特约评述

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乳酸菌的合成生物学工具及在合成益肤因子中的应用

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摘要：乳酸菌在发酵食品中的使用有着悠久历史，一些菌种被认为是安全级微生物。乳酸菌也是人体正常的共生菌，具有调节肠道和皮肤微生态的菌群平衡、增强机体免疫力等有益作用。当前，乳酸菌及其产生的活性代谢产物添加到护肤品中产生保湿、抗氧化和减轻过敏等效果得到了消费者认可，而一些乳酸菌在缓解和治疗皮肤疾病方面的作用也有越来越多的数据支持。基于此，利用乳酸菌作为底盘生产护肤活性物质或作为皮肤修复的生物治疗载体具有广阔的应用前景。本文首先总结了在乳酸菌中建立的遗传操作系统，着重关注遗传可及性、基因表达系统和基因组编辑技术，然后总结了乳酸菌作为细胞工厂生产保湿因子和抗氧化产物的研究进展，最后介绍了工程乳酸菌针对皮肤损伤进行靶向递药的可行性，旨在为乳酸菌应用于皮肤健康领域提供借鉴。

关键词：乳酸菌；合成生物学；遗传操作系统；皮肤修复；活菌药物

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Advances in synthetic biology tools for lactic acid bacteria and their application in the development of skin beneficial products

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Abstract: Lactic acid bacteria (LABs) are a group of Gram-positive bacteria that metabolize soluble carbohydrates to produce lactate as the main metabolite. Certain LABs have a long history of use in fermented foods and are generally considered as safe. On the other hand, LABs are commensal bacteria of the human body and have been shown to exert beneficial effect on the host, such as regulating the balance of intestinal and skin microecology and enhancing the body's immunity. At present, the addition of LABs and their active metabolites to skin care products could enhance moisturizing, antioxidant, and allergy-reduction effect, which have been accepted by consumers. The roles of LABs in treating skin diseases are also supported by more and more experimental data. With the rapid development of genetic tools, LABs have been explored to effectively produce value-added food and biomedical products such as organic acids, alcohols, and exopolysaccharides. Moreover, recent advances in synthetic biology have been used to engineer LABs for delivering therapeutic molecules in response to disease signals, showing attractive application prospect for

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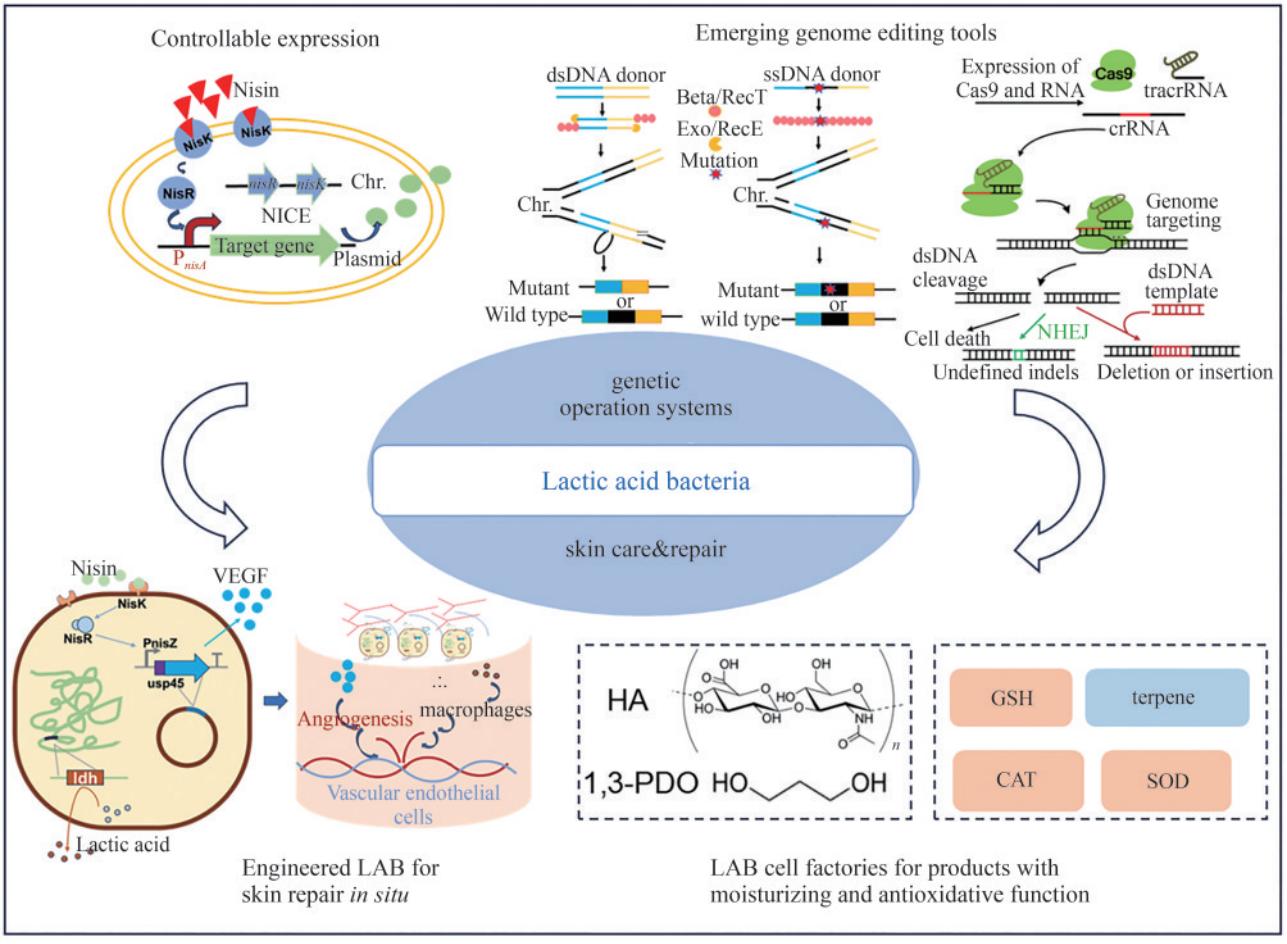
disease therapy *in situ*. In the field of skincare, LABs are an attractive chassis for being engineered to produce bioactive substances for skincare and also as biotherapy carriers for wound treatment, as the food-safe status of LABs. In this review, we summarize the genetic operation systems established for LABs, from genetic accessibility to gene expression systems, and highlight the emerging genome editing techniques for manipulating their genomes. In addition, we comment research progress in producing moisturizing factors and antioxidants using LABs as cell factories. Finally, we expect the feasibility of targeted drug delivery by engineered LABs for healing skin wound and the prevention of infection by pathogenic bacteria, aiming at providing a reference for applying LABs in skin health.



