Role of *Lactobacillus helveticus* on Flavor Formation in Cheese: Amino Acid Metabolism

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Abstract

Lactic acid bacteria, mainly lactobacilli, play an important role in cheese making. Their role can be divided into starters and non-starters or secondary microorganisms. *Lactobacillus helveticus*, an obligately homofermenter and thermophilic bacterium, has unique properties as a starter because of its ability to induce strong impact on cheese flavor. The bacteria are known to be prototrophic for 5 amino acids and auxotrophic for 13 amino acids. It is interesting that the conversion of aromatic amino acids, branch chain amino acids, and methionine into volatile and nonvolatile compounds by *L. helveticus* is thought to represent the rate-limiting step in the formation of mature flavor and aroma in cheese. The addition of a highly autolytic *L. helveticus* to a starter system could significantly increase the formation of flavor precursor and some volatile compounds during cheese ripening. This article focuses on the contribution of *L. helveticus* to flavour compound formation in cheese with particular emphasis on amino acid metabolism.

Keywords: *Lactobacillus helveticus*, amino acids catabolism, aromatic amino acid, branched-chain amino acid, sulphur compound, cheese flavor

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Introduction

Lactobacillus helveticus is one of the Generally Recognized as Safe (GRAS) organism and is among the potential lactic acid bacteria (LAB) extensively used in milk-based food production. *L. helveticus* was firstly isolated from sour milk and cheese, and was first described by Orla-Jensen in 1919 (Kandler & Weiss, 1986; Hammes & Vogel, 1995). Thereafter, *L. helveticus* strains have been widely used in the manufacturing of several types of cheeses (Table 1) and used widely both as starter and adjunct (Khalid & Marth, 1990; Fortina *et al*., 1998; Beresford *et al*., 2001; Gatti *et al*., 2003; Helinck *et al*., 2004; Kenny *et al*., 2006; Hannon *et al*., 2007; Sheenan *et al*., 2007). Strains of *L. helveticus* have become more popular in cheese making for a variety of new applications, such as to reduce browning in Mozzarella and Swiss cheeses by diminishing concentration of residual galactose (Oberg & Broadbent, 1993) and to improve stretchability and melting in Mozzarella and Swiss-type cheeses (Oberg *et al*., 1991). It is also known that *L. helveticus* have significant role in the production of specific flavor compounds in Italian cheese types (Gatti *et al*. 2003; Rossetti *et al*., 2008) and debittering of cheese (Fernández *et al*., 1994; Soeryapranata *et al*., 2007).

Effort to improve the quality of cheese by producing cheese with specific flavor has been of increasing interest and several research studies focusing on amino acids catabolism and its related aspects have been widely carried out. *L. helveticus* undoubtedly play very important role in generating specific cheese flavor derived from its amino acids catabolism. Review about the features of *L. helveticus* cell envelope proteinase (CEP) (Sadat-Mekmene *et al*., 2011), the mechanisms of lysis of *L. helveticus* (Lortal & Chapot-Chartier, 2005) and the availability information of *L. helveticus* genome sequencing (Cremonesi *et al*., 2013) are very helpful to gain better understanding of flavor generation from amino acid catabolism by *L.*

helveticus. The objective of this review article is to discuss the contribution of *L. helveticus* to flavor generation in cheese with particular emphasis on its amino acid metabolism.

Table 1. The use of *L. helveticus* as cheese starter (Gobetti *et al*., 2007).

No	Cheese product	Type of starter
1.	Asiago	Natural whey and milk culture
2.	Canestrato Pugliese	Natural whey culture
3.	Emmental	Commercial culture
4.	Grana Padano	Natural whey culture
5.	Gruyère	Commercial culture
6.	Montasio	Natural whey culture
7.	Mozzarella	Natural culture and Commercial culture
8.	Parmigiano Reggiano	Natural culture
9.	Pecorino Romano	Natural culture in scotta
10.	Pecorino Sardo	Natural whey and milk culture
11.	Pecorino Siciliano	Natural whey culture
12.	Provolone Italiano	Natural whey culture
13.	Sbrinz	Commercial culture
14.	Taleggio	Commercial culture

