SUPPLEMENTARY INFORMATION

Title

Metagenomic insights into functional and taxonomic compositions of an activated sludge microbial community treating leachate of a completed landfill: a pathway-based analysis

Authors

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FIGURE LEGENGS

Table S1 Classification and application of valuable products.

Table S2 Carotenoids and terpenoids biosynthesis related functional genes.

Fig. S1 Fifty most abundant microbial species in the activated sludge sample and read numbers.

Fig. S2 Terpenoid backbone biosynthesis pathways. An arrow of continuous line shows biosynthesis pathways. Chemical substances in boxes are generated materials by metabolism. Boxes colored in green indicates that the metabolism reaction is taken place. Functional genes and the hit number are shown sideways of the arrow. Circle size indicates sizes of the hit number.

Fig. S3 Gene-mapping to KEGG reference pathways for Ectoine.

Fig. S4 Gene-mapping to KEGG reference pathways for PHB, Acetoin, and 2,3-butanediol.

Fig. S5 Gene-mapping to KEGG reference pathways for Zeaxanthin, Astaxanthin, and Lutein.

Fig. S6 Gene-mapping to KEGG reference pathways of Terpenoid backbone biosynthesis

Table S1

Valuable products	Classification	Application	Citation
PHB (polyhydroxybutylate)	Biocompatible and biodegradable resin	Bottles Suture threads	Anderson et al, 1990 Levett et al, 2016
ectoine	Osmolytes	Cosmetics	Berry et al, 2003 Cantera et a, 2016
lutein	Carotenoids	Supplements for eyes	Zhang, 2018
zeaxanthin	Carotenoids	Supplements for eyes	Zhang, 2018
astaxanthin	Carotenoids	Supplements for eyes	Zhang, 2018
acetoin	Hydroxyketone	Food additives	Xiao and Lu, 2014
2,3-butanediol	Diol	Platform chemical (Raw materials of MEK and 1,3-butadiene)	Celińska et al, 2009

Table S2

Catergory	KeggID	Gene Name	Name	EC No.
Carotenoid	K02291	*crtB*	15-cis-phytoene synthase	EC:2.5.1.32
	K02293	*PDS*	15-cis-phytoene desaturase	EC:1.3.5.5
	K09836	*crtW*	beta-carotene/zeaxanthin 4-ketolase	EC:1.14.99.641.14.99.63
	K15746	*crtZ*	beta-carotene 3-hydroxylase	EC:1.14.15.24
	K01823	*idi*	isopentenyl-diphosphate Delta-isomerase	EC:5.3.3.2
	K00795	*ispA*	farnesyl diphosphate synthase	EC:2.5.1.10 2.5.1.1
	K13789	*GGPS*	geranylgeranyl diphosphate synthase, type II	EC:2.5.1.292.5.1.10 2.5.1.1
	K13787	*idsA*	geranylgeranyl diphosphate synthase, type I	EC:2.5.1.292.5.1.10 2.5.1.1
Terpenoids	K01662	dxs	1-deoxy-D-xylulose-5-phosphate synthase	EC:2.2.1.7
	K00099	ispC	1-deoxy-D-xylulose-5-phosphate reductoisomerase	EC:1.1.1.267
	K00991	ispD	2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase	EC:2.7.7.60
	K12506	ispDF	2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase / 2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase	EC:4.6.1.12 2.7.7.60
	K00919	ispE	4-diphosphocytidyl-2-C-methyl-D-erythritol kinase	EC:2.7.1.148
	K01770	ispF	2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase	EC:4.6.1.12
	K03526	gcpE	(E)-4-hydroxy-3-methylbut-2-enyl-diphosphate synthase	EC:1.17.7.3 1.17.7.1
	K03527	ispH	4-hydroxy-3-methylbut-2-en-1-yl diphosphate reductase	EC:1.17.7.4
	K00626	E2.3.1.9	acetyl-CoA C-acetyltransferase	EC:2.3.1.9
	K01641	E2.3.3.10	hydroxymethylglutaryl-CoA synthase	EC:2.3.3.10
	K00021	HMGCR	hydroxymethylglutaryl-CoA reductase (NADPH)	EC:1.1.1.34
	K00054	mvaA	hydroxymethylglutaryl-CoA reductase	EC:1.1.1.88
	K00869	E2.7.1.36	mevalonate kinase	EC:2.7.1.36
	K00938	E2.7.4.2	phosphomevalonate kinase	EC:2.7.4.2
	K01597	MVD	diphosphomevalonate decarboxylase	EC:4.1.1.33
	K01823	idi	isopentenyl-diphosphate Delta-isomerase	EC:5.3.3.2


