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乳酸菌生理功能的系统解析与代谢调控

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摘要:作为工业化的细胞工厂,乳酸菌广泛应用于食品、农业和医药等行业。然而在乳酸菌的工业生产中以及作为益生菌在人体胃肠道系统中都会面临多种环境胁迫,这些胁迫环境严重影响乳酸菌的生理功能,从而影响食品微生物制造的效率。近年来,随着代谢工程和系统生物学的发展,为乳酸菌生理功能的改造带来了前所未有的机遇。本文综述了系统生物学和代谢工程在乳酸菌生理功能的优化和调控中的具体应用。

关键词: 乳酸菌, 生理功能, 代谢工程, 系统生物学

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乳酸菌(lactic acid bacteria, LAB)是一类可发酵碳水化合物并以乳酸为主要产物的细菌,广泛存在于自然环境中并对人体发挥着重要的生理功能。同时,作为一类重要的工业微生物,乳酸菌菌体及其代谢产物广泛应用于食品、医药、饲料、精细化学品等工业领域中。尤其在食品工业中,乳酸菌广泛应用于生产奶制品、香精香料、氨基酸、多糖、生物活性物质(叶酸和共轭亚油酸)、防腐剂等(表1)。乳酸菌具有营养需求复杂、能量合成效率低以及在生长过程中存在产物抑制等生理特性。此外,由于乳酸菌属于分类地位差异很大的细菌类群,对其生理功能解析、调控的研究进展缓慢。因此,在深入解析乳酸菌生理功能的基础上,全局优化、调控其代谢能力对提升乳酸菌食品微生物制造效率具有重要的理论和应用价值。

表1 乳酸菌在食品工业中的应用

Table 1 Applications of lactic acid bacteria

Application	Product	LAB	References
Dairy	Hard Cheese	*Lactococcus lactis*	[1]
		Leuconostoc spp.	
	Yoghurt	*Streptococcus thermophilus*	[1]
		Lactobacillus delbrueckii	
Flavour	Acetaldehyde	*Lactococcus lactis*	[1]
ingredients	Diacetyl	*Lactococcus lactis*	[2]
Amino acid	L-Alanine	*Lactococcus lactis*	[3]
	γ-aminobutyric acid	*Lactococcus brevis*	[4]
Bio-activate	Folic acid	*Lactococcus lactis*	[5]
substance	Linolenic acid	*Lactobacillus plantarum*	[6]
Preservative	Nisin	*Lactococcus lactis*	[1]

作为食品微生物制造的主体,乳酸菌用于食品制造中首先要求细胞能够定向、高效地生产目标代谢产物,因此必须干扰或改变微生物原有的调控体系,改善细胞的生理功能,从而将细胞改造成高效地"细胞工厂"。此外,乳酸菌在食品制造中还会面临包括酸、碱、冷冻干燥、饥饿、低温、高渗透压等恶劣的物理、化学或营养环境因素胁迫作用,从而造成细胞结构、基因转录和蛋白表达发生改变,酶原的激活以及代谢途径的调整,导致细胞生理功能缺失,抑制

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细胞的活力甚至细胞死亡,从而降低食品微生物制造的效率。因此,基于乳酸菌为主体的食品微生物制造过程中,需要:(1) 对乳酸菌的代谢能力进行重构与优化;(2) 从鲁棒性(Robustness)和对营养环境条件的适应性(Fitness)两方面调控和优化乳酸菌的生产性能,以实现目标代谢产物的产量、产率和生产强度的提高。

过去的几十年间,人们运用传统诱变和生化工程等手段对乳酸菌进行改造,优化其代谢特性,在一定程度上显著提升了乳酸菌的生产效率。近年来,随着乳酸菌全基因组序列测序工作的不断推进以及代谢工程操作手段的不断更新,使得全局上理解、阐释乳酸菌的生理功能,并采用代谢工程或生化工程手段更为有效地定向调控乳酸菌的生理功能成为现实。本文在总结乳酸菌基因组工程技术研究进展的基础上,对乳酸菌生理功能的解析和调控进行了总结。

1 基于基因组工程技术的乳酸菌生理功能解析

以基因组序列为基础的系统生物学的发展,为从基因表达、蛋白质组的时序变化、代谢物含量及代谢流量比率等方面全局、深度解析工业微生物在合成目标代谢产物的过程中,发生在基因、酶、生化反应、代谢网络等层次上的时序变化提供了强有力的工具,从而为代谢途径的重构与优化、生产性能的调控与优化奠定了坚实的基础[7]。