Taxonomy and Growth Characteristics of *L. helveticus*

L. helveticus is obligately homo-fermentative and thermophilic LAB. It is one species under the extremely diverse genus *Lactobacillus* that has over 188 recognized species (Euzeby, 1997). Based on the phylogeny of *Lactobacillus* lineages, *L. helveticus* belong to subgeneric group A (Claesson *et al*., 2008) which also contain *Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus johnsonii* and *Lactobacillus gasseri. L. helveticus* and *L. acidophilus* are in the same group because they are phylogenetically very closely related. Furthermore, comparative phylogenomic study using available information of 18 genomes of *Lactobacillus* strains subjected to an array of whole-genome and single-marker phylogenetic approach showed that *L. helveticus* DPC4571 is in the same cluster with *L. acidophilus* NCFM in the phylogenetic tree based on core protein sequence (Claesson *et al*., 2008). Although *L. helveticus* DPC4571 and *L. acidophilus* NCFM share remarkable genomic homology (16S rRNA sequence shares 98.4% identity) and conserved gene synteny, they occupy distinctly different niches. *L. helveticus* DPC4571 is a dairy organism while *L. acidophilus* NCFM is a gut organism (Callanan *et al*., 2008; O'Sullivan *et al*., 2009; Slatterry *et al*., 2010). Recently within *L. helveticus,* information on complete genome sequence of strains DPC4571 (Callanan *et al*., 2008); DSM 20075 (by direct sequence submission), H10 (Zhao *et al*., 2011), MTCC5463 (Prajapati *et al*., 2011) and R0052 (Tompkins *et al*., 2012) were available in the public databases and two other strains (CNRZ 32 and H9) are not available yet (Cremonesi *et al*., 2013). The genome of *L. helveticus* was characterized by its size of 1.8-2.1 Mbp; 36.7-37.1 G+C mol %; 1,838-2,148 genes and 1,610-2,239 protein in the genome (Cremonesi *et al*., 2013).

L. helveticus is one of the most exploited bacteria in the dairy industry that play crucial roles in the flavor characteristics of cheese products. It has a dairy adapted niche culture and is well known as a specialist dairy culture (Callanan *et al*., 2008; Slaterry *et al*., 2010). Large diversity of *L. helveticus* strains have been used for research on cheese flavor and some of them represent industrial strains. *L. helveticus* is nutritionally fastidious and it is one of the most auxotrophic LAB. When grown in milk, lactose is used as the main carbon source for energy and addition of citrate in the medium enhanced the rate of lactose consumption by *L. helveticus* ATCC 15807 (Torino *et al*., 2005). *L. helveticus* requires many free amino acids which are not available sufficiently in the milk. Using single-amino acid omission method in a chemically defined medium *L. helveticus* CNRZ32 showed auxotrophy for Arg, Glu, His, Ile, Leu, Lys, Met, Phe, Pro, Thr, Trp, Tyr, and Val and

either Asp or Asn, because it lost the ability to synthesize essential amino acids that are required for growth and metabolisms (Christiansen *et al*., 2008).

L. helveticus strains also showed more extensive amino acid requirements than most LAB (Hebert *et al*., 2000; Christensen & Steele, 2003), but they possess a complex system of proteinases and peptidases (Smeianov *et al*., 2007) that enable them to liberate amino acids from the caseins in milk. *L. helveticus* has characteristic of powerful proteolytic system (Griffiths & Tellez, 2013) and ability to grow rapidly in milk is supported by an efficient CEP activity due to subtilisin-like serine proteases (Sadat-Mekmene *et al*., 2011). Christiansen *et al*. (2008) confirmed this character by reconstruction and screen for genes encoding enzymes involved in amino acid biosynthesis from genome sequence for *L. helveticus* CNRZ 32 using bioinformatics software. Their analysis revealed that amino acid auxotrophy of *L. helveticus* was due primarily to gene absence and revealed good agreement between gene content and phenotypic amino acid requirements. In addition, experiments confirmed a genome-based prediction that Asp (or Asn) auxotrophy could be alleviated by the addition of citrate. However, the results did not support another prediction that *L. helveticus* CNRZ 32 could catalyze the conversion of ornithine to putrescine (1,4-diaminobutane or butanediamine), a volatile biogenic amine. On the other side, *L. helveticus* CRL 1062 and CRL 974 were found to be prototrophic for Ala, Gly, Asp, Glu and Cys when tested using simplified chemically defined medium. In addition, *L. helveticus* CRL 974 also showed prototrophy for lysine and serine (Hebert *et al*., 2000). *L. helveticus* lyses early and releases their intracellular enzymes into the system. The mechanism of lysis in *L. helveticus* is different, involving autolysins rather than induction of prophage-encoded endolysins (Deutsch *et al*., 2003). Autolytic property of *L.helveticus* strains is one of important factor in flavor generation of Cheddar cheese (Kenny *et al*., 2006).

