calculated. The top 34 enriched proteins were considered to be glyoxysomal protein markers. While the majority of these proteins were previously known to be glyoxysome markers, 6 proteins were not previously known to be found in peroxisomes. These 6 proteins could be novel glyoxysome proteins.

Glyoxylate Cycle and β-Oxidation



The first step of oil breakdown in seeds is hydrolyzing triacylglycerides (TAG) into glycerol and fatty acids. The fatty acids then enter the glyoxysomes, where they undergo β-oxidation. The resulting Acetyl-CoA from β-oxidation enters the glyoxylate cycle, producing succinate, which is then transported to the mitochondrion and turned into malate for gluconeogenesis. Enzymes in above diagram were identified in our maize protein atlas. The figures above are from http://www.uky.edu/~dhild/biochem/21/fig10_68.png and http://3.bp.blogspot.com/-jwVHy4biomQ/T5IrWScMIiI/AAAAAAAAhM/QlZvNP6ZtvE/s1600/glyoxylatecycle.jpg

Methods



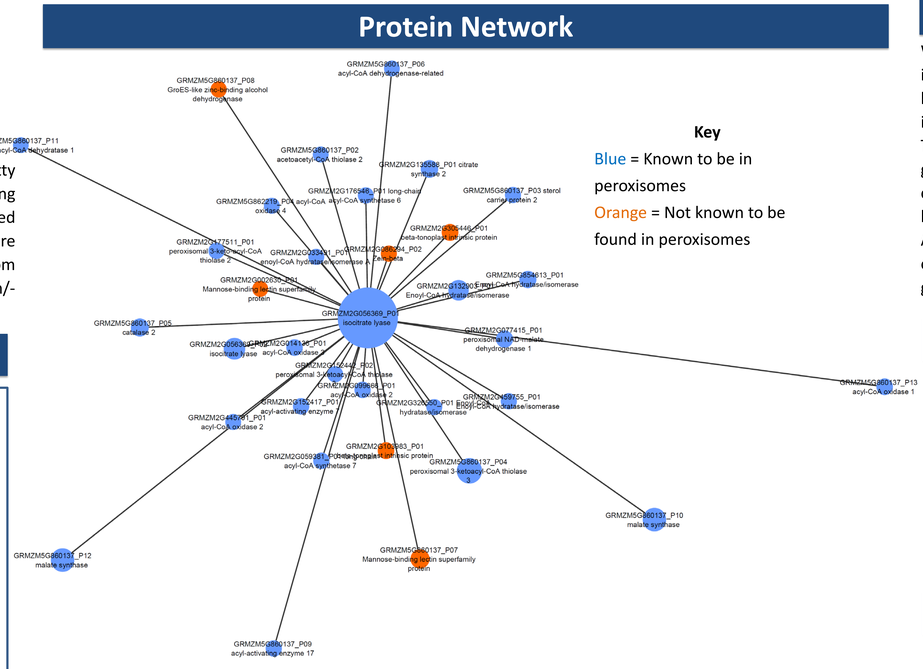
Glyoxysomes were purified from 3 day seedlings using ultracentrifgation in a sucrose gradient. Proteins were extracted, denatured, reduced, alkylated, and then digested by trypsin. Peptides were separated and analyzed using 2D nanoLC-MS/MS. Proteins/peptides were identified using Spectrum Mill. For relative quantitation, spectral counting was used. For protein functional analysis, we used MapMan ontology. Cytoscape was used to visualize the 34 marker proteins.

