**Additional file 2**

Supporting Information for:

**Genome-scale Metabolic Model for *Lactococcus lactis* MG1363 and its Application to the Analysis of Flavor Formation**

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Flavour forming pathways have been intensively studied over the past years as *Lactococcus lactis* and lactic acid bacteria (LAB) present a high interest in dairy fermentation. Here we summarized the relevant enzyme and reactions that has been identified in *L. lactis* MG1363 thanks to molecular biology and comparative genomic studies ([Ardö, 2006](#_ENREF_1), [Smit et al., 2005](#_ENREF_9), [Yvon, 2006](#_ENREF_15), [Liu et al., 2008](#_ENREF_5)). Volatiles compounds have been characterized depending the substrate used *i.e*. different amino acids leads to different flavor compounds as well as the type of metabolic routes used for their production *e.g.* elimination and transamination reactions.

The methionine metabolism holds a specific enzymatic route where methanethiol can be formed by an elimination reaction catalyzed by cystathionine β-lyase / cystathionine γ-lyase (MetC). MetC has been characterized in *L. lactis* MG1363 and found to have a dual catalytic activity ([Dobric et al., 2000](#_ENREF_4)). Indeed, MetC is able to perform both α,β- and α,γ-elimination reactions leading to H2S and methanethiol formation. Methanethiol is particularly an important flavor compound as it is a precursor for dimethyl sulfide (DMS), dimethyl disulfide (DMDS) and dimethyl trisulfide (DMTS). These compounds with a low olfactorial detection threshold are important for flavor formation as they may have desired or undesired odor characteristics depending on the type of fermentation product ([Weimer et al., 1999](#_ENREF_14)).

The transamination route starts with formation of the α-keto acid corresponding to the specific deaminated amino acids. *L. lactis* possesses several aminotransferases with characterized activities. The main enzymes are the branched-chain amino transferase (BcAT) and the aromatic amino transferase (ArAT). The BcAT has been characterized in different lactococcal strains for its activity on branched-chain amino acids and methionine as a substrate ([Rijnen et al., 2003](#_ENREF_6)). The α-keto acids which are formed play a key role in flavor formation as they are the starting point for three different pathways leading to end products with different olfactory properties. These have all been incorporated in the model as described below.

The first enzymatic pathway for α-keto acids degradation is the formation of hydroxy acids catalyzed by the improperly annotated PanE, which now has been characterized as a hydroxy acid dehydrogenase (HycDH) ([Chambellon et al., 2009](#_ENREF_3)) and is active on the branched-chain α-keto acid with NADH as a cofactor. For the leucine pathway, α-keto-isocaproate is converted into L-2-hydroxyisocaproate, concomitant with NADH oxidation . This also illustrates the influence of redox balancing on flavor formation in *L. lactis*.

The second type of compounds formed from the branched-chain α-keto acids include the well-described aldehydes 3-methylbutanal (leucine metabolism), 2-methylpropanal (valine metabolism), and 2-methylbutanal (isoleucine metabolism). These three aldehydes have been identified as products in our fermentation experiments (supplementary material file 4) and are normally formed by decarboxylation. A major difference with other LAB, including *L. lactis* IL1403, however, is that *L. lactis* MG1363 lacks the α-keto-acid decarboxylase (KdcA, EC 4.1.1.72) ([Smit et al., 2004b](#_ENREF_8)). The formation of 3-methylbutanal during the chemostat experiments can be explained in two ways. First, an unknown decarboxylase can be present in *L. lactis* MG1363 and second, a non-enzymatic reaction leading to the aldehyde formation may occur. Analysis of the genome sequence for alternative known decarboxylase gene sequences did not succeed in the identification of a putative enzyme involved in the formation of these aldehydes in *L. lactis* MG1363. Therefore, a decarboxylase reaction has not been integrated in the model.

