益生菌功能开发及其应用性能强化

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摘要 随着人们居住环境、饮食习惯和生活节奏的改变, 许多新型疾病开始出现并严重威胁着人类健康. 在“大健康”理念及其产业化背景下, 人们的健康意识逐渐提升, 而益生菌凭借其优良的益生性能受到了越来越广泛的关注. 益生菌(probiotics)指可有效增强食用者身体健康水平的活菌制剂及其代谢产物, 具有改善便秘、缓解腹泻、降胆固醇、增强免疫力、预防和治疗自闭及抑郁症, 抗肿瘤和保护口腔健康等功效, 其主要产品益生菌膳食补充剂和益生菌非处方药等已在医药领域得到广泛应用. 随着对益生菌与疾病关系研究的不断深入, 益生菌在新型及重大疾病防治领域的新功能不断刷新着人类的认知. 因此, 学术界需进一步加强益生菌的基础研究, 开发益生菌在疾病防治领域的新功能. 同时, 在益生菌产品的制备和应用过程中, 多种严峻的环境胁迫会影响菌株正常的代谢和存活能力, 抑制其益生作用的发挥. 因此, 利用多种手段, 对其应用性能进行强化显得尤为重要. 本文综述了益生菌功能开发和性能强化的主要方法和原理, 概括了其在多种疾病防治过程中的应用效果, 为加深对益生菌功能的认识, 进一步促进益生菌产业升级提供了可借鉴的思路.

关键词 益生菌, 大健康, 菌株筛选, 性能强化, 疾病防治

“大健康”是随着时代发展、社会需求和疾病谱的改变提出的一种涉及生理、心理、社会、环境和道德等方面的全局健康理念^[1], 美国著名经济学家保罗·皮尔泽认为其是继IT产业之后的全球“财富第五波”^[2], 具有广阔的市场前景. 随着《“健康中国2030”规划纲要》的颁布^[3], 以“共建共享、全民健康”为战略主题, 推进健康中国的建设, 预示着中国大健康产业时代的来临^[4].

大健康时代, 健康产业的概念已然从依赖抗生素等药物的单一救治模式转向“防-治-养”一体化模式^[1], 缓解因抗生素滥用对人类健康和生态环境造成的严重威胁^[5]. 益生菌(probiotics)是通过摄取适当的量、对食用者的身体健康能发挥有效作用的活菌^[6].

近年来, 益生菌凭借其安全、可靠、性能优良等特点在疾病的预防、治疗和重症修复过程中受到越来越多的关注. 最新数据显示, 全球益生菌市场规模每年增速在15%~20%之间, 2017年达到360亿美元; 我国益生菌市场发展前景巨大, 预计2020年产品市场规模将达到850亿元^[7]. 为加强益生菌的基础研究并促进产业化升级, 科学技术部在“十三五”期间部署了多项重点相关项目, 其中益生菌与人体健康和疾病防治的关系等研究已成为学术研究领域的重点方向^[8].

1 益生菌功能研究概述

益生菌, 这一概念最早起源于希腊语, 即“对生

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命有益”(for life). 现在, 益生菌多指食用后可改善宿主肠道菌群生态平衡, 发挥有益作用, 提高宿主健康水平的活菌制剂, 主要定殖在人体的口腔、肠道、皮肤和阴道中. 益生菌主要包括乳杆菌、双歧杆菌、肠球菌、乳球菌、链球菌、魏斯氏菌和明串珠菌等, 其在竞争消耗病原菌能源物质的同时, 代谢生成的抑菌肽等可有效抑制病原菌繁殖, 在口腔疾病、肠道疾病、过敏性疾病、三高疾病、精神学疾病、癌症和女性阴道疾病等的预防、治疗和修复过程中发挥着重要作用^[9-11](图1).

微生物与人体互利共生、协同进化, 一旦微生物与机体之间的平衡被打破, 人体健康就会受到威胁. 益生菌凭借其优良的益生功能和在多种疾病防治过程中的作用效果被广泛应用于现代医学的多个领域(表1).

近年来, 越来越多的证据表明, 人体肠道内的微生物种群变化与人类自身的健康与疾病存在密切关系^[45], 而益生菌在提升人体健康水平中扮演着举足轻重的角色, 可维护肠道菌群的健康稳态, 刺激宿主的免疫系统, 增强宿主的免疫应答反应, 从而帮助宿主抵抗各类疾病的发生.