Alphaproteobacteria, Methyloceanibacter caenitepidi
Alphaproteobacteria, Rhodopseudomonas palustris
Gammaproteobacteria, Pseudomonas stutzeri
Gammaproteobacteria, Pseudomonas fluorescens
Gammaproteobacteria, Ralstonia solanacearum
Gammaproteobacteria, Burkholderia cenocepacia
Polyangia, Sorangium cellulosum
Gammaproteobacteria, Pseudomonas putida
Actinobacteria, Amycolatopsis mediterranei
Alphaproteobacteria, Rhizobium leguminosarum
Alphaproteobacteria, Bradyrhizobium diazoefficiens
Alphaproteobacteria, Rhodobacter sphaeroides
Gammaproteobacteria, Burkholderia cepacia
Alphaproteobacteria, Azospirillum brasilense
Gammaproteobacteria, Pseudomonas aeruginosa
Alphaproteobacteria, Mesorhizobium ciceri
Alphaproteobacteria, Methylorubrum extorquens
Gammaproteobacteria, Achromobacter xylosoxidans
Gammaproteobacteria, Burkholderia multivorans
Alphaproteobacteria, Blastochloris viridis
Myxococcia, Anaeromyxobacter dehalogenans
Myxococcia, Myxococcus fulvus
Nitrospira, Nitrospira moscoviensis
Thaumarchaeota, Nitrosopumilus adriaticus
Alphaproteobacteria, Azospirillum lipoferum
Chlamydiia, Simkania negevensis
Gammaproteobacteria, Bordetella bronchiseptica
Thaumarchaeota, Candidatus Nitrosopumilus sediminis
Gammaproteobacteria, Stenotrophomonas maltophilia
Gammaproteobacteria, Burkholderia ubonensis
Alphaproteobacteria, Rhizobium etli
Gammaproteobacteria, Variovorax paradoxus
Alphaproteobacteria, Oligotropha carboxidovorans
Alphaproteobacteria, Bradyrhizobium japonicum
Actinobacteria, Streptomyces hygroscopicus
Actinobacteria, Streptomyces albus
