

SUPPLEMENTARY MATERIALS

Metabolomic analysis of *Trichophyton rubrum* and *Microsporum canis* during keratin degradation

Anita CIESIELSKA¹, Anna KAWA¹, Katarzyna KANAREK¹, Adrian SOBÓŃ¹, Rafał SZEWCZYK²

¹ Department of Molecular Microbiology, Faculty of Biology and Environmental Protection, University of Łódź, Poland

² LabExperts sp. z o.o., Gdańsk, Poland

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Table S1 List of metabolites used in this study

Table S2 LC-MS/MS parameters used for analysis of intracellular metabolites of *T. rubrum* and *M. canis*

Table S3 MS/MS parameters and LC-MS/MS method performance for compound standards

Table S1 List of metabolites used in this study

1	5-aminovalerate	21	FAD	41	Inosine	61	L- α -Amino adipic acid	81	Tartrate
2	Acetyl-CoA	22	Fumarate	42	Kynurenic acid	62	Malate	82	Thiamine
3	Adenine	23	G6P	43	L-Alanine	63	Malonate	83	Tocopherol
4	Adenosine	24	GABA	44	L-Arginine	64	Melatonin	84	UDP
5	Adipate	25	GDP	45	L-Asparagine	65	Myoinositol	85	UDP-glucose
6	ADP	26	Gluconate	46	L-Aspartic acid	66	NAD	86	UMP
7	AMP	27	Glucuronate	47	L-Cysteine	67	NADP	87	Uracil
8	ATP	28	Glutathione oxidized	48	L-Glutamic acid	68	Nicotinic acid	88	Uridine
9	Betaine	29	Glutathione reduced	49	L-Glutamine	69	Ornithine	89	UTP
10	Biotin	30	GMP	50	L-Histidine	70	Oxaloacetate	90	α -ketoglutarate
11	CDP	31	GTP	51	L-Leucine	71	P5P		
12	Cis-aconitate	32	Guanidine acetate	52	L-Lysine	72	PABA		
13	Citrate	33	Guanosine	53	L-Methionine	73	Pantothenic acid		
14	CMP	34	Homocysteine	54	L-Phenylalanine	74	PEP		
15	CTP	35	Homocysteine thiolactone	55	L-Proline	75	Pyridoxine		
16	Cyanocobalamin	36	Homocysteine	56	L-Serine	76	Quinate		
17	Cystine	37	Homoserine	57	L-Threonine	77	R5P		
18	Cytidine	38	Hydroxy-L-proline	58	L-Tryptophan	78	Riboflavin		
19	Diaminopimelic acid	39	Hypoxanthine	59	L-Tyrosine	79	Succinic acid		
20	F16bP	40	IMP	60	L-Valine	80	Succinyl-CoA		

Table S2 LC-MS/MS parameters used for analysis of intracellular metabolites of *T. rubrum* and *M. canis*

LC parameters				
	Column 1: Eksigent 3C8-EP-120	Column 2: Eksigent C318-AQ-120	Column 3: Synergi Hydro-RP	
Injection volume	2 µl	2 µl	10 µl	
Column temperature	45°C	40°C	40°C	
MS/MS parameters				
Polarity	positive	negative	negative	
CUR (Curtain gas)	25	25	30	
CAD (Collision gas)	medium	medium	medium	
IS (Ion spray voltage)	5000 V	-4500 V	-4500 V	
TEM (Source temperature)	400°C	400°C	500°C	
GS1 (Nebulizer gas)	28	28	50	
GS2 (Drying gas)	40	40	40	
ihe	ON	ON	ON	
GRADIENT ANF FLOW OF THE MOBILE PHASES				
step	running time [min]	flow [µl/min]	mobile phase A [%]	mobile phase B [%]
Column 1				
1	0	50	98	2
2	0.2	50	98	2
3	2.2	50	5	95
4	3.4	50	5	95
5	3.5	50	98	2
6	4	50	98	2
Column 2				
1	0	50	98	2
2	0.1	50	98	2
3	0.5	50	70	30
4	2.5	50	2	98
5	3.5	50	2	98
6	4	50	98	2
Column 3				
1	0	600	100	0
2	0.2	600	100	0
3	5	600	5	95
4	7	600	5	95
5	7.1	600	100	0
6	15	600	100	0