Keywords: lactic acid bacteria; synthetic biology; genetic operation systems; skin repair; live biotherapeutic product

皮肤是人体防御外界刺激和损伤的最大器官，其表面栖息着多种活跃的微生物群体，即皮肤微生物组。皮肤微生物组的分布与身体部位、年龄和卫生习惯密切相关，常见微生物类群包括棒状杆菌、丙酸杆菌、葡萄球菌、微球菌、链球菌、短杆菌和假单胞菌等[1-2]。在正常生理条件下，皮肤微生物组具有防止病原菌定殖、调节免疫反应和增强皮肤屏障的作用。当受到内外因扰动时，皮肤微生物组发生变化，例如免疫异常、气候变化、生活方式改变等可能降低皮肤微生物多样性，增加以金黄色葡萄球菌为代表的病原菌的物种丰度[3]，皮肤微生物组失调还会引发特应性皮炎、湿疹、银屑病等皮肤疾病[4-6]。

乳酸菌是利用碳水化合物产生乳酸为主要代

谢产物的革兰氏阳性细菌的统称，一些乳酸菌长期应用于食品发酵，是公认的安全级（generally regarded as safe，GRAS）微生物[7]。同时，乳酸菌是人体肠道天然的共生菌，在调节菌群平衡、促进机体免疫等诸多方面具有益生作用[8]。据报道，口服乳杆菌、双歧杆菌、嗜热链球菌等乳酸菌，通过“肠-肤”轴作用可降低皮肤敏感性、改善角质层屏障功能、调节皮肤内炎症细胞因子的释放[9]；在皮肤局部涂抹乳酸菌制品能够促进伤口愈合、抑制痤疮丙酸杆菌生长和金黄色葡萄球菌生物膜形成[10-12]。近几年，乳酸菌及其代谢产物作为护肤原料已得到广泛应用和认可，例如乳酸不仅是天然保湿因子，还能够抑制酪氨酸酶的活性从而减少色素沉积[13]。为提高乳酸菌有效化合物的生产能力，以乳酸菌为细胞工厂的合成生物学技术发展迅速，并建立了较为完善的遗传操作系统；同时为了强化、靶向益生菌的作用效果，利用合成生物技术改造乳酸菌针对皮肤伤口原位递送修复因子，具有非常诱人的应用前景。本文概述目前乳酸菌合成生物学研究中构建的遗传操作系统以及利用乳酸菌为底盘生产护肤活性产物和工程乳酸菌活菌药用于修复皮肤损伤的研究进展，预期为工程乳酸菌在护肤领域的应用奠定基础。

1 乳酸菌的遗传操作系统

1.1 乳酸菌底盘的基本特性

乳酸菌包括乳球菌属（*Lactococcus*）、乳杆菌属（*Lactobacillus*）、乳酪杆菌属（*Lacticaseibacillus*）、黏液乳杆菌属（*Limosilactobacillus*）、乳植杆菌属（*Lactiplantibacillus*）、链球属（*Streptococcus*）等，目前报道较多且易于改造的菌种主要有乳酸乳球菌（*Lactococcus lactis*）、副干酪乳酪乳杆菌（*Lacticaseibacillus paracasei*）、植物乳杆菌（*Lactiplantibacillus plantarum*）、短乳杆菌（*Levilactobacillus brevis*）、罗伊氏黏液乳杆菌（*Limosilactobacillus reuteri*）和嗜热链球菌（*Streptococcus thermophilus*）等。

乳酸乳球菌是生产奶酪的重要发酵剂菌种，也是第一个完成全基因组测序的乳酸菌[14]。因基因组小、代谢简单、遗传背景清晰，乳酸乳球菌成为乳酸菌研究的模式菌种[15]。基于生理代谢特性研究发现，当血红素存在时乳酸乳球菌启动有氧呼吸，使菌体获得更多能量且缓解了氧化胁迫损伤，因而发酵密度和菌体存活性显著提高[16]。因此，乳酸乳球菌作为细胞工厂生产食品添加剂、医药分子和精细化工品的报道较多[17-19]，也是目前最常用的乳酸菌合成生物学底盘（如乳酸乳球菌NZ9000）。