1.1 乳酸菌全基因组测序研究进展及其在乳酸菌生理功能解析中的应用

随着第一株乳酸菌—乳酸乳球菌乳酸亚种(*Lactococcus lactis* ssp. *lactis* IL1403)全基因组测序工作于2001年完成[8],在世界范围内掀起了乳酸菌全基因组测序的浪潮,至2010年,超过34株乳酸菌基因组测序工作相继完成并向国际公共数据库(www. uniprot. org)递交了全基因组序列(表2)。

由表2可知,乳酸菌的基因组具有以下特点:(1)全基因组的长度在1.8–2.9 Mb之间,但干酪乳杆菌ATCC334和植物乳杆菌WCFS1的基因组达到了2.95和3.35 Mb;(2)G+C含量通常为50%左右,最高为60.1%(双歧杆菌),最低为32.9%(唾液乳杆菌UCC118和德式乳杆菌ATCC BAA-365)。乳酸菌全基因组测序的完成,为全面分析和阐释其生理功能奠定了坚实的基础[7]:(1)乳酸菌具有完整的碳源转运和代谢系统;(2)能量代谢途径:乳酸菌主要通过糖酵解途径来获取能量,但基因组分析发现乳酸乳球菌IL1403基因组中还有编码有氧呼吸的酶类,表明存在其他产能途径[8];(3)生长因子合成途径:基因组分析发现不同种属乳酸菌的氨基酸合成途径存在不同程度的缺失,如嗜酸乳酸菌、约氏乳杆菌等缺乏维生素和嘌呤核苷酸合成必需的关键酶,而植物乳杆菌则可合成除亮氨酸、异亮氨酸、缬氨酸以外所有的氨基酸[17];(4)理解两菌生理关系:如保加利亚乳杆菌和嗜热链球菌是发酵乳生产中常用菌种,保加利亚乳杆菌几乎不具备氨基酸的合成能力,而嗜热链球菌基因组中具有除组氨酸以外所有与氨基酸合成相关的酶,保加利亚乳杆菌利用较强的蛋白水解能力为嗜热链球菌提供生长所需的氨基酸和短肽[10];(5)细菌素合成基因:研究人员在乳酸乳球菌6F3中,发现了编码nisin合成酶的具有11个基因,大小为15 kb的完整基因簇;(6)细胞表面多糖合成基因:乳酸菌基因组分析发现与胞外多糖产生相关的一个EPS基因簇,包括14个基因,编码高度保守的蛋白EpsA、EpsF、EpsJ、EpsI和5种糖基转移酶、多糖合成酶[9]。

1.2 转录组及蛋白质组解析乳酸菌生理功能

转录组学数据在解析乳酸菌生理功能的进展包括:(1)解析抵御环境胁迫的生理机制:Broadbent等[28]发现在酸胁迫条件下*L. casei* ATCC 334组氨酸合成中的8个基因簇(LSEI_1426-1434)和组氨酸渗透酶基因显著上调,通过外源添加组氨酸,使*L. casei*酸胁迫(pH 2.5)条件下的存活率提高了100倍。另一方面,Pieterse等研究*L. planturum* WCFS1在乳酸/乳酸盐、pH、渗透压等胁迫下的基因表达差异,发现了一组编码细胞表面蛋白并高表达的基因在乳酸响应环境胁迫过程中发挥重要作用;(2)解析乳酸菌糖代谢机理:Barrangou等[29]研究了*L. acidophilus* NCFM以葡萄糖、果糖、蔗糖、半乳糖、海藻糖、棉籽糖和低聚果糖为碳源时的全基因表达谱,发现摄取单糖(葡萄糖、果糖)和二糖(海藻糖和蔗糖)时需要PTS;而多糖(棉籽糖和低聚果糖)的利用需要ABC转运系统,乳糖和半乳糖的摄取需要GPH转运系统;(3)挖掘代谢调控因子:Azcarate等[30]发现嗜酸乳杆菌NCFM的组蛋白激酶的双组