Table S3 MS/MS parameters and LC-MS/MS method performance for compound standards

Metabolite Name	Q1	Q3	DP	EP	CE	CXP	RT (min)	Linearity Range (ng/ml) ¹	R	Column*
L- α -Aminoadypic acid_1	162.0	98.1	51	10	25	8	0.42	0.5-100	0.9996	1
L- α -Aminoadypic acid_2	162.0	127.1	51	10	21	8	0.42			1
L- α -Aminoadypic acid_3	162.0	144.1	51	10	21	6	0.42			1
Adenosine_1	268.0	136.0	66	10	35	10	0.55	0.25-100	0.9960	1
Adenosine_2	268.0	131.2	66	10	21	6	0.55			1
Adenosine_3	268.0	119.1	66	10	35	4	0.55			1
ADP_1	426.0	78.8	-95	-10	-23	-11	0.54	10-1000	0.9916	3
ADP_2	426.0	95.0	-95	-10	-13	-9	0.54			3
ADP_3	426.0	158.6	-95	-10	-29	-9	0.54			3
L-Alanine_1	90.1	44.0	31	10	19	6	0.39	25-250	0.9974	1
L-Alanine_2	90.1	49.0	31	10	21	5	0.39			1
L-Alanine_3	90.1	45.0	31	10	31	6	0.39			1
AMP_1	345.9	78.8	-100	-10	-21	-13	0.64	5-1000	0.9946	3
AMP_2	345.9	79.0	-100	-10	-47	-21	0.64			3
AMP_3	345.9	96.8	-100	-10	-14	-29	0.64			3
L-Arginine_1	175.1	70.2	46	10	13	8	0.34	1-100	0.9988	1
L-Arginine_2	175.1	70.1	46	10	19	12	0.34			1
L-Arginine_3	175.1	60.1	46	10	25	6	0.34			1
L-Asparagine_1	133.1	87.0	46	10	15	8	0.39	2.5-100	0.9951	1
L-Asparagine_2	133.1	85.0	46	10	39	6	0.39			1
L-Asparagine_3	133.1	74.1	46	10	15	4	0.39			1
L-Aspartic acid_1	134.1	88.1	51	10	39	12	0.39	2.5-100	0.9972	1
L-Aspartic acid_2	134.1	81.1	51	10	13	8	0.39			1
L-Aspartic acid_3	134.1	74.0	51	10	21	6	0.39			1

ATP_1	506.0	158.7	-15	-10	-15	-5	0.51	25-1000	0.9928	3
ATP_2	506.0	102.1	-15	-10	-27	-10	0.51			3
ATP_3	506.0	78.8	-15	-10	-17	-21	0.51			3
Betaine_1	118.0	58.0	76	10	41	16	0.41	1-50	0.9972	1
Betaine_2	118.0	50.1	76	10	21	12	0.41			1
Betaine_3	118.0	59.1	76	10	55	4	0.41			1
Cis-aconitate_1	172.8	85.0	-20	-10	-15	-5	0.95	2.5-1000	0.9995	2
Cis-aconitate_2	172.8	98.1	-20	-10	-15	-8	0.95			2
Cis-aconitate_3	172.8	129.0	-20	-10	-33	-7	0.95			2
Citrate_1	190.8	84.9	-15	-10	-35	-9	0.48	5-250	0.9889	3
Citrate_2	190.8	75.2	-15	-10	-19	-4	0.48			3
Citrate_3	190.8	86.9	-15	-10	-23	-7	0.48			3
CMP_1	321.9	78.8	-70	-10	-17	-1	0.51	10-1000	0.9965	3
CMP_2	321.9	100.2	-70	-10	-26	-4	0.51			3
CMP_3	321.9	96.9	-70	-10	-19	-7	0.51			3
L-Cysteine_1	122.0	59.1	61	10	27	6	0.4	1-100	0.9974	1
L-Cysteine_2	122.0	73.9	61	10	25	6	0.4			1
L-Cysteine_3	122.0	76.1	61	10	21	6	0.4			1
Cytidine_1	244.0	112.1	16	10	15	6	0.39	0.25-100	0.9963	1
Cytidine_2	244.0	96.1	16	10	39	12	0.39			1
Cytidine_3	244.0	94.9	16	10	27	12	0.39			1
Diaminopimelic acid_1	191.1	128.1	61	10	13	12	0.36	1-100	0.9998	1
Diaminopimelic acid_2	191.1	78.3	61	10	47	17	0.36			1
Diaminopimelic acid_3	191.1	82.0	61	10	21	6	0.36			1
F16bP_1	338.9	78.8	-15	-10	-15	-5	0.47	5-1000	0.9916	3
F16bP_2	338.9	96.5	-15	-10	-19	-19	0.47			3
F16bP_3	338.9	96.8	-15	-10	-39	-7	0.47			3