Gammaproteobacteria, Aeromonas hydrophila
Gammaproteobacteria, Burkholderia thailandensis
Alphaproteobacteria, Hyphomicrobium denitrificans
Gammaproteobacteria, Azotobacter vinelandii
Alphaproteobacteria, Rhodovulum sulfidophilum
Gammaproteobacteria, Burkholderia vietnamiensis
Gammaproteobacteria, Pseudomonas citronellolis
Gammaproteobacteria, Burkholderia glumae
Gammaproteobacteria, Serratia marcescens
Alphaproteobacteria, Sinorhizobium meliloti
Thermoleophilia, Conexibacter woesei
Polyangia, Haliangium ochraceum
Actinobacteria, Mycobacterium avium
Gammaproteobacteria, Pseudomonas chlororaphis
0.0
0.3
0.6
0.9
1.2
Relative abundance [%]

Figure S1

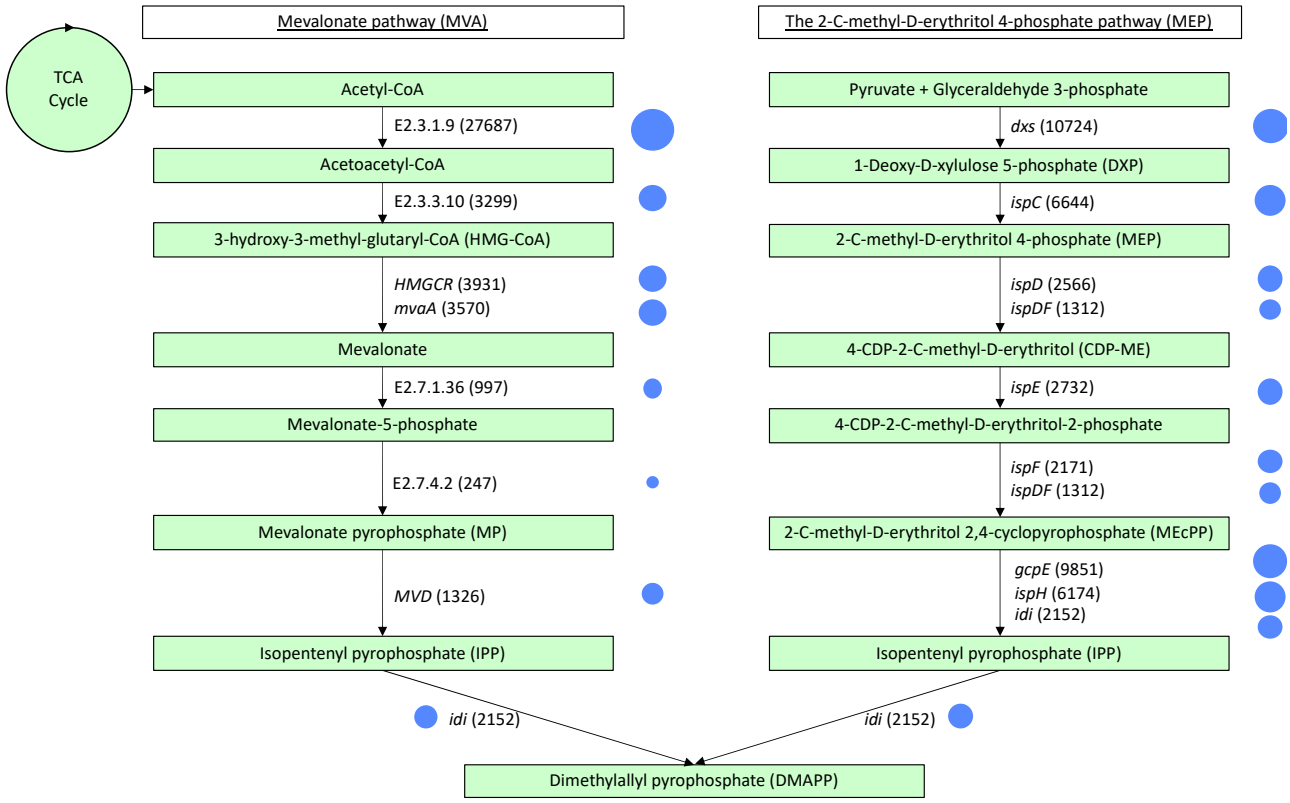
Mevalonate pathway (MVA)
The 2-C-methyl-D-erythritol 4-phosphate pathway (MEP)
TCA Cycle
Acetyl-CoA
E2.3.1.9 (27687)
Acetoacetyl-CoA
E2.3.3.10 (3299)
3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA)
HMGCR (3931)
mvaA (3570)
Mevalonate
E2.7.1.36 (997)
Mevalonate-5-phosphate
E2.7.4.2 (247)
Mevalonate pyrophosphate (MP)
MVD (1326)
Isopentenyl pyrophosphate (IPP)
idi (2152)
Pyruvate + Glyceraldehyde 3-phosphate
dxs (10724)
1-Deoxy-D-xylulose 5-phosphate (DXP)
ispC (6644)
2-C-methyl-D-erythritol 4-phosphate (MEP)
ispD (2566)
ispDF (1312)
4-CDP-2-C-methyl-D-erythritol (CDP-ME)
ispE (2732)
4-CDP-2-C-methyl-D-erythritol-2-phosphate
ispF (2171)
ispDF (1312)
2-C-methyl-D-erythritol 2,4-cyclopyrophosphate (MEcPP)
gcpE (9851)
ispH (6174)
idi (2152)
Isopentenyl pyrophosphate (IPP)
idi (2152)
Dimethylallyl pyrophosphate (DMAPP)