嗜热链球菌是继乳酸乳球菌之后第二大重要的乳制品发酵剂，它利用乳糖产生乳酸、乙醛和胞外多糖等代谢产物形成风味和质地独特的发酵乳制品[20]。从进化关系上嗜热链球菌属于链球菌，与无乳链球菌亲缘关系最近，长期的乳基质环境生长造成其毒性基因丢失或衰退，成为食品级安全菌株，同时它具有链球菌的自然转化能力且基因组上携带多种CRISPR/Cas系统，便于建立遗传操作系统，成为一种新兴的合成生物学底盘[21]。

乳杆菌属是一个大的生理类群，包含200多个物种，其生理代谢、遗传基因和生态分布的多样性显著。乳杆菌对于醇、盐、酸和温度通常具有比较好的耐受性，并且可利用的碳源种类多，因此乳杆菌作为细胞工厂受到关注[22]。目前报道较多的乳杆菌底盘有副干酪乳杆菌、植物乳杆菌和罗伊氏乳杆菌等[23-24]，由于乳杆菌菌株之间的差异，导致遗传操作工具的通用性较低，往往需要配备相应的遗传操作工具。

1.2 遗传可及性

遗传可及性（genetic accessibility）是指生物吸收外源遗传物质的能力。在自然状态下，乳酸菌中由结合型质粒、转座子和噬菌体转导引起的遗传物质水平转移现象普遍存在，然而在实验室条件下尚未广泛应用[25-27]。对于自然转化，由于多数嗜热链球菌和部分乳酸乳球菌携带了必要的遗传元件，包括用于DNA结合和重组的自然感受态调节因子ComX，这两种菌可以利用自然转化吸收外源DNA并发生基因重组[28-29]。此外，在一些没有ComX编码基因的乳酸乳球菌中异源表达嗜热链球菌的*comX*也能够建立自然感受态并实现自然转

化[30]。值得注意的是，虽然自然转化方法获得基因重组的效率较低，但是这种重组菌株被认为是非遗传修饰的生物（non genetically modified organism，non-GMO）[31]，在食品、药品制造领域具有更大的应用潜力。随着乳酸菌自然转化机制的深入揭示，有望提高转化效率且应用到更多的乳酸菌中。

为了提高乳酸菌的转化率，实验室往往采用电转化人工感受态细胞的方法。在制备人工感受态细胞时，首先在含有弱化细胞壁成分（甘氨酸、苏氨酸或山梨醇）的培养基中培养乳酸菌细胞至对数生长期，然后使用高浓度糖溶液洗涤菌体细胞[32-33]。利用人工制备的感受态细胞，乳酸菌的电转化效率提高至 10^3～10^6 CFU/μg。进一步优化试剂浓度、培养时期和电转化条件等影响因素，多数乳酸菌可以实现高效的人工转化[34-35]。

1.3　基因表达系统

广义上的基因表达系统包含多种重要的遗传元件，如启动子、核糖体结合位点、选择性标记、蛋白质定位系统和终止子等。本文重点关注在乳酸菌中控制基因表达的组成型启动子和诱导型启动子，其他遗传元件的研究进展可参考近期综述文章[36]。乳酸菌的组成型启动子常来源于细胞内持续性高水平表达基因的启动子，例如在乳酸菌中广泛应用的组成型启动子 P21、P32 和 P59 等以及乳酸脱氢酶基因启动子[37-38]。此外，根据原核生物启动子保守区和可变区核酸序列组成的特点，设计简并引物对启动子进行随机突变，可以获得强弱呈现梯度变化的启动子库，在乳酸菌的代谢调控方面有较多应用[39-40]。

相比组成型启动子，诱导型表达系统能够对表达强度和时间实现更理性的控制。乳酸菌研究中应用最为广泛的可控表达系统是乳链菌素控制的表达系统（nisin-controlled expression system，NICE）。NICE 系统包含启动子 P_{nisA} 和双组分调控元件 *nisR/K* [图 1(a)]。当添加亚致死量的 nisin（0.1～10 ng/mL）

