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·综 述·

生物被膜：益生菌肠道定植的新策略

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摘 要：益生菌可改善机体微生态平衡，在促进营养吸收、控制肠道感染和调节免疫功能等方面具有特殊的功效，但存在胃肠道环境难定植、口服生物利用度低等问题。生物被膜是多个细菌黏附于非生物或生物表面，分泌胞外聚合物 (extracellular polymeric substances)，并将自身包裹其中形成的一种有组织的细菌集团，包含胞外多糖 (exopolysaccharides, EPS)、蛋白质、胞外 DNA (extracellular deoxyribonucleic acid, eDNA) 和脂质等多种组成成分，是一个具有三维立体空间结构的聚集体。被膜状态的益生菌较浮游菌在抗逆性、对抗病原菌和调节免疫功能等方面具有明显优势，这些特点为新型益生菌的开发提供了新的研究思路。本文阐述了被膜状态益生菌的优势，重点介绍了促进益生菌生物被膜形成的活性物及其形成机制，简述了益生菌生物被膜的安全性问题。当前，益生菌生物被膜的研究尚处于起步阶段，希望本文能为该领域未来的研究提供参考。

关键词：益生菌；生物被膜；定植；双组分系统；群体感应

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Bacterial biofilms: novel strategies for intestinal colonization by probiotics

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Abstract: Probiotics can improve the microecological balance of the body and have special effects in promoting nutrient absorption, controlling intestinal infections, and regulating immune function. However, there are problems such as difficult colonization in the gastrointestinal environment and low oral bioavailability. Bacterial biofilms are organized bacterial cells that adhere to an abiotic or biotic surface and are enclosed in extracellular polymeric substances of exopolysaccharides (EPS), extracellular DNA (eDNA), proteins and lipids, with a three-dimensional spatial structure. Probiotics with the help of bacterial biofilms have obvious advantages over planktonic bacteria in stress resistance, combating pathogens and modulating the host's immune function, which provides a new research idea for the development of probiotics. This paper expounded on the advantages of probiotics with the help of bacterial biofilms, and focused on introducing substances that could promote the formation of probiotic biofilms and the mechanisms, and the safety of probiotic biofilms. Currently, research on probiotic biofilms is still in its infancy, and this paper is expected to provide references for future research in this field.

Keywords: probiotic; bacterial biofilm; colonization; two-component system; quorum sensing

联合国粮食及农业组织与世界卫生组织将益生菌定义为“当摄入足够量时，可以为宿主带来益处的活微生物”[1]，其常见的益生作用是预防和治疗疾病，主要通过 4 种机制实现：抑制病原菌或与其竞争、增强肠道上皮的屏障功能、调节宿主免疫功能和参与肠-脑轴调节[1]。机体大部分益生菌存在于肠道，故其发挥益生作用的关键是能够在肠道内成功定植。研究发现，小鼠灌胃丁酸梭菌 (*Clostridium butyricum*) 一周，使该菌数量在接下来 6 d 内维持定植水平，期间肠道微生物群中厚壁菌门 (Firmicutes) 与拟杆菌门 (Bacteroidea) 比值升高 (肠道健康的标志)，结肠中有益的短链脂肪酸也明显增多[2]。益生菌定植的重要步骤是黏附于肠道粘液。肠道黏膜上皮层的杯状细胞分泌粘液以覆盖肠道，形成粘液层，含有粘蛋白、营养物质等，是细菌在肠道定植的重要位点[3-5]。

有报道指出，大多数益生菌在口服后和停止食用后不久随粪便排出结肠[4]，临床效果往往不佳，主要与其不能在肠道中有效定植有关。然而，定植并非易事。口服是一种常见的益生菌摄入方式，但由于胃肠道环境恶劣，导致其生物利用度普遍偏低。对此，生物被膜 (biofilm) 作为一种新策略引起了很多学者的关注。生物

被膜最早由 Costerton 等[6]提出，是细菌种群产生以黏附于某一界面的封闭基质，其内部细菌互相附着，成为一种细菌群体共同生存的形式。该基质由细菌自身分泌的胞外聚合物组成[7]，主要有胞外多糖 (exopolysaccharides, EPS)、蛋白质、胞外 DNA (extracellular deoxyribonucleic acid, eDNA)，任何界面都可能有生物被膜产生[8]。