The chemical conversion of branched-chain α-keto acids may occur according to a Strecker reaction with α-keto isocaproic acid in the presence of manganese and oxygen ([Smit et al., 2004a](#_ENREF_7)). However, as all growth experiments have been performed in the absence of oxygen, the chemical reaction explaining the presence of 3-methylbutanal in our fermentation must be anaerobic (supplementary material file 4). Recently, it was shown that 3-methylbutanal can be formed as a direct reaction between free leucine and ribose ([Bi and Ma, 2006](#_ENREF_2)). The suggested mechanism is a reaction between leucine’s carboxylic group with ribose’s aldehyde group. The Schiff base formed between the two reactive group leads to 3-methylbutanal. The CDM used during our experiments contains high amounts of leucine and the formation of 3-methylbutanal, identified independently of the growth condition, was found to be enhanced by acidic conditions and temperature. The presence of a lower amount of 3-methylbutanal in blank samples containing only CDM supports this explanation (data not shown). The aldehyde group counterpart in this reactions has not yet been identified nonetheless acetaldehyde represents a plausible candidate in biotic conditions. Thus no reaction accounting for the branched-chain aldehydes formation has been integrated in the model.

The third catabolic pathway for branched-chain α-keto acids leads to carboxylic acids formation *via* oxidative decarboxylation. It has extensively been characterized in *Enterococcus faecalis* ([Ward et al., 1999](#_ENREF_13)) and is catalyzed by an α-keto acid dehydrogenase composed of several subunits. Comparative studies ([Liu et al., 2008](#_ENREF_5)) identified the pyruvate dehydrogenase complex (PDH) as the enzyme presenting a high similarity to the α-keto acid dehydrogenase complex in *E. faecalis*. In *L. lactis* MG1363, PDH is assumed to catalyze the formation of acyl-CoA with NADH regeneration, while the phospho-transacetylase (*ack*) and an acyl kinase (*pta*) lead to carboxylic acid formation, as identified by GC-MS ([Steinhaus et al., 2009](#_ENREF_12), [Ward et al., 1999](#_ENREF_13)) with the production of an ATP. The importance of this part of the catabolic pathway is related to the odor properties of those compounds. Indeed, in the case of leucine, 3-methylbutanoic acid has been characterized for it cheesy odor ([Smit et al., 2005](#_ENREF_9)). Thus, the reactions termed OIVD1, OIVD2, OIVD3 can be expected to occur in *L. lactis* MG1363 and have been integrated in the genome-scale model (supplementary material file 1). As PDH is assumed to hold the activity on BrAA α-keto acids, and as PDH is known to be inactive under anaerobic condition ([Snoep et al., 1993](#_ENREF_10)), the pathways leading to formation of carboxylic acids are only active under aerobic conditions ([Snoep et al., 1992](#_ENREF_11)). Moreover, it reflects the coupling between flavor formation and energy and redox metabolism and growth conditions. The interplay between amino acid metabolism and flavor forming pathways for the branched-chain amino acids, methionine and cysteine is illustrated in the figure 3 in the main text.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **New annotation associated functions** | **Gene** | **Protein** | **Abbreviation reaction** | **Reaction's name** | | **Equation** | **Protein Classification** | | | |
