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乳酸菌蛋白酶与发酵乳制品质量相关性研究进展

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摘要:乳酸菌可合成蛋白酶,尽管乳酸菌蛋白酶活性较弱,但可作用于酪蛋白,不仅对乳酸菌在乳中的生长至关重要,也对发酵乳制品风味质地的形成和品质具有重要的影响。本文综述了乳酸菌蛋白水解系统、酪蛋白的降解产物、乳酸菌蛋白酶的水解性质、乳酸菌蛋白酶对发酵乳制品质量影响的研究现状和进展。

关键词:乳酸菌,蛋白酶,酪蛋白水解,发酵乳品质

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乳的发酵是乳成分的预消化,如乳糖分解、蛋白质和脂肪的降解[1]、可溶性钙和磷的含量提高,与普通乳相比,其营养价值更高,可以降低乳糖不耐症的发生几率[2],具有一定的益生作用[3-4]。同时,发酵也赋予了乳制品特殊的品质,其中凝乳是发酵乳品质的重要特征。据报道凝乳特性与乳酸菌的产酸能力和产酸速率密切相关[5-6],然而产酸能力相接近的乳酸菌凝乳特性存在显著差异,这说明凝乳过程与凝乳特性可能与乳酸菌蛋白酶的性质有关。

1 乳酸菌蛋白水解系统

乳酸菌在乳中的生长依赖于其自身的蛋白水解系统降解乳蛋白,主要是酪蛋白来满足自身的生长需要[7]。乳酸菌蛋白水解系统依据其功能可分为3个部分:(1)将大分子酪蛋白水解成多肽的胞外蛋白酶;(2)位于细胞膜的转移系统将降解产物转运过细胞质膜;(3)将转运进入细胞的多肽进一步水解形成自由氨基酸的多种肽酶,这些氨基酸最终进行代谢或者被用于合成蛋白质[8]。酪蛋白最初由细胞壁附着的胞外蛋白酶(CEP蛋白酶)降解,产生大量的大分子寡肽;随后经由乳酸菌外肽酶进一步降解来满足必需氨基酸和刺激生长氨基酸;最后细胞内蛋白酶如氨基肽酶、二肽酶、内肽酶、胱氨酸肽酶等,将寡聚多肽最终水解为氨基酸,供给生长代谢需要。

目前对乳球菌蛋白水解系统的研究最为透彻,已了解大部分降解酪蛋白和转运降解产物所需要的酶,其次是对乳杆菌属,如瑞士乳杆菌、德氏乳杆菌和干酪乳杆菌的研究,但是对这些乳酸菌酪蛋白降解产物的转运没有做深入研究[9]。而且研究认为酪蛋白降解、肽/氨基酸转移系统的生物化学途径在其他乳酸菌种属中是与此相一致的[10]。

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2 酪蛋白的降解产物

酪蛋白在乳中多数是以酪蛋白胶束的形式呈胶体状分布的，具有一定的流动性。β-酪蛋白含有大约12%的α-螺旋结构可能与蛋白酶不能降解N末端区域有关。乳酸菌蛋白酶的底物特异性范围很宽，但不能明确其切割位点，酶水解作用时优先断裂特异键，特异位点与酪蛋白种类性质及他们的存在形式（纯品酪蛋白或者乳中酪蛋白胶束）有关[11]，见表1所示。

2.1 β-酪蛋白

不同乳酸乳杆菌、乳酸乳球菌和瑞士乳杆菌纯化蛋白酶体外降解β-酪蛋白时，只有部分β-酪蛋白被降解为100多种4-30个氨基酸残基的寡肽和苯基丙氨酸，没有形成游离氨基酸、二肽和三肽，50%以上的肽来自C末端，其余的肽有一半来自60-105区域。