大部分益生菌具有形成生物被膜的特性，利用生物被膜可使得益生菌在体内存活、定植更有优势，从而提高疗效与抗病菌的能力，展现出良好的研究与应用前景，对进一步探明益生菌发挥作用的机制、开发高效益生菌产品等具有重大意义。本文围绕益生菌生物被膜，将浮游状态与生物被膜状态下的益生菌进行比较，阐明生物被膜为益生菌带来的优势，并分类总结可以促进益生菌生物被膜形成的活性物质，还介绍了部分益生菌生物被膜形成的相关机制，最后简述其安全性问题，希望为该领域研究提供参考。

1 益生菌在生物被膜状态下的优势

生物被膜是细菌一种区别于浮游态的生活方式，该状态下的益生菌在对抗病原菌、抗逆性、调节免疫功能等方面具有优势。

1.1 增强抗病菌效果

生物被膜状态下益生菌的抗致病菌能力被广泛报道。例如，Wang 等[9]对枯草芽孢杆菌 (*Bacillus subtilis*) 用生物被膜进行自我包被，并用浮游态的该菌作为对照。动物实验中，采用口服给药，分别对已有金黄色葡萄球菌 (*Staphylococcus aureus*) 定植的小鼠灌胃。结果显示，仅在给药后第 2 天，生物被膜包裹的枯草芽孢杆菌就可显著减少体内病菌数量，减少量是对照组的 10 倍，且治疗效果可延长至第 6 天，这同时表明益生菌在肠道内的充分定植。

这些效果主要通过 4 种途径实现。第一，由于生物被膜提供的保护作用，益生菌在体内的停留时间延长，这显然意味着它们能够更充分地发挥抗菌等益生作用。如，罗伊氏乳杆菌 (*Lactobacillus reuteri*) 在生物被膜状态下不仅保留了浮游态所具有的抗菌与免疫调节能力，并能产生更多的抗菌物质罗伊氏菌素[10]。第二，益生菌在形成生物被膜定植肠道的过程中，也排除了致病菌的定植。如在生物被膜形成的初始阶段，黏附过程中，人源植物乳杆菌 (*Lactobacillus plantarum*) 299 和 299v 与产肠毒素大肠杆菌 (Enterotoxigenic *Escherichia coli*, ETEC) 竞争黏附位点甘露糖，以定植肠上皮[11-12]。第三，研究发现益生菌生物被膜的成分之一 EPS 具有抗致病菌生物被膜的活性[13]。第四，生物被膜促进有益细菌共存。Yahav 等[14]尝试利用枯草芽孢杆菌的生物被膜，开发出包含植物乳杆菌的共培养系统，提高后者对 pH、温度的抗性，多种益生菌共同使用也被证明对疾病有更佳的治疗效果[15]。

1.2 提高抗逆能力

生物被膜有物理稳定性、表面可感应性、内部环境分布梯度性与多样性等特点，这保证了体系内水分、营养的保持与循环利用[8]。基于这些特征，生长模式从浮游向生物被膜的转变使得包括益生菌在内的细菌能够抵御不利条件，如抗生素及 pH、温度、养分的变化[16]。日本学者对植物乳杆菌 JCM1149 使用各种有机酸进行处理，发现无论是处于稳定期还是对数生长期的浮游细菌，其对乙酸、乳酸等的抵抗力都不如生物被膜中的细菌[17]。生物被膜还使细菌群体能够忍受“饥饿”：在碳源不足时，生物被膜的粗糙程度会增加，进而比表面积 (表面积与体积之比) 增大，这有助于细菌在营养缺乏的条件下尽可能获取养分[18]；在此期间，生

物被膜还会以一个较低的水平释放浮游细胞与基质，在碳源供应恢复正常时生物被膜可以快速生长，证明这种细菌群体生存形式还具有促进细菌增殖的作用[19]。

益生菌在体内抗逆能力提高，意味着定植成功率增加，许多研究也表明，生物被膜与益生菌定植关系密切。如乳酸杆菌 (*Lactobacillus*) 形成生物被膜后可增强其在宿主黏膜中存在和定植的持久性[20]。Everett 等[21]认为，宿主免疫系统在一定程度上支持肠道生物被膜的形成，并将益生菌维持在被膜中，帮助益生菌定植。黏膜免疫系统的重要物质，分泌型免疫球蛋白 (secretory immunoglobulin A, SIgA) 和粘蛋白参与了这一过程，与免疫排斥一同作用，保持肠道健康。相应地，定植于胃肠道的细菌往往也在生物被膜中生长良好[22]。