研究表明, 肠道益生菌群存在时, 能够限制促炎症的Th1和Th17细胞扩增、促进Treg细胞分化, 从而抑制对肠道中食物和菌群抗原的炎症性应答^[46]. 研究还发现, 源自肠道微生物菌群的色氨酸分解代谢物在免疫稳态、肠道屏障功能中发挥作用, 可以用于针对患有炎症性肠病(IBD)的个体开发新的治疗药物^[24]. 此外, 益生菌通过免疫排斥、免疫排除和免疫调节, 促进肠黏膜免疫系统的发育, 增强肠道屏障功能, 促进免疫耐受的建立^[29]. 而肠道中分布有大量淋巴细胞, 免疫系统又与中枢神经系统密切相关, 因而肠道菌群亦可通过血脑屏障、Toll like 受体和IL-1来调节脑的活动和功能^[47,48]. 肠道菌群与胰岛素抵抗、胰高血糖素样多肽-1(GLP-1)、短链脂肪酸(SCFA)、胆汁酸等的关系的研究为肠道菌群作为防治老年II型糖尿病(T2DM)的新靶点提供可能^[49]. 近年来, 随着宏基因组数据的不断更新, 研究人员发现利用粪便中的肠道微生物进行宏基因组标记可进行相关疾病如结直肠癌的早期诊断或癌症筛查, 且通过PCR定量的特异性标志物可以对疾病状态进行分类^[50], 并通过基因标志物(如*Fusobacterium nucleatum*)的水平预测患者的存活能力^[51], 为肠道菌群特别是

图1 益生菌与疾病防治之间的关系

Figure 1 The relationship between prbiotics and diseases prevention

表1 常见疾病与适用益生菌

Table 1 Common diseases and applicable probiotics

疾病	常用菌株	作用效果	文献
口腔疾病	唾液乳杆菌K12、短乳杆菌、发酵乳杆菌、罗伊氏乳杆菌	益生菌治疗使益生菌组的牙龈指数(GI)和探诊出血(BOP)亦显著降低	[12~15]
肠道疾病	鼠李糖乳杆菌、乳双歧杆菌、嗜酸乳杆菌、副干酪乳杆菌、鼠李糖乳杆菌、罗伊氏乳杆菌、干酪乳杆菌、 <i>Escherichia Coli</i> Nissle 1917 (ECN)、植物乳杆菌、 <i>Saccharomyces cerevisiae</i> var. <i>boulardii</i>	减轻服用抗生素的腹泻患者的腹泻症状;辅助药物治疗可降低治疗引发的副作用;可有效缓解IBS患者腹痛、腹胀等症状,减少呕吐发生的频率,抑制小鼠中的霍乱弧菌感染. ECN增强了Caco-2单层的完整性,并恢复了其通透性,抵消了与肠炎正相关的生态失调;对成年兔进行口服接种可以消除霍乱感染的腹泻症状;改善各种腹泻状况	[16~24]
过敏性疾病	鼠李糖乳杆菌、干酪乳杆菌、肠膜明串珠菌、发酵乳杆菌、德氏乳杆菌保加利亚亚种等	促进肠黏膜免疫系统的发育、增强屏障功能、促进免疫耐受的建立、增强肠道黏膜免疫功能	[25~29]
“三高”疾病	双歧杆菌、戊糖乳杆菌、罗伊氏乳杆菌、嗜酸乳杆菌、尿肠球菌和乳双歧杆菌等	生成的降血压肽、 γ -氨基丁酸等具有优良的降压效果,有效改善糖尿病患者体内血糖和胰岛素水平,吸附胆固醇并随菌体沉淀	[30~33]
精神疾病	瑞士乳杆菌、长双歧杆菌	精神疾病和相应药物及抗生素的使用会造成肠道菌群紊乱,破坏肠道屏障,导致相应代谢并发症的发生. 同时肠道通过产生神经递质来直接调节神经活动,并且有益菌通过激活免疫系统,产生抗炎因子治疗肠胃疾病来间接治疗精神疾病	[34~38]
癌症	嗜热链球菌、双歧杆菌、嗜酸乳杆菌、植物乳杆菌	癌症,化疗及其药物会加剧肠道紊乱,导致其他并发症的发生. 益生菌通过维持肠屏障;产生抑制病原体生长的微生物因子;与有害微生物竞争营养物质;诱导毒素降解,调节肠道中酶的活性以及激活免疫应答来辅助治疗	[39~42]
女性阴道疾病	卷曲乳杆菌	乳杆菌会消耗碳水化合物生成乳酸,抑制其他酵母和病原菌的繁殖,保障阴道的健康	[43,44]

部分益生菌的功能开发与应用奠定了基础.