2.2 κ-酪蛋白

乳酸乳球菌蛋白酶降解κ-酪蛋白产生大量小的寡肽，主要来自于96-106区域和C末端。各菌种蛋白酶都能水解κ-酪蛋白，但是水解情况不同，有的甚至需要24 h。κ-酪蛋白多数序列区域能被所有酶水解，其中也存在特异性水解位置。产物中可能都存在相同的肽，但产量不同。

2.3 α_{S1}-酪蛋白

鉴定α_{S1}-酪蛋白水解产生的25种寡肽，证实其中一半的肽来自于C末端区域，各种酶的水解产物中一些小的寡肽连接在特异性的化学键位置。各个种属的乳杆菌都能水解Glu148-Leu149位点，而其他的水解位点都具有菌株特异性[8]。

表1. 乳酸菌CEP蛋白酶性质[12]

Table 1. Main properties of cell-envelope proteinases identified in LAB[12]

Strains	Molecular weight/kDa	Substrates	Type	Optimum temperature/℃ (pH)	Reference
Lc. lactis subsp. *cremoris* AM1	-	κ-, β-casein	S/PIII	-(-)	[13]
Lc. lactis subsp, *cremoris* H2	180	κ-, β-casein	S	-(5.5)	[14]
L. casei subsp. *casei* HN14	-	β-casein	S/PI	-(-)	[15]
L. delbriickii subsp. *bulgaricus* CNRZ 397	170	α_{S1}-, β-casein	S	42(5.5)	[16]
L. helveticus CP790	45	α_{S1}-, β-casein	S/PIII	42(6.5)	[17]
L. helveticus L89	180	α_{S1}-, β-casein	S	50(7.0)	[18]

S: serine-proteinase; -: not available.

3 乳酸菌蛋白酶的生化特性及分类

不同种属的乳酸菌菌株所分泌的蛋白酶分子量差异很大（表1），介于30-200 kDa，最适温度范围一般为40-50 ℃，最适pH接近中性pH5-8[19-20]。本课题组采用超滤、弱阴离子交换层析和弱疏水层析相结合的方法分离三株保加利亚乳杆菌蛋白酶[21]，分子量分别为39、40和52 kDa，最适反应温度均为42 ℃；在4-50 ℃比较稳定，热变性温度68-71 ℃；最适反应pH均约为6.0，在pH5.5-7.0比较稳定；是金属离子蛋白酶和丝氨酸蛋白酶。

乳酸菌蛋白酶由大约200个残基组成的蛋白前体构成，各种属乳酸菌蛋白酶具有同源性，N端依次包括前体区域（PP）对应一个分泌信号序列（40残基），催化区域（PR）催化去掉的原序列（150残基），催化区域（PR）（500残基），插入区域（I）（150残基）调节CEPs底物特异性，A区域（400残基）功能未知，B区域（500残基）参与稳定CEP活力/特异性，螺旋区域（H）（200残基）参与A区域和B区域定位，亲水W域（100残基）。多数CEPs具有B区域，但嗜热链球菌PrtS蛋白酶没有B区域；嗜热链球菌PrtS蛋白酶W区域包含革兰氏阳性菌细胞壁结构典型的氨基酸，但与CEPs没有同源性；H区域只存在于PrtP（210氨基酸）、PrtS（367氨基酸）和PrtH（72氨基酸）；PrtP、PrtR和PrtS蛋白酶的W区域连接绑定区域（AN）；瑞士乳杆菌和保加利亚乳杆菌CEPs没有绑定区域（AN），W区域与细胞壁相连；瑞士乳杆菌PrtH蛋白酶可能通过S层蛋白与细胞壁相连；鼠李糖乳杆菌PrtR蛋白酶与发酵剂乳酸菌CEPs不同，B区域偏小，没有螺旋区域（H）和插入区域（I），其W区域和细胞表面抗原蛋白具有同源性[22-23]（图1）。