1.3 参与肠道免疫调节

人体具有强力的肠黏膜免疫系统，即使其暴露于微生物和外来抗原，仍能保持平衡状态。它由 SIgA、黏膜免疫细胞 (如辅助性 T 细胞、调节性 T 细胞)、肠相关淋巴组织 (gut-associated lymphoid tissues, GALT) 等组成[23]。肠黏膜免疫系统与肠道微生物相互作用，参与构成了肠道屏障[24]。益生菌作为肠道微生物，其含有的微生物相关分子模式 (microbial-associated molecular pattern, MAMP) 与黏膜免疫系统细胞特定的模式受体相互作用，从而产生益生作用[25]。由于生物被膜保证了肠道菌群与宿主肠黏膜密切接触，促进了益生菌 MAMPs 与宿主相关受体的结合，故可以认为生物被膜参与了肠道免疫调节，增强了宿主的防御能力[3]。另一方面，如前文所述，肠黏膜中的免疫系统参与了生物被膜的维持。因此，益生菌生物被膜也是与肠道免疫系统有相互作用的，为宿主带来益处。文献报道，植物乳杆菌 LM3 能够产生烯醇化酶 EnoA1，诱导人结肠腺癌细胞系 (human colon adenocarcinoma cell line, Caco-2) 产生促炎因子白介素-6、抗炎细胞因子白介素-10、转化生长因子-β 和人 β-防御素-2 (human β-defensin-2, HBD-2)，参与机体免疫调节，而其突变株 (Δ*enoA1*) 的诱导效果则较弱。同时发现，突变型形成生物被膜的能力比野生型降低了 65%，从侧面表明益生菌的免疫调节能力与生物被膜形成能力有相关性[26]。

2 促进益生菌生物被膜形成的活性物

除了益生菌制剂的改良，有关促进其生物被膜形成的活性物的研究也在近年快速发展。本文从活性物类别、益生菌种、效果等角度将其归纳总结，分为内源性活性物、含甘油的组合、糖类物质、植物源活性物 4 类，见表 1。

内源性活性物质一般指人类和哺乳动物体内天然存在的具有生理活性的物质，其中肝素、胆汁、乳清蛋白水解物等被报道具有促益生菌生物被膜形成的活性。研究还发现共同添加甘油与硫酸镁使吡咯伯克霍尔德氏菌 (*Burkholderia pyrrocinia*) JK-SH007 (一种植物益生菌) 获得了更好的生物被膜形成能力[33]。食品科学中有关蔗糖、葡萄糖、乳糖等常见碳源促进生物被膜形成的研究较多，如以果糖作为碳源培养鼠李糖乳杆菌 (*Lactobacillus rhamnosus*)，增加了生物被膜量和总表面抗原性，加入酪蛋白胨后可以更好地发挥作用[36-37]。另外，多糖中的膳食纤维可以与益生菌相互作用，成为益生菌定植的支架，例如用果胶或明胶为原料制作包衣、微珠等益生菌制剂可提高其体内定植率与抗菌活性，也是目前的研究热点[43-44,70]。植物代谢物是仅次于其他天然来源和合成化合物的新开发药物来源[71]，其在促益生菌生物被膜研究中也有重要的实用价值，如研究