表 2 已完成基因组测序的乳酸菌

Table 2 Summary of genome sequencing of lactic acid bacteria

Strain	Size/Mb	Genes	Pseudo genes	Proteins	RNAs	(G + C) mol%	Origin/use	References
Lactobacillus								
acidophilus NCFM	1.99	1938	None	1864	74	34.7	probiotic	[9]
brevis ATCC 367	2.29	2314	50	2185	82	46.2	beer	[10]
casei ATCC334	2.76	2906	82	2748	76	46.7	sourdough	[10]
casei BL23	2.94	3090	None	3015	75	46	probiotic	[11]
casei Zhang	2.86	2906	27	2804	75	46	koumiss	[12]
delbrueckii subsp. *burgaricus* ATCC 11842	1.78	2184	270	1529	122	49.7	sourdough	[10]
delbrueckii subsp. *burgaricus* ATCC BAA-365	1.77	2033	192	1715	127	32.9	sourdough, cheese	[10]
fermentum IFO 3956	2.00	1912	None	1843	69	51	plant	[13]
gasseri ATCC 33323	1.95	1898	43	1755	98	35	human, probiotic	[14]
helveticus DPC4571	1.98	1838	155	1610	73	37	cheese	[15]
johnsonii NCC533	1.99	1918	None	1821	97	34.6	probiotic	[16]
plantarum JDM1	3.20	3029	3	2948	78	44	probiotic	[17]
plantarum WCFS1	3.31	3135	39	3007	86	44.5	human	[18]
reuteri JCM 1112	1.94	1901	None	1820	81	38	human, probiotic	[13]
reuteri DSM20016	1.91	2027	39	1900	88	38	human	—
sakei 23K	1.90	1963	30	1879	84	41.2	meat starter	[19]
salivarius subsp. *salivarius* UCC118	1.83	1864	73	1717	99	32.9	human, probiotic	[20]
Lactococcus								
lactis subsp. *cremoris* MG1363	2.53	2597	81	2434	81	35.8	cheese	[21]
lactis subsp. *cremori* SK11	2.33	2610	153	2384	82	36	cheese	[10]
lactis ssp. *lactis* IL1403	2.37	2425	1	2321	79	35.4	cheese	[8]
Bifidobacterium								
adolescentis ATCC 15703	1.99	1702	None	1632	70	59	probiotic	–
animalis subsp. *lactis* AD011	1.84	1603	17	1527	59	60	probiotic	[22]
animalis subsp. *lactis* DSM 10140	1.85	1629	None	1566	63	60	probiotic	[23]
animalis subsp. *lactis* BI-04	1.85	1631	None	1567	64	60	probiotic	[23]
longum DJO10A	2.27	2061	None	1989	73	60.1	probiotic	[24]
longum subsp. *longum* NCC2705	2.26	1798	None	1727	70	60.1	human, probiotic	[25]
longum subsp. *infantis* ATCC 15697	2.70	2588	80	2416	80	59	human	[26]
Streptococcus								
thermophilus LMG18311	1.8	1973	180	1888	85	39.1	sourdough	[27]
thermophilus LMD-9	1.86	2002	206	1709	87	39.1	sourdough	[10]
thermophilus CNRZ1066	1.8	2000	182	1915	85	39.1	sourdough	[27]
Leuconostoc								
mesenteroides subsp. *cremoris* ATCC 19254	1.56	1903	None	1847	56	37	–	–
mesenteroides subsp. *mesenteroides* ATCC 8293	1.94	2073	19	1970	85	37	kimchi	[10]
Oenococcus								
oeni PSU-1	1.78	1864	122	1691	51	37	wine	[10]
Pediococcus								
pentosaceus ATCC 25745	1.75	1847	20	1755	72	37	fermented food	[10]

分调控系统(LBA1524HPK)是其在环境改变时与蛋白水解相关的重要调控因子之一,影响 80 个基因的表达。在基因组研究热潮的推动下,乳酸菌蛋白质组学研究近年来也取得了令人鼓舞的进展,根据蛋白质组数据解析乳酸菌生理功能已有许多成功的报道。2009 年 Wu 等[31] 在国际上首次提供了我国第一株具有自主知识产权的乳酸菌(*Lactobacillus casei* Zhang)的蛋白质组参考图谱,研究结果发现,蛋白在不同生长期具有不同的表达量,如与对数生长期相比,稳定期有 33 个蛋白表达上调 2.5 倍以上,其中包括许多分子伴侣、胁迫应激蛋白如 Hsp20,DnaK,GroEL 等;通过对这些关键蛋白的研究,为进一步增强乳酸菌酸胁迫抗性提供了可借鉴的方法。Sánchez 等[32] 通过差异蛋白质组比较了酸胁迫条件下两株不同酸耐受性的双歧杆菌蛋白表达差异,发现支链氨基酸(BCAA)合成途径中的 IlvC2,IlvD 和 IlvE 在高耐受菌中显著上调,而且细胞具有较高的氨浓度,基于这一发现,作者认为抵御酸胁迫的机理是支链氨基酸通过脱氨基生成氨的方式中和胞内的 H^+,维持胞内 pH 处于最适状态,从而减少酸胁迫