Table of 34 Glyoxysome Protein Markers

Accession	Glyoxysome	Scutella	Glyoxysome enrichment	Scutella enrichment	MapMan Term
GRMZM2G086294_P02	198.8	0.0	100.0%	0.0%	'not assigned.unknown'
GRMZM2G099666_P01	251.5	0.1	99.7%	0.0%	'lipid metabolism.lipid degradation.beta-oxidation.acyl CoA DH'
GRMZM2G056369_P01	12283.0	80.8	99.3%	0.7%	'gluconeogenese/ glyoxylate cycle.isocitrate lyase'
GRMZM2G033491_P01	160.7	0.5	99.3%	0.3%	'amino acid metabolism.degradation.aspartate family.lysine'
GRMZM2G152442_P02	177.0	0.8	99.2%	0.4%	#N/A
GRMZM2G152417_P01	270.0	0.9	99.1%	0.3%	'lipid metabolism.FA synthesis and FA elongation.acyl coa ligase'
GRMZM2G056369_P02	1687.8	19.1	98.9%	1.1%	'gluconeogenese/ glyoxylate cycle.isocitrate lyase'
GRMZM2G305446_P01	377.3	5.0	98.7%	1.3%	'transport.Major Intrinsic Proteins.TIP'
GRMZM2G326550_P01	155.3	0.5	98.7%	0.3%	#N/A
GRMZM2G014136_P01	314.4	1.4	98.6%	0.4%	'lipid metabolism.lipid degradation.beta-oxidation.acyl CoA DH'
GRMZM2G103983_P01	349.5	5.0	98.6%	1.4%	'transport.Major Intrinsic Proteins.TIP'
GRMZM2G077415_P01	474.6	4.6	98.5%	1.0%	'gluconeogenesis.Malate DH'
GRMZM2G132903_P01	1374.5	6.1	98.3%	0.4%	'lipid metabolism.lipid degradation.beta-oxidation.multifunctional'
GRMZM2G059381_P01	265.4	1.1	98.2%	0.4%	'lipid metabolism.FA synthesis and FA elongation.long chain fatty acid CoA ligase'
GRMZM2G176546_P01	178.9	0.6	97.3%	0.3%	'lipid metabolism.FA synthesis and FA elongation.long chain fatty acid CoA ligase'
GRMZM2G002630_P01	159.1	1.6	97.1%	1.0%	'misc.myrosinases-lectin-jacalin'
GRMZM5G862219_P04	136.6	0.2	96.7%	0.2%	'lipid metabolism.lipid degradation.beta-oxidation.acyl CoA DH'
GRMZM5G854613_P01	462.3	6.2	96.6%	1.3%	'lipid metabolism.lipid degradation.beta-oxidation.multifunctional'
GRMZM2G177511_P01	222.6	3.8	96.3%	1.6%	#N/A
GRMZM2G135588_P01	582.4	4.4	96.1%	0.7%	'gluconeogenese/ glyoxylate cycle.citrate synthase'
GRMZM2G459755_P01	208.8	2.8	96.0%	1.3%	'lipid metabolism.lipid degradation.beta-oxidation.multifunctional'
GRMZM2G445791_P01	136.4	3.5	95.7%	2.5%	'lipid metabolism.lipid degradation.beta-oxidation.acyl CoA DH'
GRMZM5G860137_P02	132.1	2.4	95.7%	1.8%	'amino acid metabolism.degradation.aromatic aa.tryptophan'
GRMZM2G150656_P03	207.5	7.8	94.5%	3.5%	'lipid metabolism."exotics"' (steroids, squalene etc)'
GRMZM5G848768_P02	2704.6	108.6	94.4%	3.8%	'lipid metabolism.lipid degradation.beta-oxidation.acyl-CoA thioesterase'
GRMZM2G088212_P01	691.3	13.3	92.5%	1.8%	'redox.dismutases and catalases'
GRMZM2G001297_P01	108.1	0.1	92.1%	0.1%	'lipid metabolism.lipid degradation.beta-oxidation.acyl CoA DH'
GRMZM2G112238_P01	1013.7	71.4	92.1%	6.5%	'misc.myrosinases-lectin-jacalin'
GRMZM2G175423_P01	221.2	15.3	91.6%	6.3%	'minor CHO metabolism.sugar alcohols'
GRMZM2G472376_P02	243.9	16.3	90.9%	6.1%	'lipid metabolism.FA synthesis and FA elongation.acyl coa ligase'
GRMZM2G102183_P02	2195.5	221.7	90.8%	9.2%	'gluconeogenese/ glyoxylate cycle.malate synthase'
GRMZM2G101457_P01	105.7	1.3	90.6%	1.1%	'lipid metabolism.lipid degradation.beta-oxidation.enoyl CoA hydratase'
GRMZM2G102183_P03	2195.5	227.9	90.6%	9.4%	'gluconeogenese/ glyoxylate cycle.malate synthase'
GRMZM5G864319_P01	318.5	0.6	90.1%	0.2%	'lipid metabolism.lipid degradation.beta-oxidation.acyl CoA DH'