表 1　促进益生菌生物被膜形成的物质

Table 1　Substances that promote probiotics biofilm formation

Category	Item	Strains	Biological action	References
Autocoids	Bile; bile salts	*Lactobacillus plantarum*; *Lactobacillus fermentum*	Form biofilms under specific concentrations of bile	[27-28]
Autocoids	Heparin	*Escherichia coli* Nissle 1917	Stimulates biofilm formation	[29]
Autocoids	Whey protein hydrolysate	*Lactobacillus acidophilus* JC1	Stimulates producing EPS	[30]
Autocoids	Epinephrine	*Enterococcus faecalis* DSM16431&OB15	Modulates the formation of biofilm	[31]
Autocoids	Norepinephrine	*Enterococcus faecium* NCIMB10415	Significantly stimulates biofilm formation	[32]
Glycerol; glyceride	Glycerol combined with magnesium	*Burkholderia pyrrocinia* JK-SH007	Stimulates biofilm formation	[33]
Glycerol; glyceride	Glycerol combined with manganese	*Bacillus subtilis*	Significantly stimulates biofilm formation	[34]
Glycerol; glyceride	Linoleic acids	*Lactobacillus casei*	Significantly increases the ability to adhere to human epithelial cells	[35]
Carbohydrate	Fructose or fructo-oligosaccharides with casein peptone	*Lactobacillus rhamnosus* GG	Increases the biofilm formation and the total surface-antigenicity	[36-37]
Carbohydrate	Fructose with sodium acetate	*Lactococcus lactis* 7-1	Give the strain stronger adhesion to porcine gastric mucin	[38]
Carbohydrate	Glucose with glycerol	*Lactobacillus reuteri* DSM17938	Stimulates both proliferation and biofilm formation	[39]
Carbohydrate	Gluconic acid and glucoheptonic acid derivatives	*Lactobacillus acidophilus*; *Lactobacillus casei*	Stimulates biofilm formation	[40]
Carbohydrate	Water extractable polysaccharides (RKBWEP) from red kidney bean (*Phaseolus vulgaris*)	*Lactobacillus plantcuum*; *Lactobacillus ferrnenturn*	Significantly stimulates the growth of strains	[41]
Carbohydrate	Konjac glucomannan (KGM)	*Bifidobacteria*	Have protective effects for strains against the damage caused by specific antibiotics	[42]
Carbohydrate	Low-methoxyl pectins (CMP-6 and CMP-8) used as coating materials	*Lactobacillus acidophilus* LMG9433; *Lactobacillus casei* LMG6904; *Lactobacillus rhamnosus* LMG25859.	Stimulates the biofilm formation of encapsulated lactobacilli	[43]
Carbohydrate	Calcium pectinate beads (CPB)	*Lactobacillus paracasei* ATCC 334	Bacteria within these microcolonies display a biofilm-like phenotype	[44]
Carbohydrate	Xylan; pectin; arabinogalactan	*Bacillus subtilis*	Stimulates biofilm formation	[45]
Carbohydrate	Semi-permeable biocompatible dextranomer microsphere	*Lactobacillus reuteri*	Enhances adherence to human intestinal epithelial cells *in vitro*	[46]
Actives of plant origin	Phthalic acid; salicylic acid; cinnamic acid	*Bacillus amyloliquefaciens* NJN-6	The transcription level two biofilm formation-related genes in strain were upregulated	[47]
Actives of plant origin	Thymol; carvacrol; eugenol; catechin; genistein; cranberry extracts	*Lactobacillus lactis* 11454; *Lactobacillus rhamnosus*	Inhibit pathogenic aggregation or enhance probiotic biofilm formation or both	[48]

(待续)

(续表 1)

Category	Item	Strains	Biological action	References
Actives of plant origin	*trans*-resveratrol	*Lactobacillus paracasei* ATCC 334	Modify physicochemical properties of the bacterial surface and thereby enhances aggregation, adhesionhance, and biofilm formation	[49]
Actives of plant origin	Polyphenols like *trans*-resveratrol	*Lactobacillus paracasei* ATCC 334	Stimulates biofilm formation	[49]
Actives of plant origin	Propolis ethanolic extract	*Bacillus clausii*; *Saccharomyces boulardii*; *Lactobacillus*; *Lactobacillus acidophilus*	Has effects on the planktonic growth and biofilm-forming ability of the probiotics	[50]
Actives of plant origin	A natural sesquiterpene ketone, 9,10-dehydrofu kinone (DHF)	*Lactobacillus* ATCCSD-5212	Stimulates biofilm formation	[51]
Actives of plant origin	Walnut oligopeptide	*Lactobacillus plantarum* Z7	Significantly increases the content of biofilm	[52]
Carrier or embedding material	Polylactic acid composite soybean meal	*Pediococcus pentosaceus*	Stimulates biofilm formation, with type I glyceraldehyde-3-phosphate dehydrogenase upregulated	[53]
Carrier or embedding material	Grape seed flour (GSF)	*Bifidobacterial*	Benefits biofilm formation	[54]
Carrier or embedding material	The resistant starch fibers of chickpea milk	*Bacillus subtilis*	Stimulates biofilm formation	[55]
Carrier or embedding material	Scaffolds of self-assembled collagen fibers	*Lactobacillus fermentum*; *Lactobacillus acidophilus*	Acts as a host to the formation of the probiotic biofilm; protects live probiotics during storage under harsh conditions; enhances the adhesion of probiotics	[56]
Carrier or embedding material	Thin films of silk and the blend	*Lactobacillus plantarum* MTCC 1746	Promote rapid biofilm growth	[57]
Metals or minerals	Manganese sulfate	*Bacillus subtilis*	It eliminates the toxic effect of fusaric acid and stimulates biofilm formation	[58]
Metals or minerals	Smectite	*Lactic acid bacteria*	Promotes LABs to form biofilm on it *in vitro* and *in vivo*	[59]
Metals or minerals	Smectite combined with Cd(II)	*Serratia marcescens* S14	Promotes biofilm development significantly	[60]
Metals or minerals	Sodium chloride	*Lactic acid bacteria*	A low concentration of sodium chloride promotes the formation of LAB's biofilm	[61]
Others	Norspermidine	*Bacillus subtilis*	Promotes biofilm formation at a specific concentration	[62]
Others	L-malic acid	*Bacillus subtilis* FB17	Promotes binding and biofilm formation of FB17 on Arabidopsis roots	[63]
Others	Putrescine and its precursor arginine	*Pseudomonas* sp. WCS365	Stimulates biofilm formation	[64]
Others	Spermidine	*Bacillus subtilis*	Promotes biofilm formation by activating the expression of the matrix regulator *slrR*	[65]
Others	Validmycin A	*Bacillus subtilis* R31	Stimulates biofilm formation	[66]
Others	Proline	*Metschnikowia citriensis*	Stimulates biofilm formation	[67]
Others	L-arginine combined with NaF	*Lactobacillus rhamnosus* GG	Stimulates biofilm formation	[68]
Others	Poly(3-(3′-N,N,N-triethylamino-1′-propyloxy)-4-methyl-2,5-thiophene hydrochloride) (PMNT)	*Escherichia coli*; *Bifidobacterium infantis*; *Enterococcus faecalis*	Efficiently promotes the initial adhesion and biofilm formation	[69]

