富硒乳酸菌及其发酵食品的研究进展

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摘　要:乳酸菌作为益生菌中的一个类群,被广泛应用于食品、医药以及化妆品行业。硒,作为人体必需的微量元素之一,在人体新陈代谢及免疫调节方面起到至关重要的作用。近年来,富硒乳酸菌因为其突出的抗氧化性、抗炎症、抗癌活性以及可以将无机硒转化成有利于人体吸收的有机硒而备受关注。然而,乳酸菌对于硒的代谢途径还有待进一步深入研究;同时,对于富硒益生菌及其发酵食品的毒性及副作用也需要更为全面的测试与评估。本文从硒元素的价态、硒对于乳酸菌生长的影响、乳酸菌对硒的有机转化、富硒乳酸菌的生物活性以及富硒功能食品这五个方面对其进行综述。

关键词:乳酸菌,硒,功能性食品

Research advance of Se-enriched lactic acid bacteria and relevant fermented foods

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Abstract: Lactic acid bacteria are a class of probiotics widely used in food, medical and cosmetics industry. As an essential trace element for human, selenium (Se) plays a critical role in metabolism and immunoregulation. In recent decades, Se-enriched lactic acid bacteria attract extensive attention due to their outstanding antioxidant, anti-inflammatory, antitumor activities and the ability to transform inorganic selenium into organic form, which is beneficial to human absorption. However, further studies about metabolic pathways of Se by LAB are needed; meanwhile, it is necessary to test and evaluate the toxicities and side effects of Se-enriched probiotics and their fermented foods. In this paper, the oxidation state of selenium, the effects on the growth of LAB by selenium, the transformation of organic selenium by LAB, bioactivities of Se-enriched LAB and Se-enriched functional foods were summarized.

Key words: lactic acid bacteria; Selenium; functional food
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乳酸菌(*Lactic acid bacteria*,LAB)是世界一般公认安全的食用细菌(General Regard As Safety,GRAS),常见的种属包括乳球菌属(*Lactococcus*)、肠球菌属(*Enterococcus*)、酒球菌属(*Oenococcus*)、片球菌属(*Pediococcus*)、链球菌属(*Streptococcus*)、乳杆菌属(*Lactobacillus*)等[1-4]。乳酸菌因其突出的益生特性而被广泛应用于食品领域,用来发酵食品及饮料,包括乳制品(酸奶和奶酪)、发酵蔬菜(橄榄、酱菜以及泡菜)和发酵肉制品(腊肠)等。从不同环境发酵食品中分离出来的乳酸菌常常作为益生菌用于功能食品材料中[5]。

硒是人体所必需的一种微量元素,在人体新陈代谢、免疫调节等方面起到重要作用。与有机硒相比,无机硒因为毒性大而难以被吸收利用。大量研究表明,通过微生物的生物转化作用,可以将无机硒变为有机态硒从而利于人体吸收并且相较于其他有机硒合成方式,既便宜又安全。目前,在富硒微生物的研究中,富硒酵母的研究最为广泛[6]。而有研究表明,乳酸菌对于硒也具备富集和转化的能力。

1　硒元素

1.1　硒的重要作用

硒,作为一种人体必需的微量元素,其重要性已被广泛认可。人体推荐摄入量为 55 μg/d,最大量不超过 400 μg/d[7]。近些年来,硒因为其突出的抗氧化特性以及抗癌特性而引起了广泛关注[8]。硒在人体内的主要功能包括新陈代谢的调节,免疫力的提

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表1 硒的价态及其化合物

Table 1 The oxidation state of selenium and selected selenium compounds

硒的价态	代表化合物名称	化合物化学式	主要特征
-2	硒化氢	H_2Se	挥发性气体,$pK_1=3.9$,$pK_2=11$
	硒化钠	Na_2Se	晶体,空气中缓慢分解
	硒化氢离子	HSe^{-1}	pH=7时为主要阴离子,不稳定
	硒化汞(Ⅱ)	HgSe	不溶沉淀物
	二甲基硒醚	$H_3C-Se-CH_3$	挥发代谢物,有类似大蒜气味
0	元素硒	Se_n	多原子链,部分为同素异形体
	胶体硒	Se_n	溶液中为橙红色同素异形体
	硫化硒	Se_nS_n	元素混合物
	硒基谷胱甘肽	GS-Se-SG	不稳定亚硒酸盐代谢物
+4	二氧化硒	SeO_2	白色晶体,水溶液为亚硒酸
	亚硒酸	H_2SeO_3	pK1=2.8,pK2=8.5
	亚硒酸盐	SeO_3^{-2}	pH=7时$HSeO_3^{-1}$为主要离子
+6	三氧化硒	SeO_3	白色晶体,水溶液为硒酸
	硒酸	H_2SeO_4	pK2=1.7
	硒酸盐	SeO_4^{-2}	pH=7时为主要阴离子