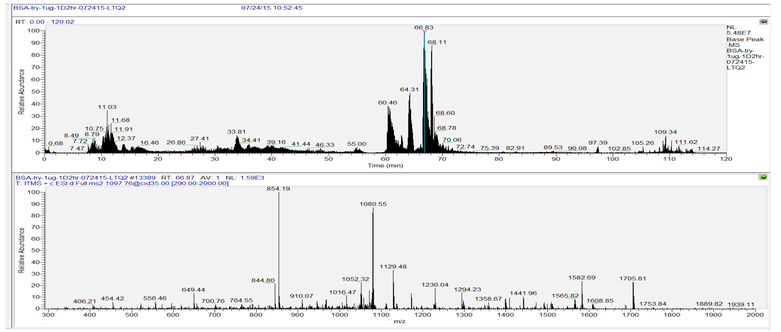
The top 34 enriched proteins in the glyoxysome are listed in the table above. Table includes the protein's accession, glyoxysome enrichment value, scutella (location in seed where glyoxysomes are found) enrichment value, and MapMan annotation. Protein must have enrichment of at least 90% among glyoxysome, chloroplast, and mitochondria to be considered protein marker for glyoxysome.

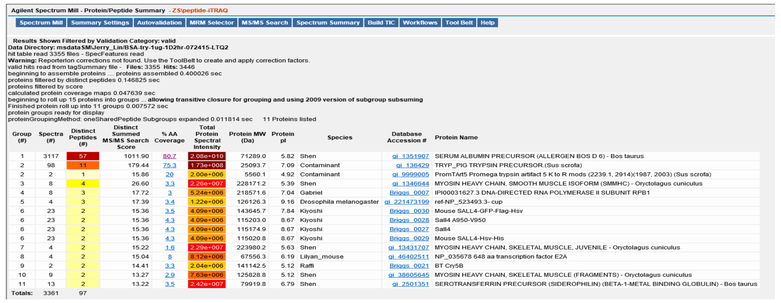
Protein Network



The protein network above uses isocitrate lyase as the marker protein for identifying the glyoxysomal proteins. Each node is labeled with a protein description and its accession. The size of the nodes corresponds with their abundance within the glyoxysome. The edge length corresponds with the glyoxysome enrichment value, computed by comparing the abundance within the glyoxysome and the abundance within the scutella. The blue nodes represent proteins previously known to be found in peroxisomes, and orange nodes represent those not previously known to be found in peroxisomes. Protein network visualized in Cytoscape.

Example LC-MS/MS Results





Agilent Spectrum Mill - Protein/Peptide Summary - ZSpeptide-iTRAQ

Spectrum Mill | Summary Settings | Autovalidation | MRM Selector | MS/MS Search | Spectrum Summary | Build TIC | Workflows | Tool Belt | Help

Results Shown Filtered by Validation Category: valid
Data Directory: msdata\MrJerry_Lin\BSA-try-1ug-1D2hr-072415-LTQ2
hit table read 3355 files - SpecFeatures read
Warning: Reporterion corrections not found. Use the ToolBelt to create and apply correction factors.
valid hits read from tagSummary file - **Files:** 3355 **Hits:** 3446
beginning to assemble proteins ... proteins assembled 0.406026 sec
proteins filtered by distinct peptides 0.146825 sec
proteins filtered by score
calculated protein coverage maps 0.047639 sec
beginning to roll up 15 proteins into groups ... **allowing transitive closure for grouping and using 2009 version of subgroup subsuming**
Finished protein roll up into 11 groups 0.007572 sec
protein groups ready for display
proteinGroupingMethod: oneSharedPeptide Subgroups expanded 0.011814 sec 11 Proteins listed