发现反式白藜芦醇是副干酪乳杆菌 (*Lactobacillus paracasei*) ATCC 334 形成生物被膜的诱导剂，促进细菌聚集而不会引起人肠上皮细胞的促炎症反应[49]。但值得重视的是，筛选活性物时，需要考察活性物对益生菌、致病菌的生物被膜是否存在无选择性的促进效果，如肝素[29,72]。针对此问题，Gutiérrez 等[48]在筛选促生物被膜形成的植物源活性物时，将其分为 3 类，即抑制病原菌聚集并增强益生菌生物被膜形成、不抑制病原体聚集但刺激益生菌生物被膜形成、对病原菌和益生菌生物被膜具有相同作用，通过这种分类，进而选择出最优的活性物。该研究思路值得学者们借鉴。

3 促益生菌生物被膜形成的机制

细菌生物被膜受外界条件、外源添加物、自身基因表达等影响，以不同途径与机制调节成膜量[62,73-74]，黏附过程是重要的调控环节。Han 等[59]发现电荷、pH 对于蒙脱石促进嗜酸乳杆菌 (*Lactobacillus acidophilus*) 生物被膜形成至关重要。蒙脱石电荷分布不均，在由产乳酸细菌创造的酸性条件下，其表面负电荷由于层间离子交换变为正电荷；革兰氏阳性菌的细胞壁含有带负电荷的磷壁酸，因此易与蒙脱石黏附；随着环境 pH 改变，蒙脱石表面电荷性质改变，提高枯草芽孢杆菌对其黏附性[75]。在一些研究中，温度甚至重力条件也被证明可以成为生物被膜形成的影响因素[76-77]。

深入研究发现，多数调控是由各种信号因子介导的[78]，如植物分泌的 L-苹果酸、腐胺可促进植物生防菌定植[63-64]。在环境治理领域，另一项有关蒙脱石的研究报道了蒙脱石与 Cd(Ⅱ)复合处理后可作为信号促进粘质沙雷氏菌 (*Serratia marcescens*) S14 生物被膜形成[60]，其效果与显著上调的 *fimA*、*bsmA* 和 *eps* 相关，其中 *fimA*、*bsmA* 分别通过群体感应 (quorum sensing, QS)、非群体感应途径发挥作用[79]。而信号因子如何被感知、传递并发挥作用，目前仍在研究中，现对已开展的信号因子介导益生菌生物被膜形成机制进行分类总结，见表 2。

3.1 胞内调控通路的起始：双组分系统

双组分系统 (two-component system, TCS) 是一种细胞内的刺激-反应偶联机制，使细菌感受环境和内部条件的变化，通过调节各种细胞功能作出相应反应。这些功能包括分子、细胞和群体 3 个水平上的现象：基因表达、第二信使转换、泳动、群体感应、生物被膜产生等[103]。经典的 TCS 由感应组氨酸激酶 (sensor histidine kinase, SHK) 及其同源反应调节子 (response regulator, RR) 组成。RR 具有可以被磷酸化的受体 (receiver, REC) 结构域，往往也有可以输出信号的效应 (effector) 结构域，二者均能发挥不同的调节作用。SHK 可分为常位于细胞膜外的传感 (sensor) 结构域及位于胞内的组氨酸激酶 (histidine kinase, HK)；从功能上，HK 包含 3 个结构域，分别负责转导胞内信号、结合三磷酸腺苷 (adenosine triphosphate, ATP)和转移磷酸基团。传感结构域与 HK 通过跨膜螺旋结构域 (transmembrane helix, TMH) 连接。SHK 在激酶状态识别并传递信号后，通过 ATP 分解反应在其保守的组氨酸残基处发生自磷酸化，并将磷酸基团转移到 RR 保守的天冬氨酸残基处，产生相应效应，同时自身转变为磷酸酶状态，催化 RR 去磷酸化[104-105](图 1)。双组分系统在细菌和植物中常见且对细胞生理功能意义重大，如 LiaRS/BsrRS 共同调控可提高益生型屎肠球菌 (*Enterococcus faecium*) 对胆盐的耐受性[106]。双组分系统中的蛋白组成及形式并不唯一，如芽孢杆菌 (*Bacillus*) 在多年前就被报道有 29 种 TCS[107]。