高,生殖能力的增强,以及显著的防癌作用等[9];此外,硒在清除自由基,延缓衰老,重金属离子的解毒以及抗地方性流行病等方面,效果也十分突出[10]。大量研究表明,硒所起到的作用与其化学形态密切相关[11],其常见价态如表1[12]。其中,零价态的纳米硒(Nano-Se)因为易于被人体吸收毒性小且能够发挥无机硒和有机硒所共有的功能性质,如抗肿瘤、抗氧化、增强机体免疫力等而成为目前的研究热门[13]。

1.2 硒的毒性及价态

超过一定限量的硒会引起中毒,硒中毒一般分为急性中毒和慢性中毒。典型的急性中毒,会在几分钟或几小时内就出现症状;慢性中毒则是因为反复摄入少量的硒积累所致,相应症状会在几天甚至更长时间后才出现,有时因个体差异而有所不同[14],不同化学结构的硒能够产生不同的毒性。一般而言,无机硒的毒性比有机硒大,有机硒的毒性比胶体状态单质硒大,纳米硒的毒性最小。Zhang 等[15]对纳米硒和亚硒酸钠的极性毒性进行比较,发现纳米硒对于老鼠生长的抑制作用更小;同时,纳米硒造成的肝功能异常程度更轻,其急性毒性是亚硒酸钠的1/7。而大鼠口服实验中,亚硒酸钠的平均致死量(LD_{50})为7 mg Se/kg,硫化硒的平均致死量为138 mg Se/kg,而单质硒的平均致死量为6700 mg Se/kg[16]。

2 乳酸菌对硒的富集及生物转化

微生物对于硒的有机转化是当前的研究热门。Sarathchandra 等[17]在进行富硒巨大芽孢杆菌(*Bacillus megaterium*)研究时,发现有红色物质的生成。此外,深红红螺菌(*Rhodospirillum rubrum*)、大肠杆菌(*Escherichia coli*)、荧光假单胞菌(*Pseudomonas fluorescens*)、枯草芽孢杆菌(*Bacillus subtilis*)、酒色着色菌(*Chromatium vinosum*)、脱硫杆菌(*Desulfovibrio desulfuricans*)、球形红细菌(*Rhodobacter sphaeroides*)等多种细菌也具有将硒酸盐或亚硒酸盐转化为这种红色物质的能力[18-20]。此外,酵母、真菌、藻类及植物对硒的生物富集转化实验中同样观察到了这种红色物质生成的现象;后续研究表明,这种红色物质主要成分为单质硒。

乳酸菌具有抗菌、产多种维生素、胞外多糖等益生特性而被广泛应用于食品工业中。随着研究深入,也发现乳酸菌具有对金属离子吸附、摄入以及进行生物转换的特性[21]。1995 年,Calomme[22]通过研究乳杆菌,发现其对于硒具有一定的富集作用,这是对乳酸菌具有富硒作用的第一次报道。保加利亚乳杆菌富硒示意图(图1)[23]如下。

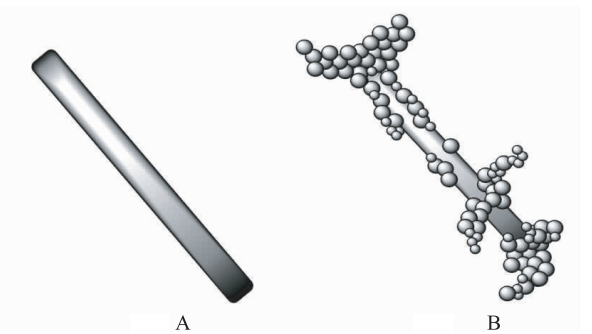

图1 乳杆菌富硒前后的对比,富硒前(A)富硒后(B)

Fig.1 Comparison of lactobacillus before and after the Se-enrichment process, before(A) after(B)

乳酸菌对于不同价态的无机硒转化效力及产生的有机硒形态也不同。与乳酸菌具有共性的动物双歧杆菌可以将无机硒转化为另一种重要的有机硒化合物——硒代蛋氨酸[24]。向酸奶中加入亚硒酸钠,乳酸菌可以将其转化为硒代胱氨酸和甲基硒代半胱氨酸,但硒酸钠不能被转化成同样的产物[25]。在无机硒溶液中,通过生物转化作用,乳酸菌可以将无机态的硒转换成不同形态的有机硒,而这些有机态硒主要是硒代胱氨酸、硒代蛋氨酸、硒代半胱氨酸、富硒生物质、二甲基硒、二甲基二硒以及单质硒等。其中,硒代半胱氨酸是转化后的主要产物,它能够进一步被合成硒蛋白。