遗憾的是,虽然益生菌为上述疾病的治疗提供了新方向,但目前益生菌的使用仍存在一定的局限性:(1)治疗效果相对有限且连续性较差,临时改善的菌群结构多随着给药过程的结束而恢复原状;(2)制剂化及人体胃肠道消化等过程会大大降低活菌数量,无法保证益生菌产品的应用效果;(3)菌株在肠道中的定殖能力较差;(4)受环境、饮食等因素的影响,益生菌具有本土化特征,目前针对中国人体质的益生菌产品还比较缺乏;(5)随机双盲对照实验中,益生菌的剂量、试验周期和评价指标等尚缺乏统一标准.因此,需要进一步筛选性能优良的益生菌菌株,强化其益生性能,完善临床应用的证据,进而推进益生菌的基础研究和产业升级.

2 益生菌选育技术

早在1857年,人们就发现了乳酸杆菌、双歧杆菌、乳酸球菌的存在.1907年俄梅切尼科夫提出“喝酸奶长寿不老学说”,人们慢慢研究并注意到了益生菌

的种类和益生性能^[52].如何获得这些功能菌株是摆在益生菌应用领域面前的一个问题.传统的菌种筛选是从形态特征、生理生化反应、血清学反应等角度出发,方法原理都是基于微生物表面受体的特异性.伴随着生物技术的进一步发展,有越来越多的生物分析手段和菌种筛选方法得以发展,并应用到了益生菌筛选中^[53].

2.1 微生物群落分析

益生菌分布广泛,主要是动物肠道正常生理性菌和非肠道菌^[53],研究其微生物群落及其动态变化并对其中的核心微生物进行分析、揭示微生物的生理特性等就需用到微生物群落分析,微生物群落分析对于功能菌种的开发具有重要意义.目前,针对微生物群落的研究方法正在从最初的传统分离培养逐渐向工作效率显著提升的高通量测序转变^[54].

(i) 传统培养方法. 传统培养的方法一般是利用多种培养基对微生物进行分离纯化,然后根据其生长特性、生理生化特征对分离菌株进行初步种属鉴

定^[55]。此方法分离出的微生物种类一般低于整个体系的10%，对于非培养的微生物无法进行分析，不能完整地反映菌群结构和丰度，且工作量较大，样本不具备时间和空间的代表性^[56]。

(ii) 生物化学方法。生物化学方法包括磷脂脂肪酸(PLFA)分析和Biolog分析，多用于土壤微生物群落分析，也应用于植物根际、空气、白酒窖泥和大曲等微生物群落研究^[57-62]。

PLFA是细胞膜重要组成部分，与细胞的生物量呈正比，以此为生物标记，根据其种类、结构的差异以及含量就可以对物种和丰度进行分析，主要针对真菌、放线菌、革兰氏阳性和革兰氏阴性细菌^[63,64]。该方法对实验要求低，成本也较低，但脂肪酸和微生物之间的对应关系还未完全确定；细胞死亡后PLFA会快速降解，影响结果的准确性^[65]。

Biolog分析是基于微生物利用不同形式的碳源进行生长代谢，选择不同的微平板(MT, GP, GN, ECO, FF等)，根据颜色反应即可分析微生物群落多样性。此方法自动化程度高、简单迅速；但仅能检测到生长迅速的微生物，且微平板的选择和培养方式会影响结果的准确性^[66]。Chojniak等人^[67]采用Biolog技术，使用GEN III平板、用于鉴定革兰氏阴性菌和革兰氏阳性菌的新测试板以及用于评估微生物群落功能多样性的ECO平板分析了酚类污染水体微生物群落结构。