Figure S2

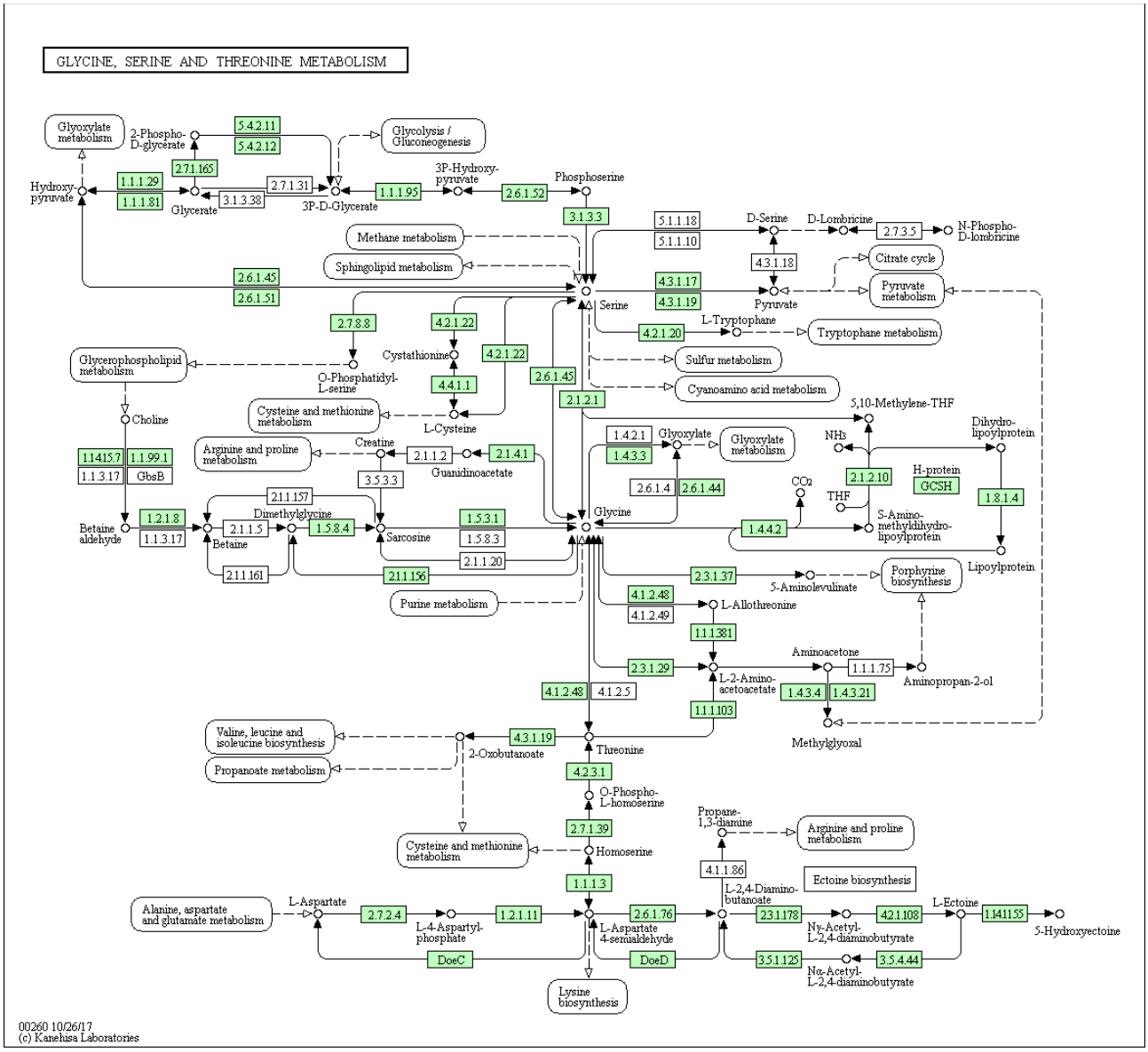
GLYCINE, SERINE AND THREONINE METABOLISM
Glyoxylate metabolism
2-Phospho-D-glycerate
5.4.2.11
5.4.2.12
Glycolysis / Gluconeogenesis
Hydroxy-pyruvate
1.1.1.29
1.1.1.81
2.7.1.165
Glycerate
3.1.3.38
2.7.1.31
3P-D-Glycerate
1.1.1.95
3P-Hydroxy-pyruvate
2.6.1.52
Phosphoserine
3.1.3.3
Methane metabolism
Sphingolipid metabolism
2.6.1.45
2.6.1.51
5.1.1.18
5.1.1.10
D-Serine
D-Lombricine
2.7.3.5
N-Phospho-D-lombricine
4.3.1.18
Citrate cycle
4.3.1.17
4.3.1.19
Pyruvate
Pyruvate metabolism
Serine
2.7.8.8
4.2.1.22
L-Tryptophane
4.2.1.20
Tryptophane metabolism
Cystathionine
4.2.1.22
Sulfur metabolism
Glycerophospholipid metabolism
O-Phosphatidyl-L-serine
4.4.1.1
2.6.1.45
Cyanoamino acid metabolism
Choline
Cysteine and methionine metabolism
L-Cysteine
2.1.2.1
5,10-Methylene-THF
Dihydro-lipoylprotein
1.4.2.1
Glyoxylate
1.4.3.3
Glyoxylate metabolism
NH3
Arginine and proline metabolism
Creatine
2.1.1.2
2.1.4.1
Guanidinoacetate
1.14.15.7
1.1.3.17
1.1.99.1
GbsB
3.5.3.3
2.1.2.10
H-protein
GCSH
2.6.1.4
2.6.1.44
CO2
THF
1.8.1.4
2.1.1.157
Dimethylglycine
1.2.1.8
1.1.3.17
Betaine aldehyde
2.1.1.5
Betaine
1.5.8.4
Sarcosine
1.5.3.1
1.5.8.3
Glycine
1.4.4.2
S-Amino-methyldihydro-lipoylprotein
2.1.1.20
2.1.1.161
2.1.1.156
2.3.1.37
5-Aminolevulinate
Porphyrine biosynthesis
Lipoylprotein
Purine metabolism
4.1.2.48
4.1.2.49
L-Allothreonine
1.1.1.381
Aminoacetone
2.3.1.29
1.1.1.75
Aminopropan-2-ol
L-2-Amino-acetoacetate
4.1.2.48
4.1.2.5
1.4.3.4
1.4.3.21
1.1.1.103
Methylglyoxal
Valine, leucine and isoleucine biosynthesis
Propanoate metabolism
4.3.1.19
2-Oxobutanoate
Threonine
4.2.3.1
O-Phospho-L-homoserine
2.7.1.39
Propane-1,3-diamine
Arginine and proline metabolism
Cysteine and methionine metabolism
Homoserine
4.1.1.86
L-2,4-Diamino-butanoate
Ectoine biosynthesis
1.1.1.3
Alanine, aspartate and glutamate metabolism
L-Aspartate
2.7.2.4
L-4-Aspartyl-phosphate
1.2.1.11
2.6.1.76
L-Aspartate 4-semialdehyde
2.3.1.178
Nγ-Acetyl-L-2,4-diaminobutyrate
4.2.1.108
L-Ectoine
1.14.11.55
5-Hydroxyectoine
DoeC
DoeD
3.5.1.125
3.5.4.44
Nα-Acetyl-L-2,4-diaminobutyrate
Lysine biosynthesis
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Figure S3