表2 乳酸菌对硒的生物转化

Table 2 Biotransformation of Selenium by lactic acid bacteria

转化前硒的形态	转化后硒的形态	代表菌株	参考文献
硒(Ⅳ)	硒代半胱氨酸	*Lb.casei*,*Lb.plantarum*,*Lb.delbrueckii subsp. Bulgaricus*,*Lb.brevis*,*Yoghurt bacteria*	[29]
	硒(0)	*Lb.rhamnosus LB3*,*Lb.fermentum LB7*,*Lb.bulgaricus*	[30]
	富硒生物质	*E.faecalis LAB14*,*E.faecium LAB1*	[31]
	有机硒	*Lb.reuteri*	[32]
	甲基硒代半胱氨酸	*Sauerkraut natural fermentative bacteria*	[33]
硒(Ⅳ)/硒代蛋氨酸	二甲基硒,二甲基二硒,硒(0)	*Lb.rhamnosus 67B*,*Lb.acidophilus L10*,*B.lactis LAFTI B94*	[34]
亚硒酸氢钠	硒(0)	*St.thermophilus*,*Lb.acidophilus LA-5*,*Lb.helveticus LH-B02*	[26,35]

同样具有富硒能力的乳酸菌还有植物乳杆菌(*Lactobacillus plantarum*)、保加利亚乳杆菌(*Lactobacillus bulgaricus*)、干酪乳杆菌(*Lactobacillus casei*)、鼠李糖乳杆菌(*Lactobacillus rhamnosus*)、发酵乳杆菌(*Lactobacillus fermentum*)、罗伊氏乳杆菌(*Lactobacillus reuteri*)、短乳杆菌(*Lactobacillus brevis*)、旧金山乳杆菌(*Lactobacillus sanfranciscensis*)以及布氏乳杆菌(*Lactobacillus buchneri*)等[26-27]。研究表明,绝大多数乳酸菌具有富集硒并能将无机硒转化为有机硒的能力;由表2可以看出,大多数乳酸菌能够将+4价的无机硒转化为单质硒以及有机态硒[28]。

3 硒对于乳酸菌生长的影响

Xia[23]等在实验中发现保加利亚乳杆菌在一定质量浓度的亚硒酸钠溶液中可以富集大量的硒,且菌体本身能很好的生长;在终浓度为1~16 mg/L含有亚硒酸钠的培养液中,保加利亚乳杆菌的富硒效果很好,其最高富硒量达到了12.45±1.04 mg/L;此外,通过调整必需元素的比例(如磷、镁、锰、锌、钙及总氨基酸)从而提高有机体的营养价值及生物作用,同时产生大量不溶于水的单质硒。曾议霆[36]在培养液中加入6 μg/mL的亚硒酸钠溶液,培养一段时间后,比较加硒和未加硒乳酸菌生物量的变化,发现加硒后的植物乳杆菌BC-25的生物量较多,达到了0.2572 g/100 mL(对照组为0.2472 g/100 mL),这说明一定浓度的硒溶液不仅不会对菌株产生负面影响,甚至还会促进其生长发育。然而,浓度超过6 μg/mL的硒溶液却对植物乳杆菌M产生了副作用,黄秀锦[37]发现保加利亚乳杆菌在富硒过程中,其生物量随亚硒酸钠浓度的增加呈下降趋势,而富硒量则呈上升趋势,在亚硒酸钠浓度超过14 μg/mL后,生物量从0.48 g/100 mL急剧下降到近0.40 g/100 mL,乳酸菌对硒的富集由原来的生理性富集转变为病理性富集。因此,对于富硒乳酸菌的筛选和驯化,硒溶液的浓度是一个重要的影响因素,大量实验表明少量的硒可以促进菌株的生长。而一些特殊菌种,如耐久肠球菌具有在硒浓度很高的培养基中生长并富集硒的能力[38]。富集的硒一部分用于硒代胱氨酸的合成,而另一部分可以被还原成零价态的单质硒,单质硒在溶液中呈现红色[23]。