Group (#)	Spectra (#)	Distinct Peptides (#)	Distinct Summed MS/MS Search Score	% AA Coverage	Total Protein Spectral Intensity	Protein MW (Da)	Protein pI	Species	Database Accession #	Protein Name
1	3117	57	1011.90	80.7	2.05e+010	71289.0	5.82	Shen	gi_1351907	SERUM ALBUMIN PRECURSOR (ALLERGEN BOS D 6) - Bos taurus
2	98	11	179.44	75.3	1.73e+008	25093.7	7.09	Contaminant	gi_136429	TRYP_PIG TRYPSIN PRECURSOR (Sus scrofa)
3	2	1	15.86	20	2.00e+005	5560.1	4.92	Contaminant	gi_9999005	ProtnTAH5 Promega trypsin artifact 5 K to R mods (2230.1, 2914(x1987, 2003)) (Sus scrofa)
3	8	4	26.60	3.3	2.26e+007	228171.2	5.39	Shen	gi_1346644	MYOSIN HEAVY CHAIN, SMOOTH MUSCLE ISOFORM (SMMHC) - Oryctolagus cuniculus
4	8	3	17.72	3	5.24e+006	218571.6	7.04	Gabriel	Briggs_0007	IPI00031627.3 DNA-DIRECTED RNA POLYMERASE II SUBUNIT RPB1
5	4	3	17.39	3.4	1.22e+006	126126.3	9.16	Drosophila melanogaster	gi_221473199	ref-NP_523493.3- cup
6	23	2	15.36	3.5	8.09e+006	143645.7	7.84	Kiyoshi	Briggs_0030	Mouse SALL4 GFP-Flag-Hsv
6	23	2	15.36	4.3	4.09e+006	115203.0	8.67	Kiyoshi	Briggs_0028	Sall4 A950-Y950
6	23	2	15.36	4.3	4.09e+006	115174.9	8.67	Kiyoshi	Briggs_0027	Sall4
6	23	2	15.36	4.3	4.09e+006	115020.8	8.67	Kiyoshi	Briggs_0029	Mouse SALL4 Hsv-His
7	4	2	15.22	1.6	2.29e+007	223980.2	5.63	Shen	gi_13431707	MYOSIN HEAVY CHAIN, SKELETAL MUSCLE, JUVENILE - Oryctolagus cuniculus
8	4	2	15.04	8	8.32e+006	67556.3	6.19	Livan_mouse	gi_46402511	NP_035678.648 aa transcription factor E2A
9	2	2	14.41	3.3	2.04e+006	141142.5	5.12	Raffi	Briggs_0021	BT Cry5B
10	9	2	13.27	2.9	7.83e+006	125828.8	5.12	Shen	gi_38605645	MYOSIN HEAVY CHAIN, SKELETAL MUSCLE (FRAGMENTS) - Oryctolagus cuniculus
11	13	2	13.22	3.5	2.42e+007	79919.8	6.79	Shen	gi_2501351	SEROTRANSFERRIN PRECURSOR (SIDEROPHILIN) (BETA-1-METAL BINDING GLOBULIN) - Bos taurus
Totals:	3361	97								

Discussion and Conclusions

We succeeded in detecting 12,702 proteins in the glyoxysome, and 34 marker proteins. Of the 34 proteins identified, 6 proteins were not known to be found in peroxisomes. Of the 6 proteins, there were 4 different kinds: zein-beta, manose-binding lectin, GroEs-like zinc-binding alcohol dehydrogenase, and beta-tonoplast intrinsic protein. One possibility that could lead to these 6 proteins appearing in the list is contamination. The proteins could have copurified while in the sucrose gradient and were eluted with the rest of the glyoxysomal proteins. The other possibility is that these proteins are novel proteins. The zein-beta protein could be in the glyoxysome, acting as a scaffold protein to regulate pathways within the glyoxysome. The lectin proteins could be a form of passive protection against predators wanting the nutritional lipid storage. Alcohol dehydrogenase may help to recycle NAD+ within the glyoxysome, which is needed for fatty acid β-oxidation. Future research will need to be done to determine whether these proteins are actually glyoxysomal , and if so, what their functions are.

References

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