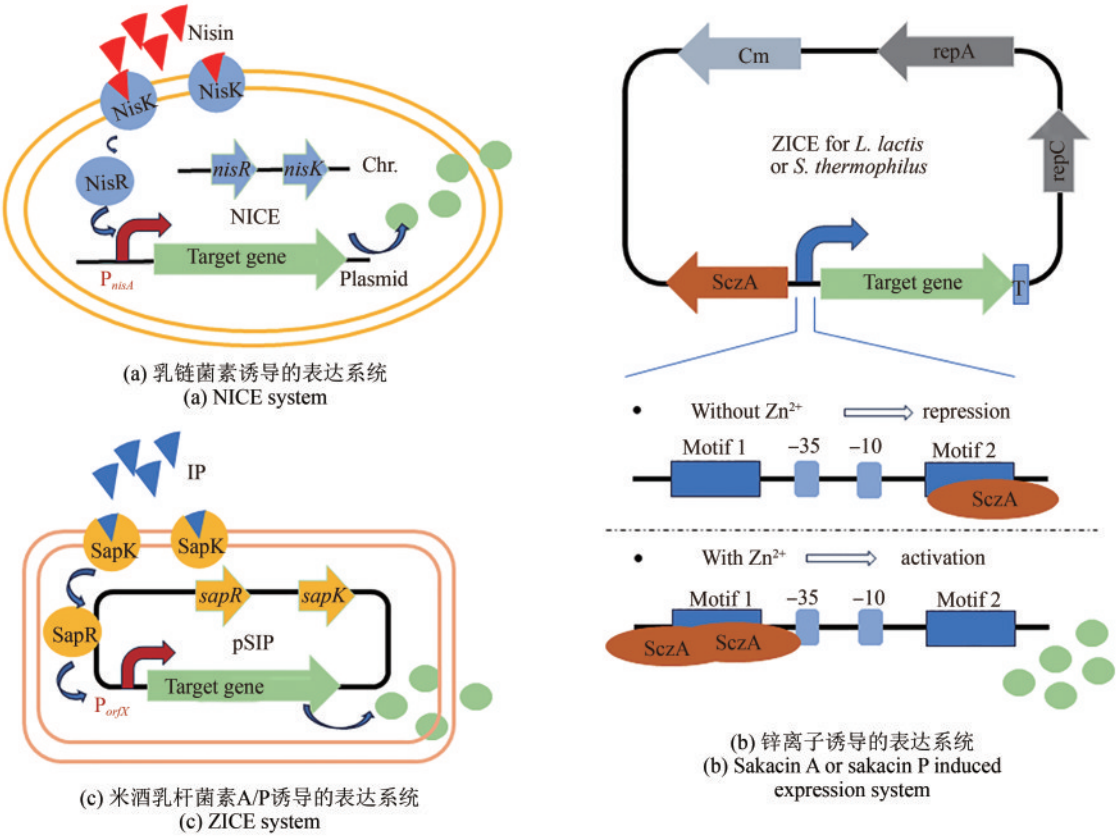


图 1　乳酸菌中常用的基因诱导表达系统工作示意图

Fig. 1　Schematic diagram of commonly used gene expression systems for lactic acid bacteria

后，目的基因即可有效表达[41-42]。NICE系统宿主范围广泛，在乳酸乳球菌、副干酪乳杆菌、植物乳杆菌和嗜热链球菌中均可使用[42-44]，此外NICE系统还有表达水平高、诱导剂符合食品级要求等优点，但是该系统也存在基因表达的渗漏现象。

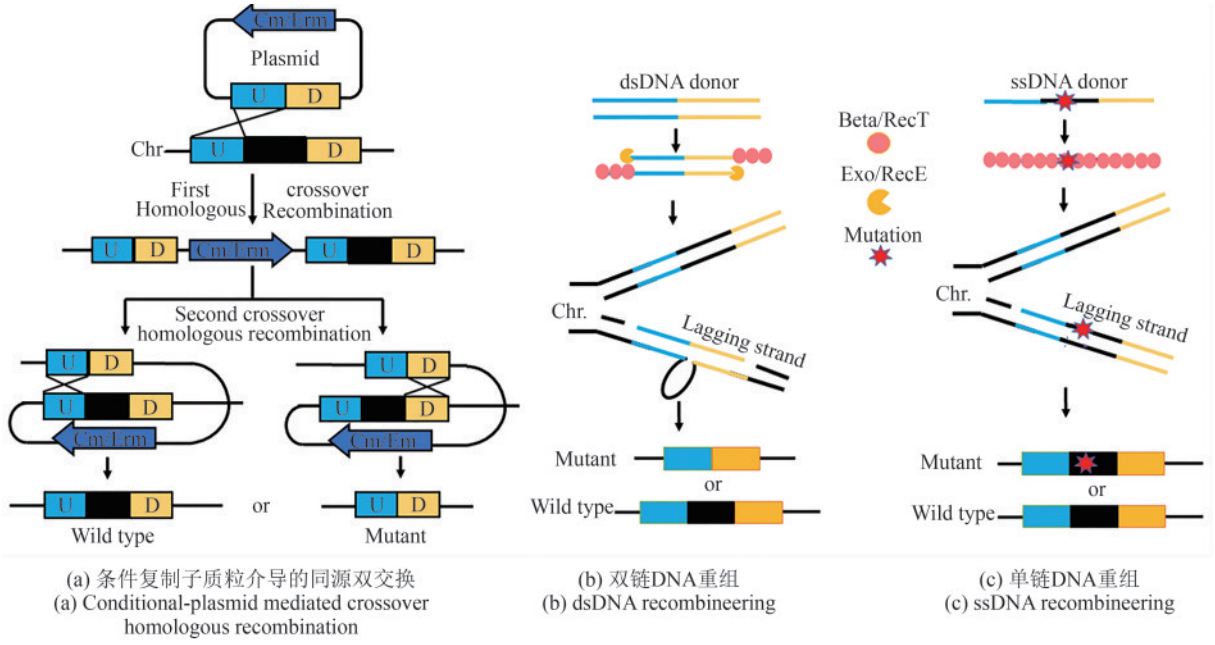
在乳酸菌中还可以利用金属离子、乳糖、木糖或pH控制基因表达[45-47]。例如，锌离子控制的表达系统（Zn^{2+}-controlled gene expression system，ZICE）包括来源于嗜热链球菌的启动子P_{czcDst}和转录调控因子$SczA_{st}$[48]。在乳酸乳球菌和嗜热链球菌中，当培养体系中没有锌离子时，$SczA_{st}$与P_{czcDst}的−10区下游结合，阻止RNA聚合酶靠近，系统关闭；当0.2～0.8 mmol/L锌离子存在时，$SczA_{st}$与P_{czcDst}的−35区上游结合，开启基因表达［图1(b)］。ZICE系统不存在渗漏，基因的表达水平约为NICE系统的一半，且两种系统可同时工作，为多基因的实时控制提供可能[45]。