(iii) 分子生物学方法。与传统方法相比，分子生物学方法能够对可培养和非培养的微生物进行研究，主要包括聚合酶链式反应变性梯度凝胶电泳(PCR-DGGE)、末端限制性片段长度多态性技术(T-RFLP)、克隆文库、核酸杂交、实时定量PCR(RT-PCR)、高通量测序等技术。

PCR-DGGE通过提取样本的总基因组，利用PCR扩增目的基因，凭借聚丙烯酰胺凝胶中不同浓度梯度的变性剂，分离大小相同、碱基序列不同的扩增产物片段得到指纹图谱，对条带进行测序、比对和物种鉴定^[68]。此方法应用范围较广泛，包括食品、口腔、肠道、土壤、活性污泥、生物膜等^[69-72]，可以迅速对多个样本同时分析，稳定性高、重复性强，但无法检测500 bp以上的DNA片段，且图谱条带数量有限，只能显示出样本中占优势的微生物，很难检测到其中的低丰度菌种^[55]。Nalepa和Markiewicz^[73]的研究利用PCR-DGGE技术分析了生牛奶和熟奶酪中的微

生物菌群，共检测73种菌株，包括明串珠菌、短乳杆菌、发酵乳杆菌、植物乳杆菌等。

T-RFLP是在特定引物的5'末端标记荧光，PCR扩增产物用限制性内切酶消化，获得带荧光标记的DNA片段，经荧光比对分析微生物群落。此方法被用于肠道、土壤、植物根围^[74,75]等微生物群落分析，效率高，特异性较强，但受PCR的影响，且获得的带荧光标记的片段可能对应多个菌种，结果不够准确^[65]。Scupham^[76]利用T-RFLP分析了火鸡盲肠内的微生物群落。

克隆文库是从样品中提取总DNA，使用特异性引物扩增基因片段，然后将扩增产物克隆到质粒载体中，构建克隆文库测序，进行GenBank序列比对、物种鉴定。此方法可以提供更详细的遗传分析多样性、物种丰度、种群结构和分类以及同源进化微生物的信息，但成本高、操作复杂且耗时^[56]，主要用于土壤、活性污泥、白酒大曲等^[77,78]。李俊平等人^[79]采用16S rRNA克隆文库分析了成人龋病唾液的微生物菌群，检测到链球菌、普氏菌等菌株。

核酸杂交主要根据碱基互补配对原则，以特异性标记(放射性元素、荧光)的DNA片段作为探针，与样品中的DNA或RNA进行杂交形成杂合分子，经仪器检测定量。其中，荧光原位杂交(FISH)应用较多，通过荧光显微镜或流式细胞仪可以直接对物种丰度进行检测。此方法检测迅速，特异性强，但自动化程度较低，探针的设计要求较高^[80]，主要应用于食品、白酒窖泥等的样本检测^[81-83]。

RT-PCR则是根据荧光染料或荧光标记检测PCR产物的荧光信号进行定量分析的方法。此方法方便快捷、特异性强，能同时分析多个样本；但由于检测体系较小，DNA质量和PCR反应条件等均会造成测定误差^[84]，目前主要应用于水体、土壤、肠道等样本检测^[85-87]。

高通量测序技术主要有454焦磷酸测序和Illumina HiSeq/MiSeq(16S rDNA, 宏基因组)测序^[88]，能够读取数百万个DNA片段并且同时分析多个样本^[56](图2)。Illumina HiSeq宏基因组测序是直接提取样本中可培养和不可培养的微生物的总基因组构建文库后进行鸟枪法测序，较于16S rDNA和454焦磷酸测序，除了可以对微生物种类和丰度进行分析以外，还包含了基因功能分析，是目前使用最广泛的测序技术，可进行食品、口腔、肠道、皮肤、土壤、海洋、沉积物等众

图2 (网络版彩色)宏基因组测序技术

Figure 2 (Color online) Metagenomic sequencing technology

多样本的检测^[89~91].

2.2 益生菌株筛选

益生菌的筛选通常是根据菌株的生长特性、生理生化特性、代谢特性及对不同人群营养与临床病理的影响分析等设计合适的筛子,将目标菌株从样本中筛选出来,并加以验证、评价和安全性分析等^[92~94].微生物菌株的高通量筛选(high-throughput screening, HTS)是一种自动化、大规模筛选目标菌株、细胞、药物等的技术,被广泛运用于菌株选育,以其筛选样本的多样性,可应用至益生菌不同种属和菌株的筛选.微型反应器、自动化移液/分选系统、自动化分析检测(酶标仪、流式细胞仪)系统是该技术的核心部件^[95](图3).