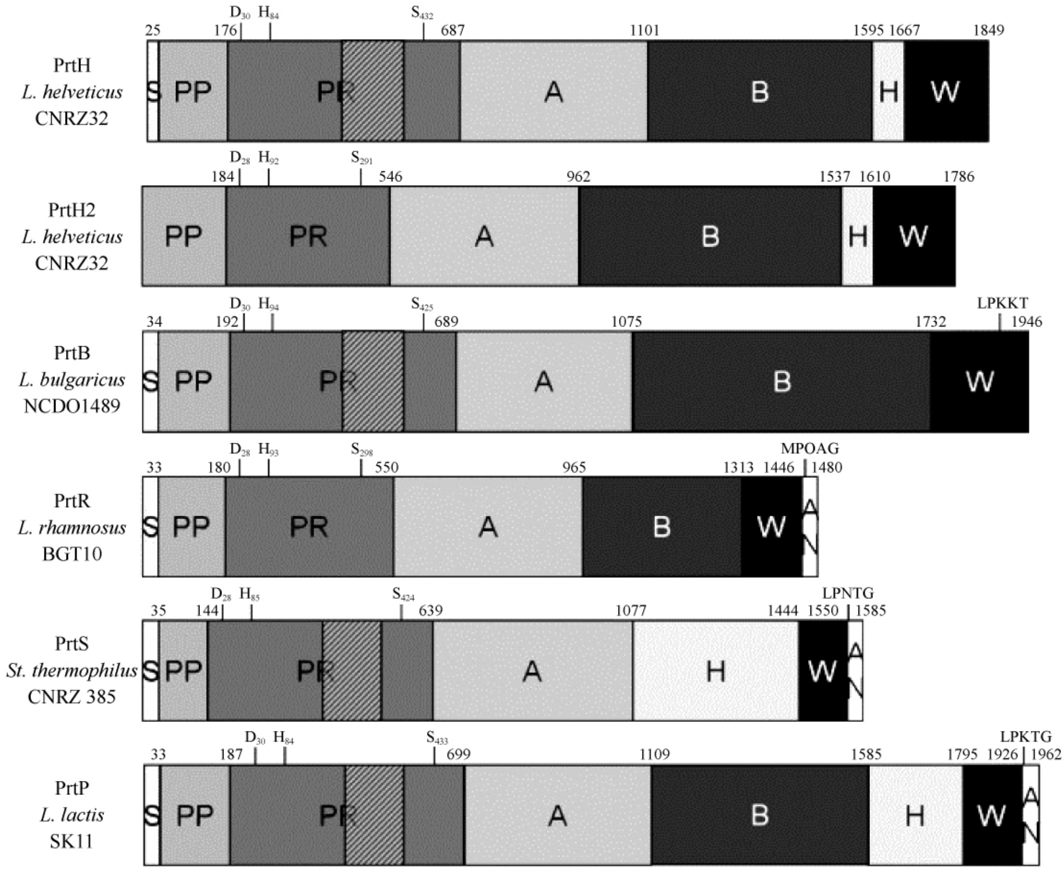

图 1. 不同乳酸菌蛋白酶功能区域[8]

Figure 1. Schematic representation of the predicted domains of CEPs from different LAB[8]. PrtH and PrtH2 from *L. helveticus* CNRZ32, PrtB from *L. delbrueckii* subsp. *bulgaricus* NCDO1489, PrtR from *L. rhamnosus* BGT10, PrtS from *St. thermophilus* CNRZ 385 and PrtP from *L. lactis* SK11. The eight main domains: signal sequence (S), prodomain (PP), catalytic proteinase domain (PR), A-domain (A), B-domain (B), helical domain (H), (cell-wall domain), anchored domain (AN). The hatched part of the catalytic domain corresponds to the insertion I domain. Numbers refer to the last amino acid of each domain. Positions of the three amino acids of the catalytic triad are indicated: D (aspartic acid), H (histidine) and S (serine).