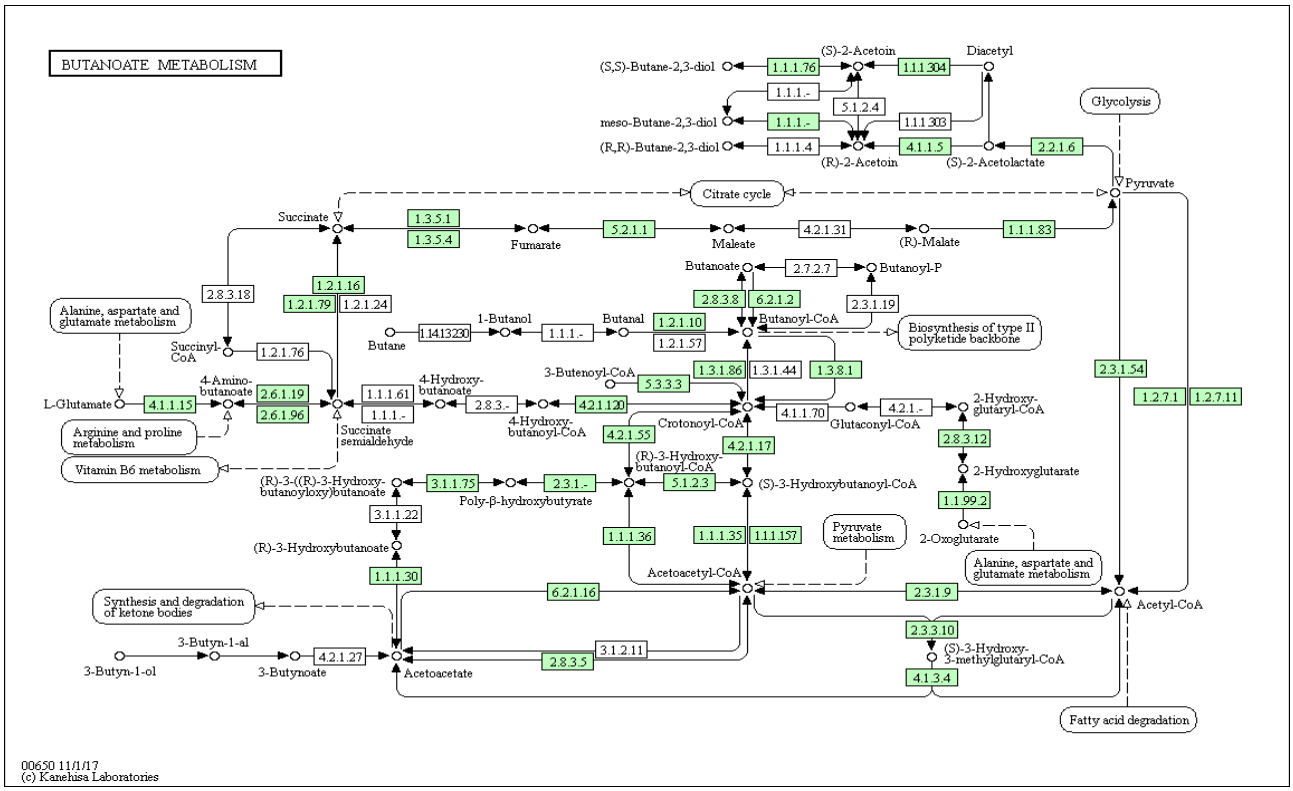
BUTANOATE METABOLISM
(S,S)-Butane-2,3-diol
(S)-2-Acetoin
Diacetyl
1.1.1.76
1.1.1304
1.1.1.-
5.1.2.4
meso-Butane-2,3-diol
1.1.1.-
1.1.1303
(R,R)-Butane-2,3-diol
1.1.1.4
4.1.1.5
2.2.1.6
(R)-2-Acetoin
(S)-2-Acetolactate
Glycolysis
Pyruvate
Citrate cycle
Succinate
1.3.5.1
1.3.5.4
Fumarate
5.2.1.1
Maleate
4.2.1.31
(R)-Malate
1.1.1.83
Butanoate
2.7.2.7
Butanoyl-P
2.8.3.8
6.2.1.2
2.3.1.19
Butanoyl-CoA
Biosynthesis of type II polyketide backbone
2.8.3.18
1.2.1.16
1.2.1.79
1.2.1.24
Alanine, aspartate and glutamate metabolism
Butane
1.14.13.230
1-Butanol
1.1.1.-
Butanal
1.2.1.10
1.2.1.57
Succinyl-CoA
1.2.1.76
3-Butenoyl-CoA
5.3.3.3
1.3.1.86
1.3.1.44
1.3.8.1
2.3.1.54
1.2.7.1
1.2.7.11
L-Glutamate
4.1.1.15
4-Amino-butanoate
2.6.1.19
2.6.1.96
1.1.1.61
1.1.1.-
Succinate semialdehyde
4-Hydroxy-butanoate
2.8.3.-
4.2.1.120
4-Hydroxy-butanoyl-CoA
4.2.1.55
Crotonoyl-CoA
4.1.1.70
4.2.1.-
Glutaconyl-CoA
2-Hydroxy-glutaryl-CoA
2.8.3.12
Arginine and proline metabolism
Vitamin B6 metabolism
4.2.1.17
(R)-3-Hydroxy-butanoyl-CoA
(R)-3-((R)-3-Hydroxy-butanoyloxy)butanoate
3.1.1.75
2.3.1.-
Poly-β-hydroxybutyrate
5.1.2.3
(S)-3-Hydroxybutanoyl-CoA
2-Hydroxyglutarate
1.1.99.2
3.1.1.22
(R)-3-Hydroxybutanoate
1.1.1.36
1.1.1.35
1.1.1.157
Pyruvate metabolism
2-Oxoglutarate
Alanine, aspartate and glutamate metabolism
1.1.1.30
Acetoacetyl-CoA
6.2.1.16
2.3.1.9
Acetyl-CoA
Synthesis and degradation of ketone bodies
2.3.3.10
3-Butyn-1-al
3-Butyn-1-ol
4.2.1.27
3-Butynoate
3.1.2.11
2.8.3.5
Acetoacetate
(S)-3-Hydroxy-3-methylglutaryl-CoA
4.1.3.4
Fatty acid degradation
00650 11/1/17
(c) Kanehisa Laboratories