| branched chain aminotransferase | llmg\_1181 | IlvE | ILETA | isoleucine transaminase | | akg + ile-L <==> 3mop + glu-L | EC-2.6.1.42 | | | |
| llmg\_1181 | LEUTA | leucine transaminase | | akg + leu-L <==> 4mop + glu-L | EC-2.6.1.42 | | | |
| llmg\_1181 | VALTA | valine transaminase | | akg + val-L <==> 3mob + glu-L | EC-2.6.1.42 | | | |
| aromatic aminotransferase | llmg\_0066 | Arat | araphe1 | aromatic amino acid aminotransferase - phenylalanine | | 4mop + phe-L <==> leu-L + phpyr |  | | | |
| llmg\_0066 | araphe2 | aromatic amino acid aminotransferase - phenylalanine | | 3mop + phe-L <==> ile-L + phpyr |  | | | |
| llmg\_0066 | araphe3 | aromatic amino acid aminotransferase - phenylalanine | | indpyr + phe-L <==> phpyr + trp-L |  | | | |
| llmg\_0066 | aratry1 | aromatic amino acid aminotransferase - tryptophan | | 4mop + trp-L <==> indpyr + leu-L |  | | | |
| llmg\_0066 | aratry2 | aromatic amino acid aminotransferase - tryptophan | | 3mop + trp-L <==> ile-L + indpyr |  | | | |
| llmg\_0066 | aratyr1 | aromatic amino acid aminotransferase - tyrosine | | 4mop + tyr-L <==> 34hpp + leu-L |  | | | |
| llmg\_0066 | aratyr2 | aromatic amino acid aminotransferase - tyrosine | | 3mop + tyr-L <==> 34hpp + ile-L |  | | | |
| llmg\_0066 | aratyr3 | aromatic amino acid aminotransferase - tyrosine | | phpyr + tyr-L <==> 34hpp + phe-L |  | | | |
| llmg\_0066 | aratyr4 | aromatic amino acid aminotransferase - tyrosine | | indpyr + tyr-L <==> 34hpp + trp-L |  | | | |
| llmg\_0066 | AKGTA3 | aromatic-amino-acid transaminase (Trp: aKG) | | akg + trp-L <==> glu-L + indpyr | EC-2.6.1.27 | | | |
| asparatic aminotransferase | llmg\_0171, llmg\_2019 | AspC, AspB1 | ASPTA1 | aspartate transaminase | | akg + asp-L <==> glu-L + oaa | EC-2.6.1.1 | | | |
| llmg\_2019 | AspB1 | ASPTA2 | aspartate transaminase | | 4hglu + akg <==> 4h2oxg + glu-L | EC-2.6.1.1 | | | |
| llmg\_0066, llmg\_2019 | AspB1, Arat | ASPTA5 | aspartate transaminase | | akg + tyr-L <==> 34hpp + glu-L | EC-2.6.1.1 | | | |
| 2-D hydroxy acid dehydrogenase | llmg\_1131 | PanE | 2H3MBDH | 2-hydroxy-3-methylbutanoate dehydrogenase | | 3mob + h + nadh <==> 2h3mb + nad | | | |  | | | |
| 2H3MPDH | 2-hydroxy-3-methylpentanoate dehydrogenase | | 3mop + h + nadh <==> 2h3mp + nad | | | |
| 2HXICDH | L-2-hydroxyisocaproate dehydrogenase | | 4mop + h + nadh <==> 2hxic-L + nad | | | |
| alcohol dehydrogenase | llmg\_0955 | Adh | ALCD19 | alcohol dehydrogenase (glycerol) | glyald + h + nadh <==> glyc + nad | | | EC-1.1.1.1 | | | |
| llmg\_0955, llmg\_2432 | AdhE, Adh | ALCD2x | alcohol dehydrogenase (ethanol: NAD) | etoh + nad <==> acald + h + nadh | | | EC-1.1.1.1 | | | |
| llmg\_1642 | ButB | BTDD-RR | (R,R)-butanediol dehydrogenase | btd-RR + nad <==> actn-R + h + nadh | | | EC-1.1.1.4 | | | |
| llmg\_1991 | AdhA | AALDH | aryl-alcohol dehydrogenase | h + nadh + pacald <==> nad + pea | | | EC-1.1.1.90 | | | |
| llmg\_2432 | AdhE | ACALD | acetaldehyde dehydrogenase (acetylating) | acald + coa + nad <==> accoa + h + nadh | | | EC-1.2.1.10 | | | |
| llmg\_0955, llmg\_2432 | ALCD2x | alcohol dehydrogenase (ethanol: NAD) | etoh + nad <==> acald + h + nadh | | | EC-1.1.1.1 | | | |