4 富硒乳酸菌生物活性研究

4.1 富硒乳酸菌抗菌活性

某些含硒化合物在不同的浓度范围内具有抗菌活性[39]。大肠杆菌产生的肠毒素能够引起人或动物的腹泻[40],而抗生素对于这类疾病的治疗并不明显,甚至有证据表明某些抗生素的使用会引起肾脏并发症[41]。益生菌因为其具有多种益生效果包括平衡肠道菌群,被认为是可行的替代方法[42]。乳酸菌在抑制某些致病菌方面尤为突出[43],Yang等通过动物实验发现,摄入了富硒嗜酸乳杆菌的小鼠,由致病性大肠杆菌所引起的死亡率相比于对照组(50.00%)、益生菌摄入组(43.75%)、亚硒酸钠摄入组(43.36%)、有机硒复合益生元摄入组(25.00%)、氧四环素摄入组(25.10%)是最低的,为18.75%。在体内以及体外实验中,富硒乳酸菌对于致病性大肠杆菌都表现出极强的抗性[44]。6×10^9 CFU的屎肠球菌与55 g有机硒混合,制成的胶囊能够减少炎症性肠病中致病菌的数量,并降低结肠癌的发病率[45]。而最近的研究表明,富硒乳酸菌还能够对某些真菌起到抑制作用,Kheradmand[46]等在实验中将二氧化硒驯化后的益生菌作用于白色念珠菌,发现其抗真菌作用突出。

4.2 富硒乳酸菌抗氧化活性

硒具有抗氧化特性。大量研究表明,不同膳食结构中的硒都能够提高人体组织的抗氧化能力。而这种功能特性主要是由于人体中的硒蛋白,如谷光氨肽过氧化物酶、硫氧还蛋白还原酶、脱碘酶以及硒蛋白P(硒蛋白的一种)的活性被显著提高而引起[47]。Penas[33]等在制作德国泡菜时,于发酵前加入0.3 mg亚硒酸钠,发酵后发现泡菜抗氧化活性因为有机硒的生成而显著提高,为对照组的1.75倍。Chen[48]等在动物实验中发现,口服富硒乳酸杆菌可以提高抗氧化酶活性,如超氧化物歧化酶等,而这一作用机制对于实验中人为造成的小鼠肝损伤具有抑制作用。而在Ren[49]等的实验中发现,动物体内这类酶的活性以及总体抗氧化能力在摄入了富硒富锌益生菌后也有显著提高。此外,从乳酸乳球菌乳酸亚种中分离得到的胞外多糖,在富硒后,能够使血清和肝脏中的谷胱甘肽过氧化物酶活性提高,从而提高了整体抗氧化活性和免疫调节能力[50];同时,这类富硒多糖可以对糖尿病起到一定的抑制作用[51]。

表3 富硒益生菌及发酵食品的健康功效

Table 3 Suggested health benefits of Se-enriched probiotics and fermented foods

编号	健康功效	富硒产品/富硒益生菌	参考文献
1	提高营养价值	富硒酸奶(有机硒),富硒克非尔乳(有机硒),富硒 Sauerkraut 泡菜(有机硒),富硒保加利亚乳杆菌(磷、镁、锰、锌、钙及总氨基酸)	[25,62]
2	提高硒的生物利用率	富硒布氏乳杆菌-26,富硒发酵乳	[27]
3	抗菌活性	富硒粪肠球菌-74,富硒组合益生菌	[53]
4	抗氧化性	富硒乳酸杆菌,富硒组合益生菌,富硒 Sauerkraut 泡菜	[33]
5	抗诱变性	富硒粪肠球菌-74,富硒长双歧杆菌	[63]
6	抗癌活性	富硒植物乳杆菌,富硒短乳杆菌	[64]
7	肠道菌群调节	富硒组合益生菌	[49]
8	生殖力提高	富硒组合益生菌	[65]
9	降胆固醇	富硒粪肠球菌-74	[66]
10	抗炎活性	富硒 Sauerkraut 泡菜	[33]

4.3 富硒乳酸菌抗癌活性

化合价不同的硒均具有不同的抗突变和抗癌作用[52]。浓度合适的硒溶液可以有效降低癌症发病率,但浓度过高就可能引起中毒。粪肠球菌-74 在一定浓度硒的刺激下,其抗氧氟沙星能力显著提高[53]。虽然硒诱导益生菌抗突变的机制还没有被完全阐明,但在生物转化的过程中,不同种类有机硒的产生成为引发这种机制的最大可能。在癌症基因治疗方面,某些细菌能够作用于实体瘤并释放治疗性分子[54]。Xu[55]等在实验中发现,与乳酸菌类似的双歧杆菌可以在实体瘤中生长和扩增,并将抗癌基因转入肿瘤中。富硒长双歧杆菌细胞能够结合重组内皮抑素和白介素 2 基因,对于 H22 肿瘤表现出抗癌效果[56]。此外,包含了纳米硒的植物乳杆菌[57]以及短乳杆菌细胞[58]也可以通过刺激作用,增加小鼠体内促炎症细胞因子的产量,从而使癌症预报过程中的免疫反应得到提高。