乳酸菌蛋白酶分为 PrtP(*L. lactis* 和 *L. paracasei*)、PrtH (*L. helveticus*)、PrtR (*L. rhamnosus*)、PrtS(*St. thermophilus*)和 PrtB(*L. bulgaricus*)五种,本课题组分离得到的保加利亚乳杆菌蛋白酶属于 PrtB。不同种属的乳酸菌克隆表达的细胞壁蛋白酶的类型不同,一般情况下乳酸菌只分泌一种细胞壁蛋白酶,但瑞士乳杆菌和保加利亚乳杆菌表达两种[7]。不同类型乳酸菌蛋白酶作用底物不同,乳球菌 PrtP 蛋白酶依据作用酪蛋白类型不同分为 PI 和 PIII 两种,PI 型蛋白酶主要降解 β-酪蛋白,也能水解少量的 κ-酪蛋白,而 PIII 型蛋白酶能同等程度水解 α_{S1}-酪蛋白、κ-酪蛋白和 β-酪蛋白。中间类型蛋白酶 PI/PIII 与 PI 型蛋白酶相似能降解 β-酪蛋白,也能水解 α_{S1}-酪蛋白。PrtP 蛋白酶依据 α_{S1}酪蛋白断裂位置(f1-23)分 7 种类型 (a,b,c,d,e,f 和 g)。乳杆菌蛋白酶(PrtH、PrtR、PrtB)分为 PI 型、PIII 型和中间类型 PI/PIII,嗜热链球菌 PrtS 蛋白酶具有中间类型 PI/PIII 蛋白酶特异性[22]。

4 乳酸菌蛋白酶对发酵乳制品质量的影响

蛋白水解作用是发酵乳制品加工过程中很重要的生物化学过程(表 2),奶酪加工中酪蛋白水解产

生的氨基酸是特定的风味化合物的主要前体[24]。水解产生的疏水性肽(如富含脯氨酸的肽)积累带来的苦味，是 Gouda 奶酪和 Cheddar 奶酪加工中严重的产品质量问题[25]，CEP 蛋白酶的特异性对苦味肽的形成具有重要作用[26]。另外，乳酸菌肽酶(包括 PepN，PepX，PepO2，PepO3)参与降解苦味肽，而这些肽直接影响乳制品的感官质量[27-28]。克隆表达 *Lb. helveticus* CNRZ32 PepO2、PepO3 和 PepN 食品级乳酸乳球菌菌株，可以减少奶酪的苦味[28]。此外，过表达某些基因片段如 *pepN* 或 *pepC*，能改善 Cheddar 奶酪的风味，提高特定游离氨基酸水平[29]。蛋白水解的平衡对于奶酪加工中风味物质的形成，尤其是防止产生苦味是十分重要的，游离氨基酸的改组控制着蛋白质形成风味物质的速率[24]，因此控制蛋白降解系统本身并不是加快奶酪风味形成的关键。通过表达瑞士乳杆菌或者德氏乳杆菌乳酸亚种肽酶的重组菌株，提高乳酸乳球菌的蛋白水解力也可以加速乳酪蛋白水解作用，进而加速奶酪成熟过程[30-31]。除蛋白水解系统外，乳酸菌菌体自溶对发酵乳制品的品质也有重要作用，发酵后期乳酸菌自溶将细胞质中的肽酶释放到发酵完成的乳凝胶中，是发酵乳制品风味形成的前提[32-33]。而乳酸乳球菌 PrtP 蛋白酶特性、位置和表达量与菌体自溶有关。本课题组从传统发酵食品中分离的乳酸菌具有不同蛋白水解度、肽酶活力和自溶率，可以作为辅助发酵剂用于奶酪加工[34]。