| aldehyde dehydrogenase | llmg\_2432 | AdhE | ACALD | acetaldehyde dehydrogenase (acetylating) | acald + coa + nad <==> accoa + h + nadh | | | EC-1.2.1.10 | | | |
| llmg\_0955, llmg\_2432 | AdhE, Adh | ALCD2x | alcohol dehydrogenase (ethanol: NAD) | etoh + nad <==> acald + h + nadh | | | EC-1.1.1.1 | | | |
| (branched-chain) ketoacid dehydrogenase complex | llmg\_0071, llmg\_0073+llmg\_0074, llmg\_0072 | PdhAB + PdhC + PdhD | OIVD1 | 2-oxoisovalerate dehydrogenase (acylating; 4-methyl-2-oxopentaoate) | 4mop + coa + nad --> co2 + ivcoa + nadh | | | EC-1.2.1.25 | | | |
| OIVD2 | 2-oxoisovalerate dehydrogenase (acylating; 3-methyl-2-oxobutanoate) | 3mob + coa + nad --> co2 + ibcoa + nadh | | | EC-1.2.1.25 | | | |
| OIVD3 | 2-oxoisovalerate dehydrogenase (acylating; 3-methyl-2-oxopentanoate) | 3mop + coa + nad --> 2mbcoa + co2 + nadh | | | EC-1.2.1.25 | | | |
| (branched-chain) phosphotransacylase | llmg\_0763 | Pta | PTAr | phosphotransacetylase | accoa + pi <==> actp + coa | | | EC-2.3.1.8 | | | |
|
| acylkinase | llmg\_2289, llmg\_2288 | ACKr | ACKr | acetate kinase | ac + atp <==> actp + adp | | | EC-2.7.2.1 | | | |
|
| serine acetyltransferase | llmg\_2042 | CysE | SERAT | serine O-acetyltransferase | accoa + ser-L <==> acser + coa | | | EC-2.3.1.30 | | | |
|
| O-acetylserine sulfhydrylase | llmg\_0508, llmg\_1775 | CysK | CYSS | cysteine synthase | acser + h2s --> ac + cys-L + h | | | EC-4.2.99.8 | | | |
|
| cystathionine beta lyase /cystathionine gamma lyase | llmg\_1776 | MetC | CYSTGL | cystathionine g-lyase | cysth-L + h2o --> 2obut + cys-L + nh4 | | | EC-4.4.1.1 | | | |
| CYSTL | cystathionine b-lyase | cysth-L + h2o --> hcys-L + nh4 + pyr | | | EC-4.4.1.8 | | | |
| cystathionine gamma synthase /O-acetylhomoserine sulfhydrylase | llmg\_2181 | MetB | SHSL1 | O-succinylhomoserine lyase (L-cysteine) | cys-L + suchms --> cysth-L + h + succ | | | | EC-4.2.99.9 | | | |
| SHSL5 | O-acetylhomoserine lysase (L-cysteine) | achms + cys-L --> ac + cysth-L + h | | | | EC-4.2.99.9 | | | |
| cobalamin-independent homocysteine methyltransferase | llmg\_1225 | MetE | MTHPTGHM | 5-methyl-tetrahydropteroyltriglutamate-homocysteine S-methyltransferase | 5mthglu + hcys-L --> met-L + thglu | | | | EC-2.1.1.14 | | | |
|
| homoserine dehydrogenase | llmg\_1332 | Hom | HSDy | homoserine dehydrogenase (NADPH) | hom-L + nadp <==> aspsa + h + nadph | | | | EC-1.1.1.3 | | | |
|
| homoserine kinase | llmg\_1331 | ThrB | HSK | homoserine kinase | atp + hom-L --> adp + h + phom | | | | EC-2.7.1.39 | | | |
|
| homoserine O-succinyltransferase /homoserine O-acetyltransferase | llmg\_2182 | MetA | HSAT | Acetyl-CoA:L-homoserine O-acetyltransferase | accoa + hom-L <==> achms + coa | | | | EC-2.3.1.31 | | | |
| HSST | homoserine O-succinyltransferase | hom-L + succoa --> coa + suchms | | | | EC-2.3.1.46 | | | |
|
| O-acetylhomoserine sulfhydrylase | llmg\_0091 | MetY | METACH | O-Acetyl-L-homoserine acetate-lyase (adding methanethiol) | achms + h2s <==> ac + h + hcys-L | | | | EC-4.2.99.10 | | | |
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Table1: Complete list of flavor forming reaction linked to the Gene-Protein-Reaction association of the genome-scale metabolic network.

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