高通量筛选过程中的分析检测主要包括:(1) 菌体生长情况及其代谢特性.如营养缺陷型、耐受性、水解圈、显色圈、抑菌圈等;(2) 目标对象的荧光性或显色性^[96].周扬等人^[97]通过活跃链霉菌产生的那西肽对藤黄微球菌具有抑制效果,根据抑菌圈的大小经高通量筛选获得了那西肽高产菌株. Cao等人^[98]通过对孢子进行碘化丙锭(PI)和二乙酸荧光素(FDA)染色,利用流式细胞仪分选活性孢子,高通量筛选了阿维菌素高产菌株. Zeng等人^[99]根据解脂耶氏酵母发酵过程中pH的变化,选择亮绿色,喹哪啶红,溴甲酚绿,百里酚蓝,橙色IV和孔雀石绿6种pH指示剂,

高通量筛选了高产 α -酮戊二酸(α -KG)的菌株.上述方法中的显色原理可进一步结合益生菌菌株细胞的生理特性进行高通量筛选方法的设计与优化.

3 益生菌功能强化

在现代工业中,生物发酵具有转化效率高、生产安全性高、原材料来源广泛等诸多优点,益生菌作为优良的微生物细胞工厂被广泛应用于相关产品的生产^[100].由于益生菌在发酵生产过程中面临着来自外界环境的多种胁迫,如生产加工过程中的酸胁迫和氧胁迫、运输储存过程中温度和水活度胁迫,以及在人体胃肠道中经历的胃酸和胆盐胁迫等,严重影响益生菌的生长、生理功能以及代谢活性,进而影响了其益生功能的发挥.因此,如何提高益生菌对环境胁迫的耐受性,强化益生菌功能,成为目前亟待解决的问题.目前主要的研究策略主要分为两大类,分别为传统进化工程策略,以及基于系统生物学和反向代谢工程的功能强化策略.

3.1 进化工程强化策略

进化工程策略是一种生物学研究中较为常用的改善生物特性的方法,主要包括诱变技术、适应性进化和基因组改组等^[101~103].随着全基因组测序的快速发展,适应性进化策略与基因表达和代谢通量分析的结合使得以遗传为基础的改良表型鉴定和这种表

图3 (网络版彩色)微生物高通量筛选流程图

Figure 3 (Color online) The process of microbial high throughput screening

型在菌株之间的传递成为可能^[104]。Demeke等人^[105]在酿酒酵母测序分析的基础上进行EMS诱变和基因组改组,获得了可利用D-木糖进行发酵且耐抑制的酿酒酵母菌株。Tilloy等人^[106]通过比较MAP激酶途径,并使用具有KCl的连续批次培养条件,获得具有更高甘油产量的酵母菌株。目前,对具有特定性质的微生物细胞进行快速、高效、大规模的筛选是现在益生菌强化过程中的关键技术。

(i) 物理和化学诱变。诱变技术主要分为化学诱变和物理诱变。大多数化学诱变剂都是碱基类似物、碱基修饰物和移码诱变剂。物理诱变法包括紫外线、 α 射线、 β 射线和 γ 射线等,常规的诱变方法在一定程度上易于操作并提高抗性突变率。何慧玲等人^[107]以干酪乳杆菌LC2W为出发菌株,分别对其进行亚硝基胍(nitrosoguanidine, NTG)、紫外(ultraviolet, UV)诱变,筛选得到的诱变菌株LC2W-NTG-12和LC2W-UV-11表现出高产酸及耐酸性能。突变菌株产酸能力分别提高了47.13%和28.20%,耐酸能力分别提高了24.38%和20.08%。常压室温等离子体诱变(ARTP)是近年来新型的物理诱变方法。与常规诱变方法相比,ARTP具有更高的突变率并可能导致多种复杂类型的DNA损伤和突变^[102]。同时,由于通过单一诱变方法难以获得高突变率来筛选有益菌株,实际应用中往往采用结合不同致突变方法的复合诱变进一步