嗜热链球菌的蛋白酶活性与其高产酸速率相关[35]，而其高产酸速率直接关系到发酵乳的凝胶质量。酸奶加工中添加保加利亚乳杆菌蛋白水解活性菌株可以缩短发酵时间并且增加坚实度[36]。流变学角度认为有限的蛋白水解能够降低乳蛋白凝胶的凝胶点 pH，使得凝胶迅速并改善凝胶过程[37]。综上所述，乳酸菌蛋白水解活性影响高蛋白乳凝胶的形成和稳定性，但是并不确定乳酸菌蛋白酶活性是否影响发酵乳的结构[38-39]。本课题组前期研究发现，同一种属的不同乳酸菌菌株的蛋白水解能力不同，蛋白水解能力的差异不会显著影响发酵乳的凝胶粒度，中等程度的蛋白水解有利于发酵乳凝胶硬度的增加，而表观黏度随着蛋白水解能力的增加而降低[40]。

表 2. 乳酸菌蛋白酶影响发酵乳制品质量

Table 2. Effects of proteinases from LAB on fermented dairy products

Strains	Enzymes (genes)	Effect
Lc. lactis	CEP	Reduce bitterness resulted from the accumulation of hydrophobic peptides in reduced-fat Cheddar cheese[26].
L. helveticus CNRZ32	Peptidases PepN, PepO2, PepO3	Involve in the degradation of bitter peptides and reduce bitterness in cheese[28].
L. delbrueckii subsp. *lactis*	Peptidases	Accelerate cheese proteolysis and, hence, the ripening process[30].
Lc. lactis subsp. *cremoris* NM1	*pepN* or *pepC*	Result in a positive effect on Cheddar cheese flavor and increases the level of specific free amino acids in cheese[29].
Lc. lactis	Autolysis of LAB	Accelerate the ripening process of cheese[32].
L. delbrueckii subsp. *bulgaricus*	Proteolytic enzymes	Reduce the fermentation time in making yoghurt and improve firmness[36].

5 展望

对于发酵乳制品而言，酪蛋白的降解对发酵质地和风味形成是至关重要的。在乳中生长时，保加利亚乳杆菌蛋白酶阴性菌株只能达到阳性菌株22%的生物量[41]，然而嗜热链球菌需要的氨基酸较少而且自身能够合成支链氨基酸，乳中的游离氨基酸和肽就足以供给菌体生长需要[42-43]，也就是说嗜热链球菌在乳中生长的营养需求比保加利亚乳杆菌少，这解释了商业发酵剂保加利亚乳杆菌蛋白水解活性较高，而嗜热链球菌的蛋白水解能力却有限[44]。从乳制品中分离的多数乳酸菌都是多重氨基酸的营养缺陷体，乳中天然的游离氨基酸和肽很少，随着菌体的生长很快就被消耗掉，而酪蛋白含有乳酸菌在乳中生长达到高细胞密度所必需的所有氨基酸，因此酪蛋白的水解对菌体生长是十分必要的。乳酸菌的蛋白水解能力很弱，但酸乳等发酵乳制品会有不同程度的蛋白水解，水解程度取决于保加利亚乳杆菌和嗜热链球菌接种比例、培养温度和时间。因此本课题组将根据前期实验结果确定具有促进凝乳质量蛋白酶的乳酸菌，提取纯化具有促进凝乳质

量的蛋白酶,并研究蛋白酶性质及其对凝乳理化性质和凝块品质的影响,发掘与凝乳品质有关的蛋白酶基因,旨在通过研究乳酸菌蛋白酶特性来选择优良酸奶生产用菌种,为高品质发酵乳的生产提供理论依据和基础。

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Lactic acid bacteria proteinase and quality of fermented dairy products – A review

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Abstract: Lactic acid bacteria (LAB) could synthesize cell envelope proteinase with weak activity, which primarily degrades casein. In addition to its crucial role in the rapid growth of LAB in milk, LAB proteinases are also of industrial importance due to their contribution to the formation of texture and flavor of many fermented dairy products. The proteolytic system, properties of proteinase, the degradation product of casein and its effect on the quality of fermented dairy products were reviewed in this manuscript.

Keywords: lactic acid bacteria, proteinase, proteolysis of casein, quality of fermented dairy products

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