对细胞的伤害。

建立在全基因组序列基础上的基因组工程技术,通过整合其他组学数据和生物信息学的研究策略,可让研究者从全基因组规模去全面理解细胞的代谢网络、调节网络以及遗传和环境扰动对细胞全局代谢的影响,并构建基因组规模的网络模型,从而可以全面深入地对乳酸菌生理功能进行解析[33],并在此基础上发展代谢工程策略,定向改善微生物细胞的表型和生产性状或优化细胞的生理功能(图 1)。

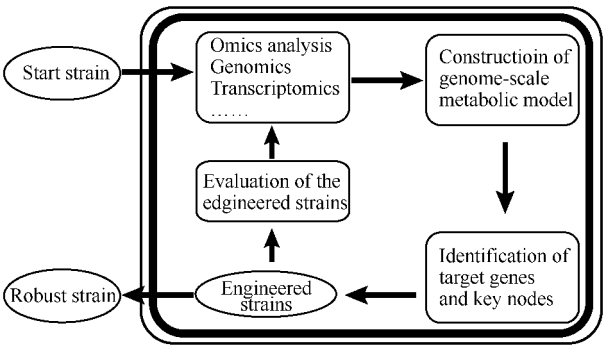

图 1 基于系统生物学策略的菌株改造流程[34]

Fig. 1 Scheme of strains improvement based on systems biology tools[34].

2 代谢工程策略优化与调控乳酸菌生理功能

2.1 代谢工程策略拓展乳酸菌底物谱和利用能力

在以乳酸菌为主体的食品微生物制造中遇到的关键问题是乳酸菌苛刻的营养需求,原料成本约占35%的生产成本,其次是产物抑制。因此,拓展乳酸菌的底物谱,以期选择廉价碳源,是提高微生物生理功能的关键问题之一。LeBlanc 等[35]在 *L. lactis* 中表达源于 *L. plantarum* 的 α-半乳糖苷酶基因,使 *L. lactis* 能够降解豆制品中难以消化的寡糖(non-digestible oligosaccharides, NDO)如棉子糖、水苏糖等,从而消除由 NDO 发酵引起的肠胃气胀和胃肠功能紊乱等疾病。

2.2 代谢工程策略强化乳酸菌代谢能力和产物分泌能力

强化乳酸菌代谢功能的研究主要包括增强主流代谢途径、削弱分支代谢途径以及提高产物的分泌能力。在正常条件下,野生型乳酸乳球菌(*Lactococcus lactis*)的代谢以同型乳酸发酵为主,若敲除乳酸脱氢酶基因 *ldh*,细胞不产生乳酸,但因为丢失了氧化 NADH 的能力,生长受到明显地抑制。Hols 等[3]在敲除了乳酸脱氢酶 Ldh 的 *L. lactis* 中表达源于 *Bacillus sphaericus* 的丙氨酸脱氢酶(L-AlaDH),使代谢由同型乳酸发酵转化为同型丙氨酸发酵,在此基础上敲除丙氨酸消旋酶基因,并补充适度氨离子,使最终代谢产物 99% 转变为 L-丙氨酸。利用乳酸菌合成叶酸的产量仅为 100 μg/L,其中90%为胞内产物,少量释放到胞外的叶酸是不易被人吸收的多聚谷氨酰叶酸。针对这一问题,Hugenholtz 等[5]在 *L. lactis* 中表达源于人的 γ-谷氨酰水解酶 cDNA,显著降低叶酸中多聚谷氨酰链的聚合度,使分泌到胞外的叶酸产量比对照组提高了6倍。此外,Hugenholtz 等[2]在 *L. lactis* NZ9050 中过量表达 NADH 氧化酶,使碳代谢流转向不需要 NADH 的代谢反应,显著提高了双乙酰产量。乳酸菌代谢工程改造的典型研究实例列于表 3 中。

表 3 代谢工程改善乳酸菌代谢能力

Table 3 Improvement of the metabolic capability by metabolic engineering

Organism	Product	Genetic modification	Substrate	Yield and/or productivity	References
L. lactis	Xylitol	Expressed *P. stipotis* XR	Xylose + glucose	2.5 mol/(mol glucose);2.7 g/(L h)	[36]
L. lactis	Mannitol	Expressed Mtl1PDH and mannitol-1-phosphatase	Glucose	0.50 mol/(mol glucose)	[37]
L. plantarum	L-ribulose	Expressed araA, ribulokinase-deficient	L-arabinose	0.70 mol/mol 14.8 g/(L h)	[38]
L. plantarum	Sorbitol	Overexpressed Stl6PDH, LDH-deficient	Glucose	0.65 mol/(mol glucose)	[39]