此外，在植物乳杆菌、罗伊氏乳杆菌和清酒乳杆菌中，由米酒乳杆菌素A（sakacin A）或米酒乳杆菌素P（sakacin P）诱导的表达系统包含调控系统元件*sapK*/*sapR*和启动子P_{orfX}，将目的基因置于P_{orfX}后，即可受到米酒乳杆菌素A或米酒乳杆菌素P的可控表达［图1(c)］，该系统渗漏水平低，体现较好的应用效果[49-51]。在鼠李糖乳杆菌、乳酸乳球菌和嗜热链球菌中利用四环素诱导表达系统实现了外源异源蛋白的表达[52-53]，在植物乳杆菌中使用T7启动子控制红色荧光蛋白的表达[54]。

1.4 基因组编辑技术

乳酸菌的基因组编辑始于20世纪90年代，利用条件复制子质粒介导同源单/双交换［图2(a)］。该方法适用范围广，但其突变频率低（约10^{-6}～10^{-4}），筛选烦琐，耗时过长[56-57]。将其与反向筛选标记如*pheS*、*oroP*和*upp*等结合使用，能够将突变菌株的筛选效率提高100倍[58-60]。另外，位点特异性重组酶或转座子系统也能够引起遗传重组，例如在乳酸乳球菌中通过*attB*与*attP*位点的特异性重组将木糖分解基因插入到染色体中[61]，通过Group-Ⅱ型内含子催化外源基因在染色体的多拷贝整合[62]。与同源双交换方法相比，位点特异性重组或转座子介导的重组效率更高，但是需要宿主基因组有特定的识别序列，限制其应用范围。

噬菌体来源的λ Red/ET重组酶系统是一种新型的基因组编辑工具，最早在大肠杆菌中报道[63-64]，目前已应用于多种细菌[65-66]。通过生物信息学分析，在乳酸菌的原噬菌体中预测到了与λ Red/ET同源的遗传元件。例如，作者课题组在



(a) 条件复制子质粒介导的同源双交换
(a) Conditional-plasmid mediated crossover homologous recombination

(b) 双链DNA重组
(b) dsDNA recombineering

(c) 单链DNA重组
(c) ssDNA recombineering

图2 乳酸菌中3种基因组改造方法原理[55]

Fig. 2 Schematic diagram of methods for genome engineering in lactic acid bacteria[55]

干酪乳杆菌BL23中鉴定了重组酶系统LCABL_13040-50-60，功能预测表明LCABL_13040是5′→3′核酸外切酶，LCABL_13050是单链DNA退火蛋白，LCABL_13060具有抑制宿主核酸酶活性的作用。在副干酪乳杆菌中过量表达LCABL_13040-50-60之后，可以完成双链DNA重组（[图2(b)]）[67]。类似地，Yang等[44]鉴定并验证了植物乳杆菌WCFS1的重组酶系统Lp_0640-41-42，并利用其建立了适用于植物乳杆菌、短乳杆菌的基因组编辑工具[44, 68]。在乳杆菌中，重组酶系统LCABL_13040-50-60或Lp_0640-41-42均与Cre-*lox*位点特异性重组酶结合，提高了重组菌的筛选效率，高效完成基因删除、点突变和整合等操作，但会在染色体上残留*lox*位点重组后的疤痕[44, 67]。

Redβ或RecT还可以单链DNA与染色体之间发生重组，称为单链DNA重组工程（ssDNA recombineering）[55]。van Pijkeren等[69]首次在罗伊氏乳杆菌和乳酸乳球菌中重组表达RecT蛋白，利用单链DNA重组完成染色体的定点突变，突变率为0.4%～19%［图2(c)］。Filsinger等[52]将RecT蛋白与相同来源的单链DNA结合蛋白（SSB）共同表达，使单链DNA重组效率提高1000倍，在乳酸乳球菌、鼠李糖乳杆菌中均得到验证。RecT与同源SSB相互匹配，有利于单链DNA与染色体结合，该策略为优化单链DNA重组效率提供了有效参考。

自2012年起，CRISPR/Cas系统应用于基因组编辑取得了巨大的成功。乳酸菌的CRISPR/Cas系统类型多样[70-72]，启动乳酸菌自带的CRISPR/Cas系统即可简便地进行基因组编辑。Ma等[73]在嗜热链球菌LMD-9中导入了靶向CRISPR阵列和DNA修复模板后，删除了785 bp的染色体DNA片段。Martel等[74]启用嗜热链球菌的CRISPR系统对烈性噬菌体2972进行了点突变、大片段删除和功能基因插入。以上工作证实了内源性CRISPR系统对嗜热链球菌或其噬菌体编辑的可能性。另外，酿脓链球菌CRISPR/Cas9系统在乳酸菌中也有应用，但是由于Cas9蛋白的双链DNA断裂活性高、乳酸菌的本底重组水平低，因而很难获得目的突变体。目前解决该问题的策略有两种：减弱Cas9蛋白的活性，或使用外源的重组酶。例如，Song等[75]将Cas9蛋白的RuvC核酸酶结构域定点失活，得到了突变体Cas^{D10A}在染色体上产生单链DNA缺口，这种染色体损伤对菌体细胞致死率低于双链DNA断裂并且还刺激了本底的同源重组水平提高，在干酪乳杆菌中编辑效率提高至20%～65%。在植物乳杆菌、乳酸乳球菌和罗伊氏乳杆菌中，Ⅱ型CRISPR/Cas9与dsDNA重组或ssDNA重组方法联合使用，其中CRISRP/Cas9作为反向筛选工具，可将编辑效率提高至100%，大大简化了筛选工作[68, 76-77]。此外，Kong等[78]在嗜热链球菌中构建了CRISPR/nCas9辅助基因组编辑系统可以作为精确基因编辑的工具包，促进嗜热链球菌产生胞外多糖和其他生物基发酵产品。Tian等[79]在乳酸乳球菌中开发了CRISPR-脱氨酶辅助的碱基编辑器，对基因组多位点精准点突变。