表 2 不同类型益生菌定植或生物被膜形成的调控机制

Table 2 Colonization by different types of probiotics and the regulatory mechanisms of biofilm formation

Probiotics types	Typical strains	Mechanisms and illustration	References
Non-spore lactobacillus	*Lactobacillus acidophilus*	Quorum sensing (AI-2 signaling) helps respond to low pH; c-di-GMP regulates the functions of EPS formation and co-aggregation	[80-81]
Non-spore lactobacillus	*Lactobacillus casei*	Two-component signal transduction system and quorum sensing system act in combination to increase low pH resistance	[82-83]
Non-spore lactobacillus	*Lactobacillus rhamnosus*	Adhesive heterotrimeric sortase-dependent pili (encoded by the *spaCBA-srtC1* gene cluster) contributes to adherence, biofilm formation and host signaling; quorum sensing (AI-2 signaling) contributes to biofilm formation and persistence capacity	[84-86]
Non-spore lactobacillus	*Lactobacillus plantarum*	Quorum sensing signal AI-2 molecules upregulated the *cysE* gene, which helps to promote biofilm formation; quorum sensing and two-component regulatory systems (AIP signaling) are related to biofilm formation	[87-90]
Lactococcus lactis	*Lactic streptococci*	Cyclic di-3′,5′-adenosine monophosphate (c-di-AMP) mediates the metabolic regulation of pyruvate carboxylase (PC)	[91]
Lactococcus lactis	*Lactococcus lactis*	Fructose metabolism can affect many other cellular processes, such as biofilm formation	[92]
Lactococcus lactis	*Streptococcus thermophilus*	Two-component systems help cells cope with changing environments such as the digestive tract; regulator gene of glucosyltransferase 3 (Rgg3) is the primary quorum-sensing regulated transcription (short hydrophobic peptides, SHP3) factors, controlling biofilm formation	[93-94]
Irregular lactobacilli	*Bifidobacterium*	ABC transporters, quorum sensing, two-component system, oxidative phosphorylation, amino acid metabolism are all related to biofilm formation	[95]
Non lactic acid bacteria	*Clostridium butyricum*	The *dlt* operon encodes the enzymes responsible for the D-alanylation of cell wall components and influences the surface properties of the cell wall, which helps colonize the digestive tract.	[96]
Non lactic acid bacteria	*Escherichia coli* Nissle 1917	The colonization and biofilm formation are related to CsgD, diguanylate cyclase YedQ, F1C fimbriae	[97]
Non lactic acid bacteria	*Sinorhizobium meliloti*	Overproducing diguanylate cyclases (DGCs) or phosphodiesterases (PDEs) affected the process of biofilm formation and EPS biosynthesis.	[98]
Non lactic acid bacteria	*Bacillus amyloliquefaciens*; *Bacillus thuringiensis*; *Bacillus subtilis*; *Bacillus licheniformis*	The formation of *Bacillus* biofilms is generally regulated by c-di-GMP	[99-101]
Non lactic acid bacteria	*Bacillus subtilis*	The formation of *Bacillus subtilis* biofilm apparently depends on quorum sensing (AI-2 signaling)	[102]

双组分系统对胞外信号因子的接收是细菌内部多种生理调节的开始，以枯草芽孢杆菌形成生物被膜的过程为例。枯草芽孢杆菌是一种农业、工业、医药卫生领域应用广泛的革兰氏阳性菌，其生物被膜组成成分有 EPS、蛋白纤维 (如 TasA)、eDNA、多聚谷氨酸、表面疏水蛋白 BslA，其对应操纵子有 *epsA-O* (调节 EPS)、*tapA-sipW-tasA* (调节 TasA) 等。SinR (抑制 EPS、TasA 产生)、AbrB (抑制 TasA 产生) 是这些操纵子的抑制因子，其抑制作用可被反抑制子 SinI 解除。组氨酸激酶 KinA-D 经由 Spo0F、Spo0B 介导 Spo0A (枯草芽孢杆菌形成生物被膜的关键调控因子) 磷酸化，开启生物被膜形成的调控。磷酸化的 Spo0A 激活 SinI，从而解除了 SinR 的抑制作用，该抑制作用的解除又激活 SlrR 合成，这是一种调控 EPS、TasA 合成的关键蛋白；同时，Spo0A 促进 AbrB 的反抑制子 *abbA* 的表达；它还可以直接抑制 AbrB 表达，