5 富硒乳酸菌及其发酵食品

硒对于人体的健康起到重要作用;因此,富硒发酵食品以及相关益生菌的研发对于人体营养需求也是十分必要的。在饮食中添加硒,尤其是富硒食品[59]以及硒的膳食补充剂[60]这一方法已经受到广泛重视与认可。目前,已报道过具有促进人体健康功效的富硒发酵食品如表 3 所示。由微生物发酵制成的富硒食品,能够为人体提供所需的硒代半胱氨酸以及硒蛋白等有机态硒,而这种由微生物转化出的硒,更为安全也更为营养;因此,食用含有富硒微生物的发酵食品,可以达到补充人体所需硒元素的目的,表 3 中给出了已报道过的富硒食品[61]。

Palomo[67]等对富硒酸奶进行分析,发现硒对于乳酸杆菌中伴侣蛋白的表达起到了重要作用,同时也减少了奶中的应激因子对于乳酸杆菌的不利影响。此外,通过对氨基酸成分进行分析,发现硒代半胱氨酸是最主要的含硒种类。而在肽谱图中,含硒氨基酸片段分析也证实了硒在硫氧还蛋白、谷氧还蛋白、清蛋白、β-乳球蛋白以及乳过氧化物酶的存在。Deng[68]等从西藏灵菇中分离出了耐硒能力极强的短乳杆菌 CGMCC No.6683,富硒培养后,与嗜热链球菌、保加利亚乳杆菌混合用来发酵脱脂乳,结果表明酸奶中硒含量显著提高,为 24 μg/g;同时,也检测到了含量较高的单质硒。

绿茶中含有丰富的必需膳食营养素和抗氧化剂[69]。Molan[70]等在研究中发现,富硒绿茶能够促进乳酸杆菌和双歧杆菌的生长。Vodnar[71]等对富硒绿茶进行研究,在体外实验中,发现 85.8~96 mg/kg 的富硒绿茶在不同浓度的壳聚糖海藻酸液中,对干酪乳杆菌和植物乳杆菌起到了保护作用。将 2 g 富硒绿茶溶于 100 mL 壳聚糖中,做成微型胶囊,在维持菌株稳定性上更加有效;同时,在 4 ℃下储藏 30 d,菌株活性也明显提高;在肠道模拟实验中,富硒绿茶也显著提高了乳酸菌的存活率,使其活菌数达到了 10^9 CFU 以上的益生水平。目前,富硒乳酸菌及其发酵食品因为突出的优良特性,已经引起了广泛关注。

6 总结

继富硒酵母之后,富硒乳酸菌也倍受关注。大量的实验结果表明,乳酸菌能够富集硒并通过生物转化作用使其成为有机态硒,从而更利于人体吸收。而这种富硒菌使得种类丰富的富硒型功能食品的出现成为可能,同时为解决地方性硒摄入量不足问题提供了新方案。通过生物转换作用,乳酸菌可以将无机硒转化为硒代胱氨酸、硒代蛋氨酸、硒甲基硒代半胱氨酸、二甲基硒、二甲基二硒以及单质硒等。对于乳酸菌自身而言,其某些生理特性,如生长状态、蛋白表达方式、抗氧化活性、矿物质及氨基酸的成分组成也会受到影响,进入到乳酸菌细胞内的硒元素,对于相关酶的活性有一定的刺激作用,这也是富硒乳酸菌生物活性相较于普通菌突出的原因之一。

近些年来,富硒益生菌及其发酵食品因为具有多种健康功效而受到了广泛的认可,这些功效包括生物利用率和抗氧化性能的提高,抗菌、抗癌、抗突变能力的加强等。一些临床实验也证实,某些富硒乳酸菌所转化出的单质硒或纯种硒化合物具有增强免疫刺激、抗衰老以及减少妊娠并发症的作用。富硒型益生菌产品在营养基因组学上的应用,正在为人类的健康带来福音。

然而,肠道微生物如何对硒进行代谢以及某些

临床表现出现的原因有待进一步深入。同时,对于富硒益生菌及其功能产品的毒性及副作用也需要更为全面的测试。因此,在这类功能食品成为大众消费品之前,更为广泛以及更加深入的调查和研究是十分必要的。而对于不同种属的乳酸菌,其对于硒的代谢途径及相关代谢模型也有待进一步确定。

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