2 乳酸菌在合成护肤活性物质中的应用

乳酸菌通过糖酵解途径将糖类转化为丙酮酸后再生成乳酸，改造丙酮酸的分支途径后可生产多元醇、醛类等高附加值食品添加剂，当引入外源基因后还可以合成多糖、萜类等结构复杂的代谢产物[80]。同时，乳酸菌作为革兰氏阳性菌没有外膜层，因而其合成蛋白类的产物不存在脂多糖的潜在危害。鉴于此，乳酸菌是生产健康相关产品的优良宿主。这里简要介绍利用乳酸菌为细胞工厂合成保湿、抗氧化产物的相关进展。

2.1 保湿因子

透明质酸由D-葡萄糖醛酸和*N*-乙酰葡糖胺为结构单元的天然高分子黏多糖，因优良的保水性和生物相容性作为保湿因子广泛应用于护肤品。据报道，透明质酸的生产菌株兽疫链球菌合成透明质酸的关键基因包括透明质酸合成酶（HasA）、UDP-葡萄糖脱氢酶（HasB）和UDP-葡萄糖焦磷酸化酶（HasC）基因[81]。在嗜热链球菌基因中鉴定到了透明质酸合成酶*hasA*基因[82]，嗜热链球菌的透明质酸产量约0.3 g/L[83]。此外鼠李糖乳杆菌FTDC 8313和格氏乳杆菌FTDC 8131产生的透明质酸浓度约为1 g/L[84]。为了获得更高的透明质酸

产量，Sunguroğlu等[85]以乳酸乳球菌CES15为宿主利用NICE系统过量表达链球菌来源的透明质酸合成酶基因*hasA*，发酵液中的透明质酸产量达到6.09 g/L。在乳酸乳球菌中，共同表达*hasA*、*hasB*和*hasC*后能够使透明质酸的产量提高2倍[86]，将这3个基因插入到乳酸乳球菌的染色体中不仅提高了重组菌的遗传稳定性，还能提高透明质酸的分子量至（3.5～4）×10^6 Da[87]，此外在培养过程中控制重组菌发酵体系中葡萄糖的供给量也能够调控透明质酸的分子量在（0.4～1.4）×10^6 Da之间变化[88]。当前，乳酸菌为细胞工厂合成透明质酸的产量较工业生产还有较大差距，但因宿主不具有致病性，因此值得后续更为深入的研究和开发。

1,3-丙二醇是一种与水、醇和脂互溶的黏性液体，具有较好的吸水性和保湿性，可作为护肤品中的保湿成分。生产1,3-丙二醇的方法主要有化学合成法和生物发酵法两种。化学合成法包括丙烯醛水解法和环氧乙烷羰基化法，因成本高、原料不可再生等已经退出市场[89-90]。生物发酵法是通过克雷伯氏菌、梭状芽孢杆菌和乳杆菌等微生物转化甘油生产1,3-丙二醇，具有条件温和、原料廉价可再生的特点[89-91]。在短乳杆菌、罗伊氏乳杆菌和双科特迪瓦乳杆菌中，通过甘油脱水酶将甘油转化为3-羟基丙醛，再经1,3-丙二醇氧化还原酶催化3-羟基丙醛生成1,3-丙二醇，同时消耗NADH[92]。Ju等[93]筛选到罗伊氏乳杆菌CH53具有较高的1,3-丙二醇合成能力，在培养过程中添加玉米浸泡液作为氮源时1,3-丙二醇的产量为68.3 g/L，生产强度1.27 g/(L·h)；进一步，利用耐酸性驯化，所得突变株JH83在纯甘油或粗甘油为底物时1,3-丙二醇达93.2g/L[94]。除了筛选高产菌株，代谢工程改造的策略同样应用到了乳杆菌生产1,3-丙二醇的研究中，例如在罗伊氏乳杆菌DSM20016中过量表达1,3-丙二醇氧化还原酶、阻断消耗NADH的竞争途径、删除引起菌体细胞自裂解的原噬菌体，使1,3-丙二醇产量较野生型提高了45.2%[95-96]。乳杆菌具备GRAS属性，对粗甘油耐受性强，体现出生产食品级1,3-丙二醇的巨大潜力，但乳杆菌在发酵后期产生大量乳酸，对1,3-丙二醇的产量造成影响，有待进一步解决。