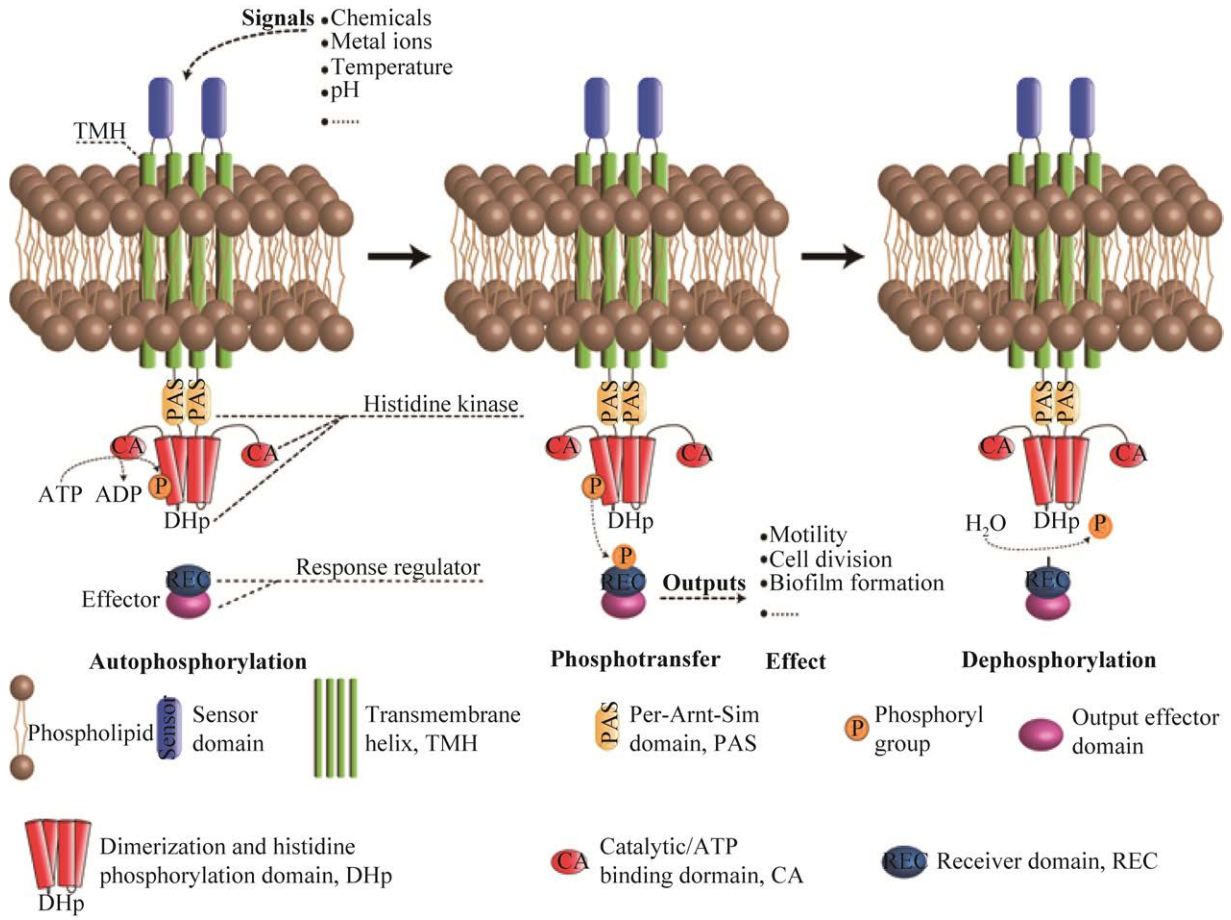

图 1　经典双组分系统识别信号的过程[104-105]