Fumarate_1	114.9	70.9	-35	-10	-31	-5	0.88	1-100	0.9970	2
Fumarate_2	114.9	96.8	-35	-10	-37	-17	0.88			2
Fumarate_3	114.9	59.1	-35	-10	-16	-5	0.88			2
G6P_1	258.9	96.8	-70	-10	-26	-7	0.5	5-1000	0.9986	3
G6P_2	258.9	88.1	-70	-10	-19	-17	0.5			3
G6P_3	258.9	78.8	-70	-10	-27	-5	0.5			3
GABA_1	104.1	86.0	30	4	39	5	0.37	5-250	0.9977	1
GABA_2	104.1	158.7	30	4	15	9	0.37			1
GABA_3	104.1	69.0	30	4	13	6	0.37			1
GDP_1	441.9	78.9	-90	-10	-27	-7	0.54	10-1000	0.9944	3
GDP_2	441.9	59.1	-90	-10	-43	-5	0.54			3
GDP_3	441.9	158.8	-90	-10	-15	-9	0.54			3
Gluconate_1	194.9	128.9	-65	-10	-23	-9	0.49	2.5-1000	0.9931	2
Gluconate_2	194.9	86.9	-65	-10	-27	-9	0.49			2
Gluconate_3	194.9	74.9	-65	-10	-67	-7	0.49			2
Glucuronate_1	192.8	112.9	-50	-10	-23	-7	0.49	2.5-1000	0.9971	2
Glucuronate_2	192.8	94.9	-50	-10	-19	-6	0.49			2
Glucuronate_3	192.8	73.0	-50	-10	-30	-5	0.49			2
L-Glutamic acid_1	148.0	84.0	46	10	45	6	0.4	1-100	0.9964	1
L-Glutamic acid_2	148.0	78.8	46	10	15	7	0.4			1
L-Glutamic acid_3	148.0	102.0	46	10	39	6	0.4			1
L-Glutamine_1	147.1	84.1	36	10	29	4	0.4	1-100	0.9924	1
L-Glutamine_2	147.1	101.1	36	10	24	2	0.4			1
L-Glutamine_3	147.1	101.1	36	10	19	8	0.4			1
Glutathione oxidized_1	611.1	305.9	-105	-10	-57	-11	0.86	1-250	0.9894	2
Glutathione oxidized_2	611.1	78.7	-105	-10	-17	-12	0.86			2
Glutathione oxidized_3	611.1	271.8	-105	-10	-39	-9	0.86			2

Glutathione reduced_1	305.9	142.8	-60	-10	-17	-11	0.75	2.5-1000	0.9966	2
Glutathione reduced_2	305.9	111.2	-60	-10	-55	-12	0.75			2
Glutathione reduced_3	305.9	271.9	-60	-10	-15	-13	0.75			2
GMP_1	361.9	78.8	-15	-10	-25	-11	0.55	5-1000	0.9947	3
GMP_2	361.9	96.0	-15	-10	-92	-10	0.55			3
GMP_3	361.9	96.9	-15	-10	-50	-7	0.55			3
GTP_1	521.9	78.9	-80	-10	-44	-13	0.48	25-1000	0.9973	3
GTP_2	521.9	57.1	-80	-10	-44	-12	0.48			3
GTP_3	521.9	423.9	-80	-10	-29	-13	0.48			3
Guanosine_1	283.9	152.1	6	10	19	8	0.66	0.1-100	0.9998	1
Guanosine_2	283.9	158.7	6	10	21	9	0.66			1
Guanosine_3	283.9	135.0	6	10	39	8	0.66			1
L-Histidine_1	156.0	110.0	61	10	27	14	0.33	2.5-100	0.9956	1
L-Histidine_2	156.0	78.9	61	10	50	12	0.33			1
L-Histidine_3	156.0	83.1	61	10	35	12	0.33			1
Hydroxy-L-proline_1	132.0	86.0	1	10	19	8	0.4	2.5-100	0.9947	1
Hydroxy-L-proline_2	132.0	73.0	1	10	25	4	0.4			1
Hydroxy-L-proline_3	132.0	68.0	1	10	21	4	0.4			1
Hypoxanthine_1	136.9	110.1	31	10	21	8	0.59	0.5-100	0.9992	1
Hypoxanthine_2	136.9	100.8	31	10	35	5	0.59			1
Hypoxanthine_3	136.9	94.0	31	10	21	6	0.59			1
IMP_1	347.1	347.0	-10	-10	-35	-13	0.58	25-1000	0.9847	3
IMP_2	347.1	57.1	-10	-10	23	-11	0.58			3
IMP_3	347.1	78.9	-10	-10	13	-7	0.58			3
Inosine_1	268.9	137.0	101	10	29	12	0.62	0.25-100	0.9981	1
Inosine_2	268.9	78.8	101	10	19	9	0.62			1
Inosine_3	268.9	119.1	101	10	21	8	0.62			1