提高突变效率^[108]。

(ii) 适应性进化。适应性进化是利用环境压力,对微生物进行逐步驯化和筛选,最终获得胁迫抗性显著增强的菌株的过程,具有较强的进化目的。适应性进化与传统的诱变技术相比需要较长的时间来积累连续突变,突变的方向和性质可以通过适应性进化方法来控制^[109,110]。通过适应性进化获得的菌株性能优良且稳定,因此该技术广泛应用于耐受性益生菌的筛选。Ming等人^[111]对*Lactobacillus casei*进行了70 d的适应性进化,分离出3株耐酸菌株。在pH 4.3条件下培养54 h,进化菌株的生物量比对照菌株增加了60%以上,同时确定了进化菌株对酸胁迫、胆盐和模拟胃肠道的耐受性均高于对照菌株。Zhang等人^[112]在pH 4.3(乳酸调节)的筛选环境条件下对*L. casei* Zhang连续驯化70 d,获得了1株耐酸菌株。在酸胁迫条件下,耐酸菌株的生物量和比生长速率较对照菌株分别提高了60%和10%,在pH 2.5条件下,胁迫培养7 h,耐酸菌株存活率达到对照菌株的4100倍。

(iii) 基因组改组。近年来基因组水平进化的研究备受关注,基因组改组(genome shuffling)是继适应性进化后新发展起来的一种定向进化技术,通过将传统的诱变与原生质体融合技术相结合,大幅度提高了微生物的正向突变频率,进而在微生物育种中得到了广泛的应用。基因组改组的原理是对微生物

全基因组进行重排,通过传统的诱变方式获得拥有多个正向突变的突变体库,以获得的突变体库为亲本进行原生质体融合,使基因组发生交换重组.经过递推式多次融合,将正向突变集中重组到最终获得的目标菌株中.基因组改组已被成功应用于原核生物和真核生物中^[113],并且选育的菌株具有遗传稳定性^[103].齐路路^[114]利用⁶⁰Co- γ 射线对*L. plantarum* CICC20765进行诱变处理,对正向变异子进行递推式原生质体融合,获得了可以高效降解亚硝酸盐的突变株.此外,突变菌株具有较强的产酸能力和耐酸性.

3.2 基于系统生物学和反向代谢工程的功能强化

进化工程策略广泛应用于提高菌株的代谢表型,然而在了解表型与基因型之间相关性方面尚存在较大的局限性.为了解决这一问题,可使用系统生物学和反向代谢工程相结合的方法快速整合形成“表型-基因型-表型”的重组菌株.首先,可以通过进化工程策略如适应性进化获得表型菌株;其次,通过系统生物学对表型相关的基因表达进行全面的分析,如基因组学、转录组学、蛋白质组学和代谢组学分析;最后,通过反向代谢工程的方法对表型的相关基因进行修饰.

常规技术包括过量表达或敲除靶基因^[115].一方面,常通过使用高拷贝质粒或强启动子增强目的基因的转录水平;另一方面,可以通过直接将目标基因弱化或敲除来降低基因的转录和表达水平.然而,当一个菌株中目标突变基因的数目较多时,有必要基于突变基因重建进化菌株,如利用CRISPR-Cas9基因编辑系统实现多达3个基因的同时编辑. Yang等人^[116]对在12%乙醇中生长的*L. acetotolerans* F28进行RNA测序(RNA-seq)以确定短期和长期适应该环境的重要遗传机制.转录组和功能分析表明,应激反应和脂肪酸生物合成增加,并且氨基酸转运和代谢的减少可能对乙酰乙酸杆菌F28适应乙醇环境起着重要作用. Desmond等人^[117]发现*L. paracasei* NFBC338在热胁迫条件下GroEL表达显著上调,通过过量表达GroESL蛋白,使工程菌对热、盐和丁醇等环境胁迫的抗性显著增强. Zhang等人^[115]利用重组酶Cre-lox系统在*L. lactis* NZ9000中构建了敲除*recO*基因的菌株*L. lactis* NZ9000 Δ *recO*.研究表明,未敲除*recO*基因的原始菌株在多种胁迫环境下的生长能力均优于敲

除菌株.