Figure S4

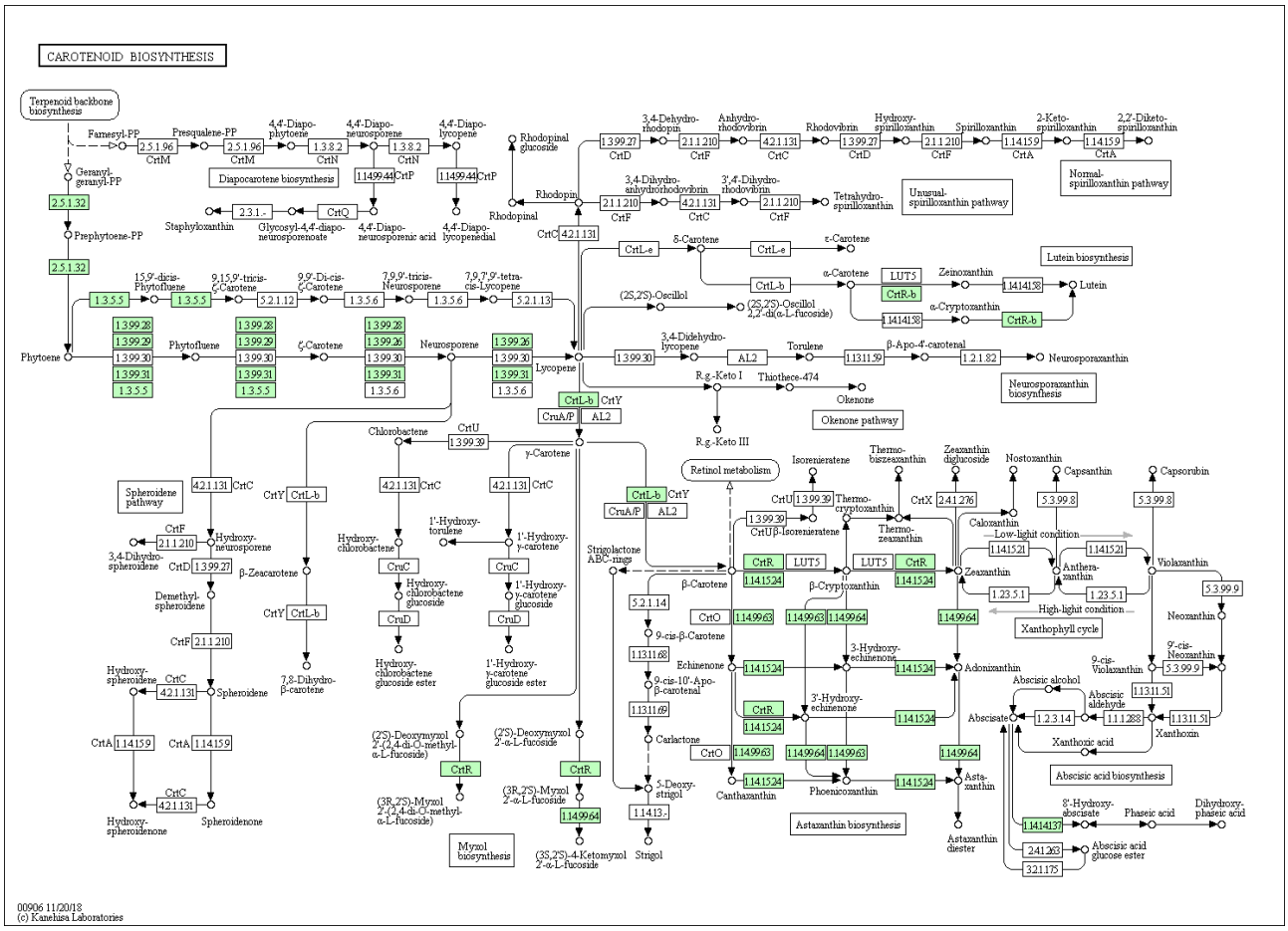
CAROTENOID BIOSYNTHESIS
Terpenoid backbone biosynthesis
Farnesyl-PP
Geranyl-geranyl-PP
Prephytoene-PP
Phytoene
Phytofluene
ζ-Carotene
Neurosporene
Lycopene
Rhodopinal
Rhodopinal glucoside
Rhodopin
Rhodopinal
Diapocarotene biosynthesis
Staphyloxanthin
Glycosyl-4,4'-diapo-neurosporenoate
4,4'-Diapo-neurosporenoic acid
4,4'-Diapo-lycopenedial
Spheroidene pathway
Hydroxy-neurosporene
3,4-Dihydro-spheroidene
β-Zeacarotene
Demethyl-spheroidene
7,8-Dihydro-β-carotene
Hydroxy-spheroidene
Spheroidene
Hydroxy-spheroidenone
Spheroidenone
Chlorobactene
γ-Carotene
Hydroxy-chlorobactene
1'-Hydroxy-torulene
1'-Hydroxy-γ-carotene
Hydroxy-chlorobactene glucoside
1'-Hydroxy-γ-carotene glucoside
Hydroxy-chlorobactene glucoside ester
1'-Hydroxy-γ-carotene glucoside ester
(2'S)-Deoxymyxol 2'-(2,4-di-O-methyl-α-L-fucoside)
(2'S)-Deoxymyxol 2'-α-L-fucoside
(3R,2'S)-Myxol 2'-(2,4-di-O-methyl-α-L-fucoside)
(3R,2'S)-Myxol 2'-α-L-fucoside
(3S,2'S)-4-Ketomyxol 2'-α-L-fucoside
Myxol biosynthesis
Retinol metabolism
Strigolactone ABC-rings
9-cis-β-Carotene
Echinenone
9-cis-10'-Apo-β-carotenal
Carlactone
3-Deoxy-strigol
Strigol
β-Carotene
β-Cryptoxanthin
Zeaxanthin
Adonixanthin