In order to select good flavor-producing strains for cheese, three stages are usually necessary including 1) isolation of strains with specific media; 2) preselection with molecular tools of strains having genotypes related to those known aroma producers and 3) analyses of their activity (Mariley & Casey, 2004). The study of Broadbent *et al*. (2011) using comparative genome hybridization (CGH) suggested strain heterogeneity in peptidase activity is not based on differences in gene content but rather it is likely due to a combination of nonsense mutations and sequence polymorphisms that affect the expression level, specificity, or activity of individual enzymes involved in the reactions. CEP paralogs are probably very important determinants of strains functionally in cheese flavor.

Flavor Formation in Cheese by *L. helveticus*

Milk provides very low concentrations of free amino acids and peptides. Therefore, LAB depend on its proteolytic system when used as starter cultures for cheese process. Degradation of caseins by the CEP and peptidases from LAB yields small peptides and free amino acids, which are important components of flavor precursor.

1. **The proteolytic system**

Degradation of casein can be carried out by the activities of the CEP and peptidases from LAB. Proteolysis is undoubtedly the most important biochemical process for flavor formation in hard and semi-hard cheese types. Contribution of proteolysis to cheese flavor is through the release of peptides and amino acids (aromatic, branched-chain and sulfur-containing amino acids). *L. helveticus* has higher proteolytic activity than most other lactobacilli and hydrolyses more casein in culture media than other species (Savijoki *et al*, 2006). It might be related to the possession of CEP which is responsible to initiate the hydrolysis of casein. *L. helveticus* CNRZ 32 was reported to have at least two CEPs that are PrtH and $PrtH_2$ (Gilbert *et al*., 1997; Griffiths & Telez, 2013), or even more, since there are two other types of CEP in *L. helveticus* are reported, namely PrtH3 and PrtH4 (Savijoki *et al*., 2006; Broadbent *et al*., 2011). This high proteolytic activity is related to the capability of *L. helveticus* to reduce cheese bitterness which is due to the further hydrolysis of hydrophobic peptides or bitter peptides (Sadat-Mekmene *et al*., 2011). The role of peptidases

in proteolytic system has been reviewed by Griffiths and Tellez (2013). Important role of peptidases in *L. helveticus* is related to the degradation of essential amino acids involving two proline specific endopeptidases, PepE, PepO; a tripeptidase PepT, four aminopeptidases, PepX, PepI, PepQ and PepR, and four dipeptidases PepD, PepV, PepC and PepN (Griffiths & Tellez, 2013). Debittering peptidases, PepE, PepO, PepO2, PepO3 and PepN of *L. helveticus* has been cloned to *E.coli* (Soeryapranata *et al*., 2007).

2. Amino acid catabolism

Amino acid catabolism is essential in cheese flavor development, since it generates flavor compounds. The pathway of amino acid catabolism is initiated by a transamination reaction (Figure 1), that requires the α-keto acid as the amino group receptor such as α-ketoglutarate (Helinck *et al*., 2004). Transamination of amino acids results many major aroma compounds. The conversion of some amino acids produced from casein degradation during cheese maturation into volatile and nonvolatile compounds by LAB is believed to represent the rate-limiting step in the development of mature flavor and aroma compounds in cheese. Aromatic amino acids (Trp, Tyr, Phe), branched-chain amino acids (Val, Ile, Leu), and sulfur-containing amino acids (Cys, Met) that are produced from casein degradation are important precursors of flavor compounds. The ability of LAB to degrade amino acids to flavor compounds is highly strain dependent (Yvon & Rijnen, 2001). Amino acids are precursors of various volatile flavor compounds (Table 2). Conversion of amino acids in many different ways by several enzymes, deaminases, decarboxylases, aminotransferases and lyases may produce several compounds including ammonia, amines, aldehydes, phenols, indole and alcohol that contribute to cheese flavor.