近年来,不同进化策略已能够帮助研究者得到理想的表型菌株.快速发展的系统生物学和反向代谢工程使人们对菌株表型的认识更加深入全面.同时,组学技术被广泛应用于揭示相关信息进化株的表型机制.采用反向代谢工程方法可以进一步强化应变性能.综上所述,这些技术都将推动进化工程的发展,为强化益生菌的功能提供技术保障.

3.3 制剂化

经过上述进化工程策略和反向代谢工程策略得到的特定表型功能强化的益生菌株不能长时间储存,且难以通过胃肠消化道中胃酸和胆碱的考验,而最终定植于肠道中发挥益生作用.因此,需要对益生菌进行制剂化研究来使其能克服重重困难,对人体达到预期的益生效果.我们针对益生菌微胶囊制剂化发展情况进行如下概述.

(i) 包封材料.微胶囊技术是指将气体、液体、固体物质利用高分子材料包裹起来,起到保护包裹物质,形成半透明或不透明壳状微粒的技术^[118].被包埋进行保护的物质称为芯材,而实现微胶囊的材料称为壁材.壁材主要分为碳水化合物类、胶类、多糖类、蛋白类和纤维素类(表2).

壁材的选择需要根据芯材益生菌、微胶囊所应用的环境、释放机制、以及储存条件来进行确定.由于是将益生菌活细胞进行封装,壁材的毒性,与芯材的相容性都需着重考虑,同时在应用于工业中时,壁材的价格以及微胶囊所采用的工艺技术也同样需要重点考虑.例如将嗜酸乳杆菌以喷雾干燥法进行微胶囊化以提高在肠胃中的存活率,宜使用脱脂牛奶和甜乳清作为包封材料,形成的微胶囊尺寸约为13 μm ^[119];将乳酸杆菌以离子凝胶法进行包埋,以耐60~70℃的高

表2 微胶囊壁材材料分类总结

Table 2 The summary of microcapsule material classification

类别	具体材料
碳水化合物类	海藻酸钠、壳聚糖、环糊精、多孔淀粉、抗性淀粉等
植物胶类	海藻胶、阿拉伯胶、果胶、k-卡拉胶
多聚糖	黄原胶、普鲁兰多糖、结冷胶
蛋白类	酪蛋白酸钠、大豆蛋白分离物、乳清蛋白、脱脂乳、明胶
纤维素类	邻苯二甲酸醋酸纤维素

表3 微胶囊制备新方法

Table 3 New methods of microcapsule preparation

制备方法	原理	优点	缺点
层层自组装技术	依据静电作用、氢键、电荷转移和疏水作用力等通过多层复合来制备微胶囊壳层 ^[121]	设备简单,可调控壳层的厚度,可选择性包埋芯材及芯材的可控释放 ^[122]	操作复杂繁琐
超临界流体快速膨胀技术	通过喷嘴快速减压膨胀使超临界溶液过饱和而析出壳层微粒,然后与被包裹的核芯微粒相互碰撞形成微胶囊 ^[123]	可在温和条件下得到特定均匀粒径的微珠且菌株活性较高	成本高,包埋量较低
蒸发诱导自组装	采用挥发性有机物做溶剂,在一定的温度下,伴随着模板剂和前驱体浓度增大,溶剂逐渐挥发,并以溶剂挥发为驱动力,诱导模板剂和前驱体自组装,形成多孔材料 ^[124]	微胶囊多孔、连续、透明且机械强度高	尺寸分布不均,效率较低
微流控技术	利用直径为数十到数百微米的微管道处理或控制微量流体的系统 ^[125]	微胶囊具有高度单分散性且直径、壁厚可控制在微米范围内 ^[126]	技术不成熟,分析芯片一次性使用,成本高

温,则宜使用褐藻酸、氯化钙和壳聚糖作为包封材料,颗粒直径为400~450 μm ^[120]。

(ii) 微胶囊制备方法。目前在国内工业中广泛应用的益生菌制剂化方法主要为传统微胶囊制备法,分别为挤压法、乳化法、喷雾干燥法和喷雾冷冻干燥法。其中喷雾干燥法,因其速度快,设备技术成熟而得到广泛应用,但由于其中的高温过程,在制剂化过程中会造成益生菌的热灭活。因此需要添加相应的保护剂来尽量减少益生菌的热灭活,常用的保护剂为低熔点脂肪、糖、脱脂牛奶、海藻糖、淀粉、纤维等。