2.2 抗氧化产物

正常生理条件下，细胞经有氧代谢或紫外线照射会产生大量活性氧（reactive oxygen species，ROS），引起脂质过氧化、细胞膜损伤、炎症和自身免疫性疾病[97]。细胞内主要的抗氧化酶是超氧化物歧化酶（superoxide dismutase，SOD），保护细胞免受ROS的损伤。Kong等[98]以嗜热链球菌为底盘，首先根据转录组数据建立了组成型启动子库，然后选择不同强度的启动子过量表达干酪乳杆菌来源的*sod*基因，所得重组嗜热链球菌最高的SOD活性达到20000 U/mg。除了SOD，过氧化氢酶（catalase，CAT）通过清除过氧化氢发挥抗氧化作用，在鼠李糖乳杆菌或干酪乳杆菌中共同表达*sod*基因和*cat*基因后二者发挥协同抗氧化的作用[99-100]。谷胱甘肽（GSH）对ROS也具有解毒作用，它由半胱氨酸、甘氨酸和谷氨酸组成，可以由谷氨酰半胱氨酸合成酶（GshA）和谷胱甘肽合成酶（GshB）催化合成。在乳酸乳球菌中，表达大肠杆菌来源的*gshA*和*gshB*基因后重组菌细胞内GSH浓度达到358 nmol/mg[101]。另外，双功能的谷胱甘肽合成酶（GshF）能够一步催化半胱氨酸、甘氨酸和谷氨酸合成GSH且不受产物反馈抑制。在乳酸乳球菌中异源表达了无乳链球菌来源的*gshF*基因，重组菌发酵17 h后细胞内GSH含量提高到17.3 mg/L[102]。此外，番茄红素含有11个共轭双键，抗氧化能力强，现已作为抗氧化剂添加到日常护肤产品中。Wu等[103]在乳酸乳球菌中异源表达番茄红素合成基因簇*crtEBI*并结合乳酸乳球菌的有氧呼吸作用，重组菌发酵后番茄红素产量达到1.09 mg/L。在后续研究中，一方面利用合成生物学手段提高抗氧化产物的产量，另一方面通过自然转化等方法获得非遗传修饰的重组乳酸菌，其细胞裂解液或代谢产物添加到护肤品中发挥抗氧化作用。

3 工程乳酸菌在原位修复皮肤损伤中的应用

微生物组对于维持机体健康具有重要作用，以肠道微生物组为靶位点利用工程化的大肠杆菌、

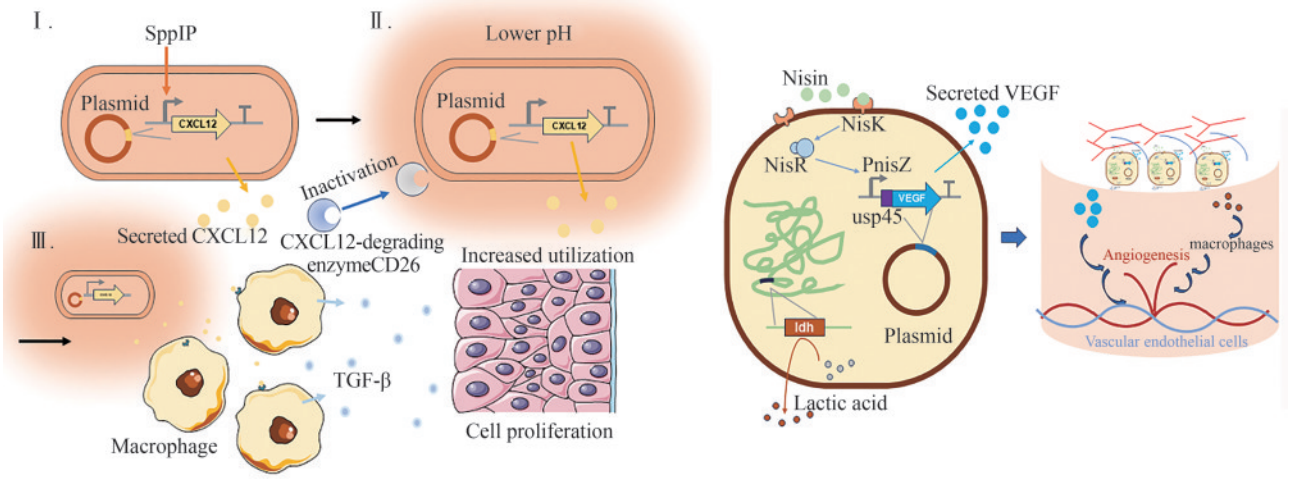
乳酸乳球菌、酵母菌有望治疗代谢性和免疫性等疾病[104-106]。相比肠道微生物组，皮肤微生物组更容易接近，工程菌可以直接涂抹或敷于皮肤患处，展现出巨大的应用前景，本文主要介绍工程乳酸菌在修复皮肤伤口和防治致病菌感染中的应用。