Genes involved in amino acid metabolism are highly conserved across the species and most of the observed differences would not be predicted to affect flavor production in cheese. Most of the *L. helveticus* CNRZ32 genes for amino acid biosynthesis and metabolism were conserved in all of the strains tested. The differences detected were the absence of enzymes in some *L. helveticus* strains tested, cystathione-β-lyase, an enzyme that converts Met into methanethiol, and *serC*, which encodes phosphoserine transaminase. Strains of *L. helveticus* lacking cystathione-β-lyase may have decreased capacity to produce sulfur-based flavor in cheese. It is suggested that there is strain heterogeneity in peptidase activity (Broadbent *et al*., 2011).

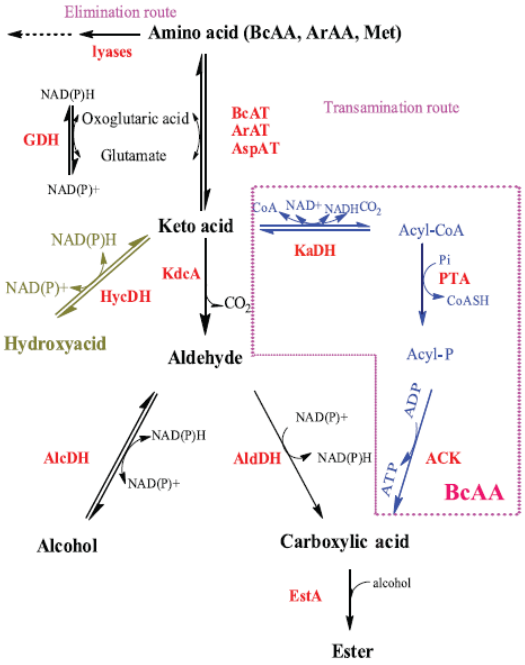

Figure 1. Generalized amino acid catabolism (aromatic, branched-chain and sulfur-containing amino acids) pathway of LAB (Liu *et al*., 2008).

Table 2. Name and chemical nature of the major aroma compounds derived from branched-chain and aromatic amino acids and methionine (Yvon & Rijnen, 2001).

Amino acids	Aldehydes	Alcohols	Carboxylic acids	Thyol/divers
Leucine	3-Methylbutanal or Isovaleraldehyde	3-Methylbutanol	3-Methylbutanoic acid or isovaleric acid	
Isoleucine	2-Methylbutanal	2-Methylbutanol	2-Methylbutanoic acid	
Valine	2-Methylpropanal or isobutyraldehyde	2-Methylpropanol	2-Methylpropanoic acid or isobutyric acid	
Phenylalanine	Phenylacetaldehyde, benzaldehyde (-2C)	Phenylethanol	Phenylacetic acid	
Tyrosine	OH-Phenylacetaldehyde, OH-benzaldehyde (-2C)	OH-Phenylethanol	OH-Phenylacetic acid	*p*-cresol, phenol
Tryptophane	Indol-3-acetaldehyde, indol-3-aldehyde	Tryptophol	Indol-3-acetic acid	Skatole, indole
Methionine	3-Methylthiopropanal, or methional	3-Methylthiopropanol	3-Methylthiopropionic acid	Methanethiol