L-Leucine_1	132.1	85.9	51	10	31	10	0.52	2.5-100	0.9962	1
L-Leucine_2	132.1	158.7	51	10	21	10	0.52			1
L-Leucine_3	132.1	69.3	51	10	47	8	0.52			1
Kynurenic acid_1	190.1	144.0	55	8	14	5	1.3	0.1-100	0.9992	1
Kynurenic acid_2	190.1	70.0	55	8	13	7	1.3			1
Kynurenic acid_3	190.1	89.0	55	8	19	7	1.3			1
L-Lysine_1	147.2	84.2	36	10	25	6	0.33	2.5-100	0.9979	1
L-Lysine_2	147.2	113.0	36	10	15	7	0.33			1
L-Lysine_3	147.2	67.0	36	10	39	10	0.33			1
Malate_1	132.9	114.9	-15	-10	15	-9	0.65	5-1000	0.9972	2
Malate_2	132.9	70.0	-15	-10	39	-4	0.65			2
Malate_3	132.9	70.9	-15	-10	13	-5	0.65			2
L-Methionine_1	150.1	104.1	51	10	21	8	0.47	1-100	0.9981	1
L-Methionine_2	150.1	96.0	51	10	15	8	0.47			1
L-Methionine_3	150.1	133.1	51	10	27	6	0.47			1
Myoinositol_1	178.8	86.9	-80	-10	17	-7	0.48	5-1000	0.9971	2
Myoinositol_2	178.8	96.9	-80	-10	41	-7	0.48			2
Myoinositol_3	178.8	71.0	-80	-10	21	-5	0.48			2
NAD_1	662.0	540.0	-75	-10	55	-7	1.39	2.5-1000	0.9968	3
NAD_2	662.0	323.0	-75	-10	15	-7	1.39			3
NAD_3	662.0	78.9	-75	-10	15	-7	1.39			3
Nicotinic acid_1	124.0	79.9	26	10	33	8	0.49	1-100	0.9990	1
Nicotinic acid_2	124.0	78.9	26	10	35	11	0.49			1
Nicotinic acid_3	124.0	53.0	26	10	19	14	0.49			1
Oxaloacetate_1	131.0	86.9	-55	-10	23	-5	0.5	50-500	0.9966	3
Oxaloacetate_2	131.0	91.0	-55	-10	17	-10	0.5			3
Oxaloacetate_3	131.0	112.9	-55	-10	26	-7	0.5			3

PABA_1	138.0	77.0	56	10	19	10	1.16	0.1-100	0.9999	1
PABA_2	138.0	137.0	56	10	27	6	1.16			1
PABA_3	138.0	94.0	56	10	19	8	1.16			1
Pantothenic acid_1	217.9	87.8	-70	-10	-18	-7	1	0.5-100	0.9980	2
Pantothenic acid_2	217.9	165.0	-70	-10	-27	-7	1			2
Pantothenic acid_3	217.9	146.0	-70	-10	-22	-7	1			2
PEP_1	166.8	78.8	-40	-10	-44	-7	0.48	2.5-1000	0.9992	3
PEP_2	166.8	122.1	-40	-10	-52	-9	0.48			3
PEP_3	166.8	62.8	-40	-10	-90	-3	0.48			3
L-Phenylalanine_1	166.1	120.1	26	10	17	10	0.64	1-100	0.9988	1
L-Phenylalanine_2	166.1	143.9	26	10	22	11	0.64			1
L-Phenylalanine_3	166.1	103.0	26	10	39	10	0.64			1
Piridoxine_1	170.0	152.1	36	10	19	6	0.43	0.5-25	0.9963	1
Piridoxine_2	170.0	78.9	36	10	45	8	0.43			1
Piridoxine_3	170.0	134.0	36	10	27	4	0.43			1
L-Proline_1	116.0	70.1	36	10	19	8	0.42	1-100	0.9981	1
L-Proline_2	116.0	86.9	36	10	53	5	0.42			1
L-Proline_3	116.0	68.0	36	10	39	4	0.42			1
R5P_1	228.9	96.9	-65	-10	-18	-5	0.5	2.5-1000	0.9944	3
R5P_2	228.9	86.9	-65	-10	-22	-9	0.5			3
R5P_3	228.9	78.9	-65	-10	-52	-11	0.5			3
Riboflavin_1	377.1	243.0	135	10	44	11	1.15	0.25-250	0.9997	1
Riboflavin_2	377.1	382.4	135	10	22	10	1.15			1
Riboflavin_3	377.1	172.1	135	10	44	11	1.15			1
L-Serine_1	106.0	60.1	1	10	15	4	0.39	5-100	0.9968	1
L-Serine_2	106.0	71.0	1	10	67	7	0.39			1
L-Serine_3	106.0	42.0	1	10	33	10	0.39			1