3-Hydroxy-echinenone
3'-Hydroxy-echinenone
Canthaxanthin
Phoenicoxanthin
Astaxanthin
Astaxanthin diester
Astaxanthin biosynthesis
Okenone
Okenone pathway
Thiothece-474
R.g.-Keto I
R.g.-Keto III
Torulene
Neurosporaxanthin
Neurosporaxanthin biosynthesis
Lutein biosynthesis
Lutein
Zeinoxanthin
α-Carotene
α-Cryptoxanthin
(2S,2'S)-Oscillol
(2S,2'S)-Oscillol 2,2'-di(α-L-fucoside)
Thermo-cryptoxanthin
Thermo-zeaxanthin
Thermo-biszeaxanthin
Zeaxanthin diglucoside
Isorenieratene
β-Isorenieratene
Caloxanthin
Nostoxanthin
Capsanthin
Capsorubin
Anthera-xanthin
Violaxanthin
Neoxanthin
9'-cis-Violaxanthin
9'-cis-Neoxanthin
Xanthophyll cycle
Low-light condition
High-light condition
Abscisic alcohol
Abscisic aldehyde
Abscisate
Xanthoxin
Xanthoxic acid
Abscisic acid biosynthesis
8'-Hydroxy-abscisate
Phaseic acid
Dihydroxy-phaseic acid
Abscisic acid glucose ester
Unusual-spirilloxanthin pathway
Normal-spirilloxanthin pathway
Tetrahydro-spirilloxanthin
3,4-Dehydro-rhodopin
Anhydro-rhodovibrin
Rhodovibrin
Hydroxy-spirilloxanthin
Spirilloxanthin
2-Keto-spirilloxanthin
2,2'-Diketo-spirilloxanthin
3,4-Dihydro-anhydrorhodovibrin
3',4'-Dihydro-rhodovibrin
00906 11/20/13
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Figure S5