2.1. Aromatic amino acid

Catabolism of Trp, Tyr and Phe by *L. helveticus* cheese flavor adjunct was studied by Gummalla and Broadbent (1999, 2001). Under simulated near cheese-ripening (pH 5.2, 4% NaCl, 15°C and no sugar) and carbohydrate starvation (pH 6.5, 37°C, no sugar) conditions *L. helveticus* cell-free extract catabolized Trp of Cheddar cheese to indole-3-lactic acid and when tested using micellar electrokinetic capillary chromatography showed that the reaction occured via succesive transamination and dehydrogenation (Gummalla & Broadbent, 1999). *L. helveticus* catabolized Tyr to *p*-hydroxy phenyl lactic acid and *p*-hydroxy phenyl acetic acid, while Phe degradation gave rise to phenyl lactic acid, phenyl acetic acid, and benzoic acid (Gummalla & Broadbent, 2001). *L. helveticus* produced mainly acids and small amount of alcohol and hydroxyacid from Phe, in the presence of α-ketoglutarate in the medium, and the enzyme involved in the α-keto acid conversion to acids is an α-keto acid dehydrogenase that produces acyl coenzymes A (Helink *et al*., 2004). When aromatic amino acid Tyr and Phe were mixed with those of branched-chain and sulfur amino acids, they were transaminated the most efficiently than the others. This indicated the presence of an efficient aromatic aminotransferase by *L. helveticus* (Klein *et al*., 2001). The formation of potential off-flavours from Tryp catabolism by this species was highlighted by Gummala & Broadbent (1999).

2.2. Branched-chain amino acid

From the study of amino acid catabolism of thermophilic LAB, *L. helveticus* was reported to produce a large quantity of acids, about 80%, from degradation of Leu in the presence of α-ketoglutarate in the medium (Helinck *et al*., 2004). Mix of Val, Ile and Leu was transaminated efficiently and three major volatile compounds detected were benzaldehyde, dimethyl disulphide and 2-methyl propanol (Klein *et al*., 2001).

2.3. Sulfur-containing amino acid

Sulfur-containing compounds such as methanethiol, methional, dimethyl sulfide, dimethyl tetrasulfide, carbonyl sulfide and hydrogen sulfide are volatile compound that contribute to the aroma of cheese (Urbach, 1995). Production of methanethiol is important since it is related to the desirable flavor of good quality Cheddar cheese. Production of methanethiol, dimethyldisulphide and dimethyltrisulphide from Met by methionine aminotransferase. Enzymes cystathione γ- and β-lyases are found to contribute to the formation of volatile sulfur compounds Dias and Weimer (1998). Formation of volatile sulfur compounds from methionine, cystathionine, and cysteine to sulfur volatiles compounds in *L. helveticus* CNRZ32 was detected in a model system using GC-MS with solid-phase microextraction (Lee *et al*., 2007). Using methionine as a substrate, cystathione β-lyase overexpression resulted in higher volatile sulfur compounds production than that of wild-type *L. helveticus* CNRZ 32 or the cystathione β-lyase-null mutant. However,

there were no differences in volatile sulfur compounds production between the wild type and the cystathione β-lyase-null mutant. With cystathionine, methanethiol production was detected from the cystathione β-lyase overexpression variant and complementation of the cystathione β-lyase-null mutant, implying that cystathione β-lyase may be involved in the conversion of cystathionine to methanethiol. With cysteine, no differences in volatile sulfur compounds formation were observed between the wild type and genetic variants, indicating that cystathione β-lyase does not contribute to the conversion of cysteine (Figure 2).

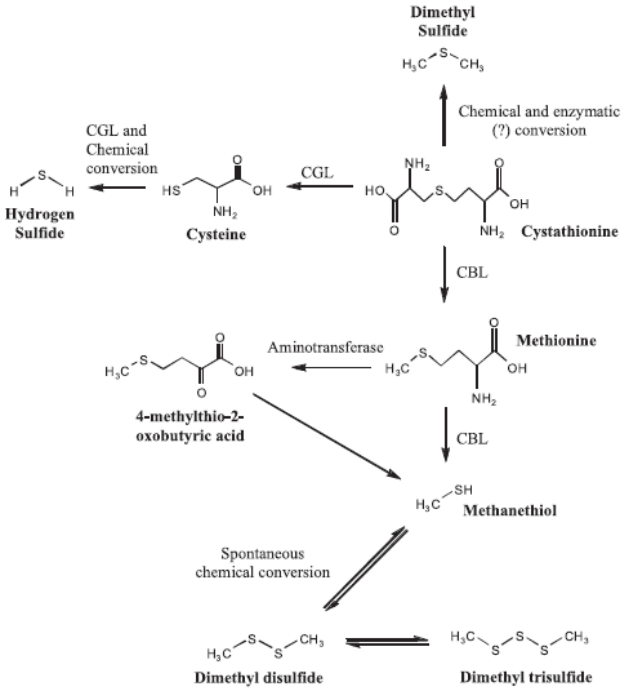

Figure 2. Proposed catabolic pathways of methionine, cystathionine, and cysteine in *L. helveticus* (Lee *et al*., 2007).