2.3 代谢工程策略提高乳酸菌环境胁迫抗性

利用传统的代谢工程手段提高乳酸菌环境胁迫抗性的方法主要有:构建新的代谢途径、拓展已有的代谢途径和削弱竞争代谢途径。例如,Fu 等[40]利用代谢工程手段在乳酸乳球菌 NZ9000 中引入谷胱甘肽(GSH)合成能力,宿主菌对氧胁迫的抗性提高了 15 倍。Zhang 等[41]研究发现,在 NZ9000 中引入 GSH 合成能力,宿主菌对酸胁迫(pH 4.0, 10 h)的抗性比对照菌提高了 18 倍。Sheehan 等[42]在唾液乳杆菌 *Lactobacillus salivarius* UCC118 中表达来自李斯特菌的甜菜碱利用基因 *betL*,研究发现,工程菌对渗透压胁迫和低温胁迫的耐受性得到了显著提高,同时也增

强了其对喷雾干燥和冷冻干燥的抗性。表 4 总结了代谢工程策略在改善乳酸菌胁迫抗性的具体应用。

传统的代谢工程手段虽然在提高乳酸菌环境胁迫抗性方面有不少成功的例子，但由于微生物代谢网络的全局调控，对单条代谢途径进行改造，往往并不能获得预期的表型。Bailey 等[43] 在 1996 年提出了另一种代谢工程策略—反向代谢工程（Inverse metabolic engineering）。这种策略最重要的两部分是：(1) 获得预期的表型；(2) 确定这一表型所对应的基因型。获得预期的表型的代谢工程方法还包括：结合高通量筛选方法的传统诱变技术、全局转录工程（Global transcription engineering，gTME）、基因组重排（Genome shuffling）和核糖体工程（Ribosome engineering）等。Patnaik 等以乳酸杆菌的野生型菌株为出发菌株，用传统诱变的手段得到酸耐受性显著增强的 pop1 和 pop2 菌株，将 pop1 和 pop2 进行五轮递归原生质体融合以后，突变株 F5 与野生型相比，酸耐受性和乳酸生产能力提高了 3 倍。通常，在获得优势表型菌株后确定其基因型的方法包括“组学”技术、人工转录因子工程（Artificial transcription factor engineering）和文库富集尺度分析技术（Scalar analysis of library enrichments，SCALEs）等。

表 4 代谢工程改善乳酸菌环境胁迫抗性

Table 4 Improving the stress resistance of lactic acid bacteria by metabolic engineering

Organism	Genetic modification	Performances	References
Oxygen stress			
L. lactis	Expressed GSH from *E. coli*	The survival increased 2.9-fold when treated in 150 mmol/L H_2O_2 for 15 min	[40]
Acid stress			
L. lactis	Heterologous expression of *E. coli dnaK*	The maximum OD_{600} increased 1.44-fold in the presence of 0.5% lactic acid (pH 5.47)	[44]
Osmotic stress			
L. salibarius	Heterologous expression of *BetL*	A significantly higher growth rate was detected when grown at 7% NaCl	[42]
L. lactis	Heterologous expression of *E. coli dnaK*	The maximum OD_{600} increased 1.28-fold in the presence of 3% NaCl	[44]
Solvent stress			
L. lactis	Heterologous expression of *E. coli dnaK*	The growth increased 1.13-fold in the presence of 5% ethanol	[44]
Temperature stress			
L. salibarius	Heterologous expression of *BetL*	The percent survival during freeze-drying increased 2-fold	[42]

3 展望

食品微生物制造所要解决的根本问题是，如何高效地改造生产菌，改善其生理功能。代谢工程和组学分析虽然大大提高了系统分析微生物代谢网络结构和生理功能的能力，但在实际应用上仍有一定的局限性。与此同时，一些基于基因组技术的新方法不断涌现，并能对微生物细胞生理功能进行定向调控，这些策略包括系统代谢工程（Systems metabolic engineering）、合成生物学（Synthetic biology）等。

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Systematic analysis and metabolic regulation of physiological functions for lactic acid bacteria—A review

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Abstract: As cell factories, lactic acid bacteria are widely used in food, agriculture, medicine and other industries, and play a great role in industrial processes. However, lactic acid bacteria encounter various environmental stresses both in industrial processes and in the gastrointestinal tract, which impair their physiological functions and food manufacture efficiency. Recently, the development of metabolic engineering and system biology brings unprecedented opportunity for the physiological modification of lactic acid bacteria. In this review, we addresses the progress of lactic acid bacterium system biology, and based on this, the metabolic engineering strategies for manipulating and optimizing lactic acid bacteria physiological function were summarized.

Keywords: lactic acid bacteria, physiological function, metabolic engineering, system biology

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