Succinic acid_1	116.8	73.0	-35	-10	-16	-5	0.89	5-500	0.9927	2
Succinic acid_2	116.8	78.8	-35	-10	-43	-7	0.89			2
Succinic acid_3	116.8	98.9	-35	-10	-14	-9	0.89			2
L-Threonine_1	265.0	122.1	36	10	25	4	0.32	0.25-100	0.9979	1
L-Threonine_2	265.0	143.9	36	10	21	12	0.32			1
L-Threonine_3	265.0	57.1	36	10	25	6	0.32			1
L-Tryptophan_1	205.1	188.0	41	10	15	8	0.98	1-100	0.9999	1
L-Tryptophan_2	205.1	86.9	41	10	34	9	0.98			1
L-Tryptophan_3	205.1	146.1	41	10	25	10	0.98			1
L-Tyrosine_1	182.0	165.0	36	10	15	6	0.57	0.5-100	0.9993	1
L-Tyrosine_2	182.0	112.1	36	10	76	7	0.57			1
L-Tyrosine_3	182.0	91.0	36	10	39	6	0.57			1
UDP_1	403.0	78.7	-60	-10	-100	-5	0.51	10-1000	0.9981	3
UDP_2	403.0	128.1	-60	-10	-67	-9	0.51			3
UDP_3	403.0	158.7	-60	-10	-32	-5	0.51			3
UDP-glucose_1	565.0	323.0	-90	-10	-32	-11	0.52	2.5-250	0.9974	3
UDP-glucose_2	565.0	437.0	-90	-10	-11	-13	0.52			3
UDP-glucose_3	565.0	78.9	-90	-10	-112	-7	0.52			3
UMP_1	322.8	78.9	-90	-10	-100	-7	0.51	10-1000	0.9973	3
UMP_2	322.8	96.8	-90	-10	-98	-5	0.51			3
UMP_3	322.8	96.9	-90	-10	-26	-7	0.51			3
Uracil_1	113.0	96.0	41	10	23	6	0.55	5-100	0.9990	1
Uracil_2	113.0	128.9	41	10	43	11	0.55			1
Uracil_3	113.0	70.0	41	10	19	16	0.55			1
Uridine_1	244.9	113.0	31	10	30	10	0.56	0.5-100	0.9988	1
Uridine_2	244.9	70.9	31	10	13	9	0.56			1
Uridine_3	244.9	70.0	31	10	45	6	0.56			1

UTP_1	482.9	158.7	-85	-10	-34	-5	0.5	10-1000	0.9954	3
UTP_2	482.9	96.8	-85	-10	-55	-8	0.5			3
UTP_3	482.9	78.8	-85	-10	-102	-5	0.5			3
L-Valine_1	118.1	57.1	66	10	39	14	0.44	5-250	0.9946	1
L-Valine_2	118.1	57.1	66	10	43	9	0.44			1
L-Valine_3	118.1	78.9	66	10	89	11	0.44			1
α-ketoglutarate_1	144.9	100.8	-5	-10	-12	-3	0.49	10-250	0.9973	3
α-ketoglutarate_2	144.9	99.3	-5	-10	-17	-11	0.49			3
α-ketoglutarate_3	144.9	73.0	-5	-10	-18	-7	0.49			3

¹ First MRM transition was chosen for quantitative analysis, second was confirmatory transition.

* Column 1: Eksigent 3C8-EP-120 (0.5 × 150 mm, 3 μ m, Eksigent);

Column 2: Eksigent 3C18-AQ-120 (0.5 × 150 mm, 3 μ m, Eksigent);

Column 3: Synergi Hydro-RP column (2 × 150 mm, 4 μ m, Phenomenex)