微胶囊技术具有广阔的应用前景,例如在医药领域中,妈咪爱益生菌粉、金双歧菌片、丽珠肠乐胶囊等都是采用微胶囊方法进行制剂化。但由于传统的制剂化技术未能够制造出完全满足人类需求的微胶囊,且操作繁琐,对益生菌损伤较大,因此需要在传统方法的基础上开发新方法来解决这些问题,表3中列出了近5年涌现出的新兴微胶囊化方法。

通过对表3中新兴微胶囊制备方法的介绍可以发现,微胶囊制剂化正向着设备精简、包覆率高、结构可调控、功能多样化等方向发展,以此来降低细胞的损伤或死亡。

4 展望

目前,越来越多的研究证实益生菌通过促进人体特别是肠道腔健康来发挥着改善精神健康状况、降低胆固醇、分泌降压物质、改善人体胰岛素水平、调节免疫因子辅助过敏治疗、癌症用药所致的肠道疾病的辅助治疗及初期病症筛查等作用。益生菌对多种疾病展现出良好的诊断、预防和治疗作用,使得益生菌临床制剂的开发成为当前研究热点,并取得了可喜的进展。但目前益生菌的使用仍存在一定的局限性,如益生菌作为活菌制剂需定殖于机体内从而发挥作用,由于地域、环境条件、饮食习惯等因素的差异,益生菌制剂在体内的定殖状况也具有较大的个体差异。在后续的研究中,可进一步通过动物模型及临床验证中肠道菌群组成、结构及功能互作分析,结合多组学联动分析,深入挖掘益生菌功能的开发与应用。同时,可进一步通过诱变、适应性进化、反向代谢工程等策略等新型技术强化益生菌优选菌株的生理性能,实现优良益生菌的高效筛选和性能强化,并结合制剂化技术保证益生菌产品的应用效果,促进益生菌相关产业的快速发展。

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Summary for “益生菌功能开发及其应用性能强化”

Functional development and performance enhancement of probiotics

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Several new kinds of diseases posing serious threat to human life have developed owing to the drastic changes in living environment, eating habits, and lifestyle. Concomitantly, there has been a gradual increase in health awareness among people, and probiotics have gained much attention for its excellent health benefits. Probiotics are live microorganisms, mainly bacteria such as *Lactobacillus*, *Lactococcus*, *Bifidobacterium*, *Leuconostoc*, and *Streptococcus*, which benefit human health, especially the digestive system. Probiotics have been reported to improve constipation, reduce diarrhea, decrease the content of cholesterol, enhance immunity, and protect oral health. Moreover, they have been associated with anti-tumor effects and positive effects towards prevention and treatment of autism and depression. Currently, probiotics dietary supplements and non-prescription drugs are widely used in the field of medicine. With the gradual improvement in research tools, newer functions of probiotics and insights into its relationship with diseases continue to emerge. Thus, it is important to further engage in the basic research of probiotics and explore new functions in the field of disease prevention. Meanwhile, inevitably, probiotics production faces a variety of stresses from the external environment, such as acid stress caused by lactic acid accumulation in the fermentation process, freezing stress during formulation, and osmotic stresses at various production stages. These adverse conditions affect the normal metabolism and survival of strains, thus inhibiting their probiotic effects. Therefore, it is essential to improve the stress tolerance of strains and strengthen the performance of probiotics. Recent developments in biotechnology, including microbial community analysis and high throughput screening, and evolutionary techniques can improve the efficiency of strain screening. Evolutionary engineering, including mutagenesis techniques, adaptive laboratory evolution, and genome evolution, is a valuable method to promote the phenotype or physiological characteristics of strains. Moreover, using systems biology, we can examine the effects of mutant strains as probiotics and even apply inverse metabolic engineering approaches to improve microbial characteristics and get the desired phenotype. This comprehensive review describes the main methods and principles used in functional development and performance enhancement of probiotics, and summarizes their effects in the prevention and treatment of various diseases. The aim of this review is to provide an understanding of the multiple functions of probiotics and provide a perspective on the challenges faced in the probiotics industry so as to trigger development of better industrial processes for probiotics production.

probiotics, health benefits, screening, performance enhancement, disease prevention

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