Limited number of reports on amino acids catabolism indicated that *L. helveticus* is less studied than *Lactococcus lactis.* Amino acids catabolism pathways for *L. lactis* are widely available and studied intensively. The reason behind this situasion is because study on amino acid catabolism by *L. lactis* was represented as model of cheese manufacture.

3. Role of *L. helveticus* on Cheese Debittering

The action of proteolytic enzymes on casein can produce bitter peptides. The bitterness in cheese is due to the partial casein hydrolysis. *L. helveticus* is recognized among LAB for its proteolytic system and its ability to reduce bitterness and accelerate flavor development in cheese (Broadbent *et al.*, 2011; Griffiths & Tellez, 2013). This ability is strain specific, where different strains vary widely in this characteristic, and *L. helveticus* CNRZ 32 is commonly used as strain marker for its active protease and peptidase toward bitterness reduction and flavor development. *L. helveticus* has a complex proteolytic system capable of degrading casein into peptides and free amino acids, thereby fulfilling their nutritional requirements when grown in milk. The hydrolysis of protein into peptides and free amino acids is an important series of events in cheese ripening and flavor development, where the balance between protease and peptidase activities is important for proper flavor generation without the formation of bitterness (Ardo *et al.*, 1989).

3.1. Source of bitterness

Bitterness is a limiting factor for milk based fermentation product such as cheese. During the enzymatic hydrolysis of proteins, bitter taste peptides are released limiting their application in food processing. This defect

seems to be one of the main concerns in cheese production process which is mainly caused by the proteolytic enzymes action on casein. Proteolysis of caseins contributes to the flavor development during ripening of semi-hard cheeses. The proteolytic system involved in casein utilization provides cells with essential amino acids for their growth in milk and is also has significant contribution to the formation of organoleptic properties of fermented milk product (Savijoki *et al*., 2006). The exploitation of casein by LAB is initiated by a CEP that degrade the protein into oligopeptides that are subsequently used by the cells through specific peptide transport systems for further degradation into rather shorter peptides and amino acids by the action of various intracellular peptides (Kunji *et al*., 1996). However, this action of proteolytic enzymes on casein might lead to the formation of bitter peptides (Soeryapranata *et al*., 2004). The relation of starter proteolysis to bitter peptides formation is indisputable. Most of bitter peptides formed is related to enzymic hydrolysates of casein rather than from cheese itself (Habibi-Najafi & Lee, 1996). Generally rennet proteolysis of casein resulted the formation of large peptide which is non bitter, but become precursors to bitter peptides due to further proteolytic cleavage caused by starter or non starter microorganisms. Bitter peptide in cheese may also formed when rennet or rennet substitutes are used at excessive level (Visser, 1977). Accumulation of bitter-tasting peptides at sufficient concentration will result in bitterness, a major taste defect in Gouda and Cheddar cheeses.

Bitterness is an off flavor which might reduce the flavor acceptance quality. It is due to the accumulation of bitter tasting peptides which usually has molecular weight (M_w) of less than 6000 D (M_w range of either 500 and 3000 or > 3000) and are composed mainly of hydrophobic amino acids (Lemieux & Simard, 1992; Saha & Hayashi, 2001). Bitterness is related to the average hydrophobicity of the peptide (Q value) which is defined as the sum of the free energies of transfer of the amino acid side chains from ethanol to water, divided by the number of amino acids residues in the peptide, i.e. $Q = \Sigma \Delta g/n$, where Δg is the transfer of free energy and n is the number of amino acid residues (Ney, 1979). A peptide is almost certainly bitter when its Q value exceeds 1400 cal/mol (Fukui *et al*., 1983). Proteins with high Q value such as casein (1605 cal/mol), and soybean protein (1540 cal/mol) would give bitter peptides. The bitterness seems to be related to a high degree of hydrolysis (DH). Small peptides have been shown to be bitter if they contain predominantly hydrophobic amino acids residues.