3.1 工程乳酸菌促进皮肤伤口愈合

在临床上，久护不愈的皮肤伤口对患者的身体健康和生活质量产生了严重影响，其原因是伤口处的蛋白水解活性高进而降低相关药物的利用度。例如，趋化因子CXCL12可以诱导伤口处的免疫细胞-巨噬细胞表现出抗感染表型，还能促进皮肤组织再生，但是外用CXCL12容易被蛋白酶水解。Vågesjö等[107]以罗伊氏乳杆菌为底盘构建了一种能够递送CXCL12的工程菌［图3(a)］。当工程菌用于小鼠的伤口后，由于巨噬细胞的抗感染能力增强并且协同提高了TGF-β的表达量，加速了肉芽组织和上皮层的形成，促进伤口愈合。进一步，该研究团队将工程菌制备成冻干粉ILP100，并在实验小猪中证实了其作用效果；一期临床试验结果表明ILP100安全无毒，伤口愈合时间缩短了6～10天，效果好于安慰剂组[108-109]。此外，研究表明570～600 nm范围内的黄光可以刺激胶原蛋白合成，将表达CXCL12的乳酸乳球菌与黄色发光二极管联合使用，上调了Wnt和Notch信号通路中的关键蛋白表达水平、降低了炎症因子IL-1β和TNF-α的表达水平，除了加速伤口愈合外，该联合治疗方法还有效减少了皮肤表面病原体的群落密度，降低感染风险[110]。为了解决皮肤表面营养贫瘠、工程菌存活性差的问题，Li等[111]使用了一种偏利共生策略，将表达CXCL12的工程乳酸乳球菌与光能自养菌聚球藻PCC7942封闭在水凝胶基质中，后者产生的蔗糖供给前者生长和合成CXCL12，在小鼠伤口上外敷这种共生菌基质第14天时愈合面积达到86.8%。除了趋化因子，血管内皮生长因子（vascular endothelial growth factor，VEGF）是促进伤口愈合的有效分子，Lu等[112]构建了一种携带VEGF编码基因的工程乳酸乳球菌［图3(b)］，并将其嵌入肝素-波洛沙姆水凝胶中，这不仅提高了VEGF在慢性伤口氧化环境中的稳定性，还利用水凝胶材质的缓释特性延长了VEGF的有效作用时间。该方法促进了内皮细胞的生长和运动，增强巨噬细胞的抗感染活性，成功应用于糖尿病患者溃烂伤口处的血管生成，加速伤口愈合。

3.2 工程乳酸菌防治皮肤致病菌感染

皮肤是抵御病原菌感染的首要防线。当皮肤受损时，传统的治疗策略是使用抗生素杀死有害菌，但抗生素的使用也会抑制有益菌的生长，破坏皮肤微生态平衡。因此，基于工程菌原位递送



(a) 工程罗伊氏乳杆菌递送细胞因子CXCL12
(a) Engineered *Limosilactobacillus reuteri* delivering cytokines CXCL12

(b) 工程乳酸乳球菌递送血管内皮生长因子VEGF
(b) Engineered *Lactococcus lactis* delivering VEGF

图3 利用工程乳酸菌原位修复皮肤伤口的示意图

Fig. 3 Strategies for repairing cutaneous wounds *in situ* by engineered lactic acid bacteria

杀菌物质的防治策略得到重视。

罗伊氏菌素是乳杆菌产生的对大肠杆菌、金黄色葡萄球菌和沙门氏菌有杀灭作用的抗菌物质，Ming等[113]将产生罗伊氏菌素的罗伊氏乳杆菌使用水凝胶微球包裹后敷在伤口处，可显著降低伤口处有害菌感染。溶葡萄球菌素作为一种选择性的抗菌酶，能够杀灭金黄色葡萄球菌，防止其传播和感染。Mierau等[114]利用NICE系统构建了一种表达溶葡萄球菌素的工程乳酸乳球菌，可有效改善婴儿的金黄色葡萄球菌感染，并且该重组菌中表达溶葡萄菌素的遗传元件整合到了乳糖筛选的质粒上，避免抗生素抗性基因的潜在风险。Lubkowicz等[115]开发了一种监测金黄色葡萄球菌的工程益生菌，在罗伊氏乳杆菌中整合了金黄色葡萄球菌的Agr群体感应（AgrQs）系统，能够实时检测到环境中nmol级的金黄色葡萄球菌自诱导肽AIP-1，是一种高灵敏度的致病菌检测方法。

目前，在国内外多家研究机构积极推动下，利用工程乳酸菌作为活菌药物治疗皮肤疾病显示出巨大潜力。上述研究实例表明，将工程乳酸菌与活性材料有机结合，不但为工程菌提供了良好的携带载体，更重要的是活性材料在一定程度上限制工程菌的扩散，避免了潜在的免疫反应和菌群失衡，为活菌药物的合理使用提供了保障。

4 总结和展望

乳酸菌在合成皮肤健康相关产物方面呈现出广阔的前景，其具GRAS属性、拥有食品级诱导表达系统、代谢途径相对简单且易于改造，为工程乳酸菌合成高值化益肤成分乃至用于原位皮肤修复奠定了基础。未来利用工程乳酸菌生产益肤因子的研究重点主要体现在以下几个方面：

（1）开发乳酸菌的合成生物学工具，特别是高效、简便、普适性和高通量的基因组编辑工具将为乳酸菌细胞工厂的构建与优化提供更大助力；

（2）进一步提高活性产物的产量、开发配套的产物提取技术，将会极大推进工程乳酸菌合成护肤因子从实验室到工业化生产的步伐；

（3）在工程菌设计时将感知、治疗和自杀遗传模块有机结合，既可控制治疗性物质的实时传递，又能避免活菌药物可能存在的生物污染；

（4）根据不同皮肤环境定制人工合成菌群，可以更好地辅助工程菌执行预设的功能，还能应对复杂微生态环境的挑战。

在皮肤修复领域，活体工程菌的构建和应用处于临床前开发阶段，相信更多智能化工程菌的出现以及法律法规的完善将快速推动这一领域的发展。

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