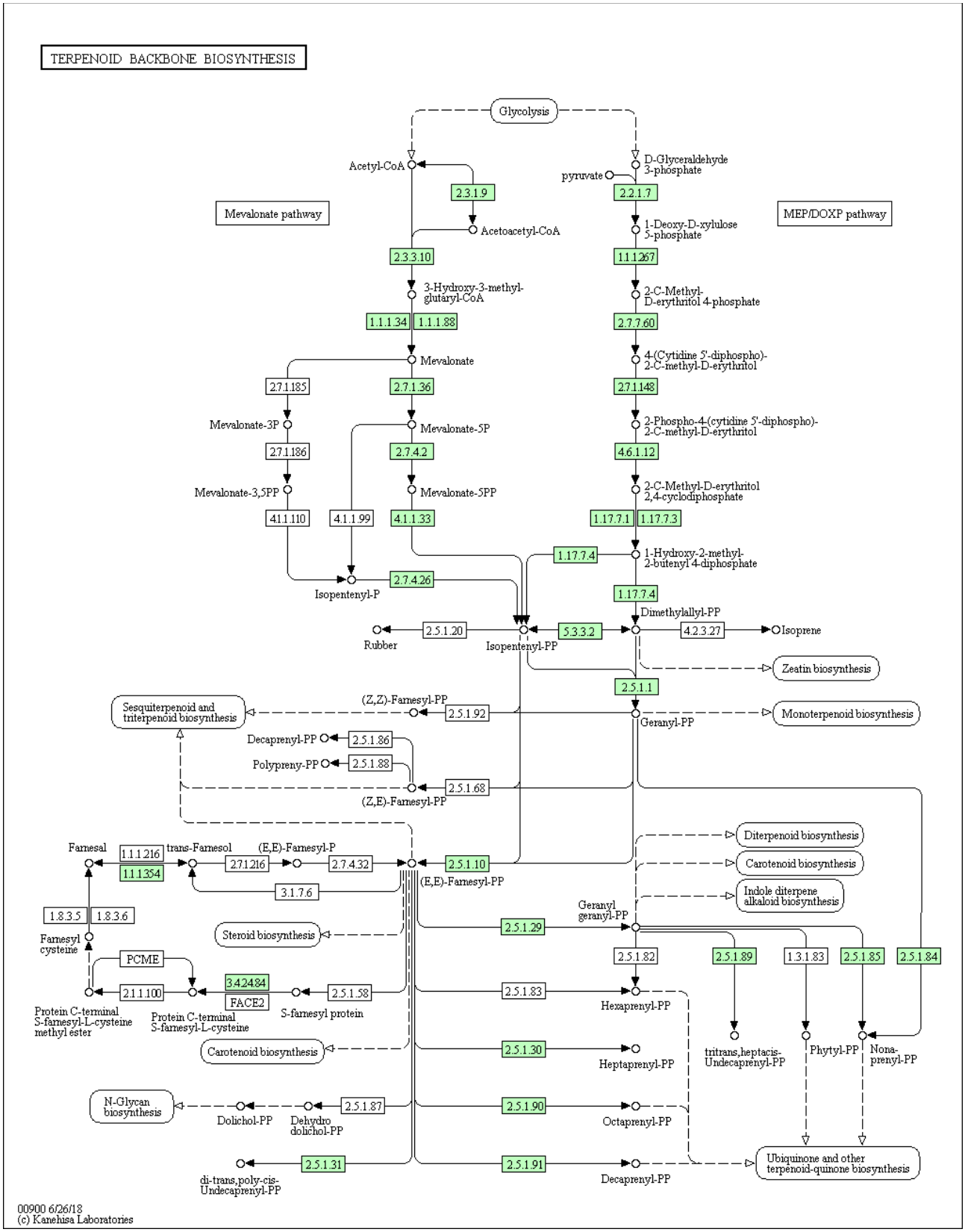
TERPENOID BACKBONE BIOSYNTHESIS
Glycolysis
Acetyl-CoA
pyruvate
D-Glyceraldehyde 3-phosphate
2.3.1.9
2.2.1.7
Mevalonate pathway
MEP/DOXP pathway
Acetoacetyl-CoA
1-Deoxy-D-xylulose 5-phosphate
2.3.3.10
1.1.1.267
3-Hydroxy-3-methyl-glutaryl-CoA
2-C-Methyl-D-erythritol 4-phosphate
1.1.1.34
1.1.1.88
2.7.7.60
Mevalonate
4-(Cytidine 5'-diphospho)-2-C-methyl-D-erythritol
2.7.1.185
2.7.1.36
2.7.1.148
Mevalonate-3P
Mevalonate-5P
2-Phospho-4-(cytidine 5'-diphospho)-2-C-methyl-D-erythritol
2.7.1.186
2.7.4.2
4.6.1.12
Mevalonate-3,5PP
Mevalonate-5PP
2-C-Methyl-D-erythritol 2,4-cyclodiphosphate
4.1.1.110
4.1.1.99
4.1.1.33
1.17.7.1
1.17.7.3
1.17.7.4
1-Hydroxy-2-methyl-2-butenyl 4-diphosphate
2.7.4.26
Isopentenyl-P
1.17.7.4
Dimethylallyl-PP
2.5.1.20
5.3.3.2
4.2.3.27
Isoprene
Rubber
Isopentenyl-PP
Zeatin biosynthesis
2.5.1.1
(Z,Z)-Farnesyl-PP
Sesquiterpenoid and triterpenoid biosynthesis
2.5.1.92
Geranyl-PP
Monoterpenoid biosynthesis
Decaprenyl-PP
2.5.1.86
Polyprenyl-PP
2.5.1.88
2.5.1.68
(Z,E)-Farnesyl-PP
Diterpenoid biosynthesis
Carotenoid biosynthesis
Indole diterpene alkaloid biosynthesis
Farnesal
1.1.1.216
1.1.1.354
trans-Farnesol
2.7.1.216
(E,E)-Farnesyl-P
2.7.4.32
2.5.1.10
(E,E)-Farnesyl-PP
3.1.7.6
Geranyl geranyl-PP
1.8.3.5
1.8.3.6
Steroid biosynthesis
2.5.1.29
Farnesyl cysteine
PCME
2.5.1.82
2.5.1.89
1.3.1.83
2.5.1.85
2.5.1.84
2.1.1.100
3.4.24.84
FACE2
2.5.1.58
2.5.1.83
Hexaprenyl-PP
Protein C-terminal S-farnesyl-L-cysteine methyl ester
Protein C-terminal S-farnesyl-L-cysteine
S-farnesyl protein
tritrans,heptacis-Undecaprenyl-PP
Phytyl-PP
Nona-prenyl-PP
Carotenoid biosynthesis
2.5.1.30
Heptaprenyl-PP
N-Glycan biosynthesis
2.5.1.87
2.5.1.90
Dolichol-PP
Dehydro dolichol-PP
Octaprenyl-PP
2.5.1.31
2.5.1.91
Ubiquinone and other terpenoid-quinone biosynthesis
di-trans,poly-cis-Undecaprenyl-PP
Decaprenyl-PP
00900 6/26/18
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Figure S6

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