Cheese manufactured without starter cultures does not posses typical flavor, it means that addition of starter culture is essential for specific flavor formation. However, starter proteinase is responsible for formation of bitter peptides, at least partially, since bitter taste appeared in cheese production is related to the proteinase systems of LAB used. While, the other part is related to rennet (Habibi-Najafi & Lee., 1996), and there is a relationship between the strain used and the level of bitterness. Proteolysis, however, plays a direct role in development of desired texture, aroma, and bitterness reduction.

Various procedures had been developed to reduce or eliminate bitter peptides causing the bitter taste, but some known procedures might be accompanied with a significant loss of essential amino acids. Basically, bitter taste could be reduced by further hydrolysis of protein hydrolysates like casein. Additional hydrolysis of casein hydrolysate by the application of exopeptidases like aminopeptidase from different sources has been used successfully to debitter protein hydrolysates (Saha & Hayashi, 2001). Exopeptidase activities have been detected in the number of lactic acid bacteria. Debittering process is related significantly to the ability of exopeptidase to hydrolyze proline-containing peptides which is often posses a bitter peptides, either by direct degradation of proline-containing peptides (Habibi-Najafi & Lee, 2007) or indirect by removing the blockage (proline residue) that blocked further degradation of such peptides by aminopeptidases (Habibi-Najafi & Lee, 1996).

3.2. Analytical tools for detecting flavor compound

Most of the studies on amino acid catabolism in cheese by LAB have been conducted using chemically defined medium by testing the presence or absence of certain amino acid of interest. The decrease in amino acid content is detected as their conversion into other compounds. However, there are

related analyses involved in the metabolic pathways of amino acids catabolisms leading to the detection of of flavor compounds produced in cheese. Enzyme activities are measured using spectrophotometer using cell free extract of LAB cultures that used in cheese production based on simulated condition. Production of organic acid during cheese ripening was detected using several chromatography including liquid and gas chromatography. Liquid chromatography was directed for study of glycolysis and fermentative pathways as well as to monitor the degradation of amino acids occured, while gas chromatography for detection of volatile compounds of amino acids degradation. Thin layer chromatography can also be applied for detection of flavor components such as organic acid and sugar (Mariley & Casey, 2004).

New Approach Based on Genome Sequence Analysis

The recent available information on genome sequences of *L. helveticus* provide an insight into all encoded proteins that potential of metabolism of amino acids. By computer simulation and by the use of bioinformatics tools, search in genomes for the different components that could contribute to flavor formation from amino acid can be predicted. The genome data have provided insights into the different sets of metabolic capabilities necessary for different species, in which for *L. helveticus* is dairy niche. It is therefore open for information on the potential of biosynthesis and metabolic routes as well as regulatory and transport system of LAB. Whole genome analysis should broaden our knowledge of the mechanisms and pathways of flavor-generating strains of *L. helveticus.*

Liu *et al*. (2012) performed a genome-wide in silico analysis to reveal the transcription regulatory interactions that control the expression of the genes encoding various key enzymes involved in cysteine and methionine metabolism in all sequenced species of the order *Lactobacillales* (40 strains) including *L. helveticus* DPC 4571. The combination of availability of next generation sequencing, systems biology and single-cell technology will ultimately reveal the complex metabolism of LAB starter culture during flavor cheese generating (Steele *et al*., 2013).

Conclusion

Cheese making is a complex process which consists of many steps, involving at least chemistry and biochemistry of milk, microbiology and enzymology. The role of *L. helveticus* to control generation of cheese flavor from amino acid catabolism has been studied using several available *L. helveticus* strains. All results from several studies using starter and adjunct cultures provide new information with its strain specificity. The available information from the new technologies is very useful to improve the cheese quality. New insight of flavor generation by *L. helveticus* is made possible in this near future.

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