Figure 1　Process of signal recognition by classical two-component system[104-105]. Shown are the domain structures and the signaling mechanisms of a prototypical two-component system (TCS). A TCS comprises a sensor histidine kinase (SHK) and its cognate cytoplasmic response regulator (RR). RR comprises a receiver (REC) domain and usually an output effector domain. SHK comprises an extracytoplasmic sensor domain flanked by transmembrane helices (TMHs), an intracellular signal-relay module, frequently a HAMP domain (a domain found in histidine kinases, adenyl cyclases, methyl-accepting proteins and phosphatases) or a Per-Arnt-Sim (PAS) domain, and a conserved module called kinase domain, composed of a dimerization and histidine phosphotransfer (DHp) domain and a catalytic-ATP binding (CA) domain. SHK can switch between kinase and phosphatase modes in a signal-dependent manner. In the kinase mode, input signals are perceived by sensor initially, then transduced and amplified by TMHs and PAS or HAMP, leading to the autophosphorylation of a conserved His residue of DHp. Later, the phosphoryl group is transfered from the His residue of the SHK to the conserved Asp residue of the REC domain of the RR, with effects output. RR would finally be dephosphorylated via hydrolysis reaction catalysised by SHK in the state of phosphatase. This diagram was not drawn to true shape or scale.

进一步促进生物被膜形成[45,65,108]，见图 2。Spo0A 的磷酸化过程可由 RicA、RicF、RicT (也称 YmcA、YlbF、YaaT 和 Y-复合体) 加速，且最新研究表明，Y-复合体可能通过一条 Spo0A 非依赖性途径参与生物被膜形成[109-110]。枯草芽孢杆菌还存在可以感知并调节 EPS 的通路，它以双

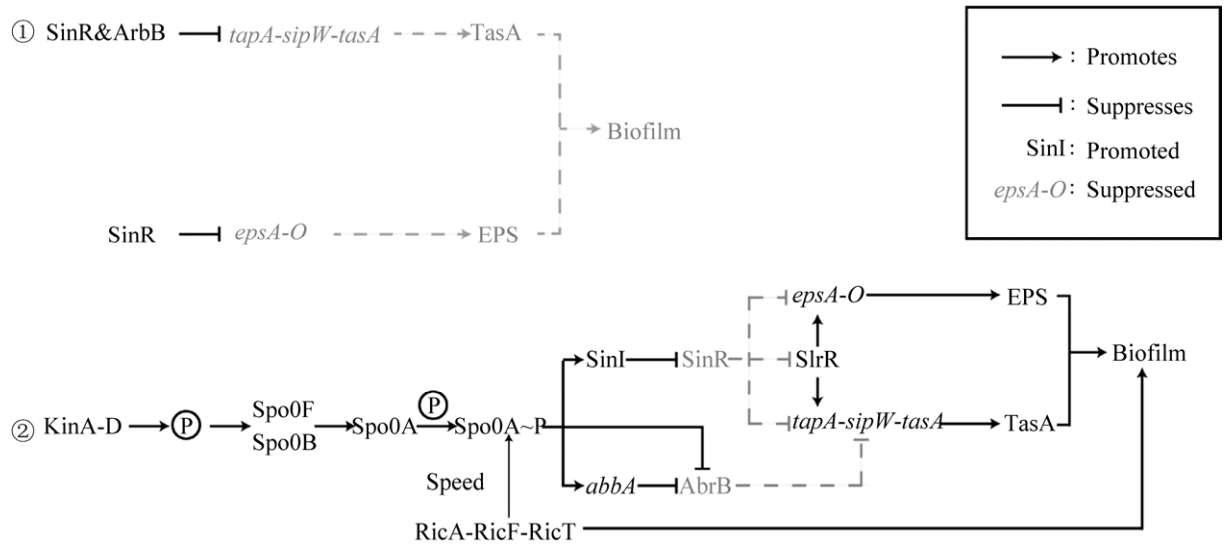

图 2　调控枯草芽孢杆菌生物被膜形成的通路[45,65,108-110]

Figure 2　Pathway regulating the formation of *Bacillus subtilis* biofilm[45,65,108-110]. ① In the general state, the formation of *Bacillus subtilis* biofilm is suppressed. Operon *tapA-sipW-tasA* regulates TasA synthesis and is repressed by negative regulators SinR and ArbB. Operon *epsA-O* regulates EPS (exopolysaccharide) synthesis and is repressed by negative regulator SinR. Both TasA and EPS are components of *Bacillus subtilis* biofilm. ② The formation of *Bacillus subtilis* biofilm is initiated by histidine kinase. A two-component system (KinA-D as the histidine kinase) initiates the phosphorylation of Spo0A, a key regulator of biofilm formation in *Bacillus subtilis* (phosphoryl group was denoted P). Phosphorylated Spo0A activates SinI and *abb*A, which suppress SinR and AbrB, respectively, and then the synthesis of EPS and TasA starts. Even the phosphorylated Spo0A can directly suppress AbrB. Also, the inhibition of SinR activates SlrR, a key protein regulating EPS and TasA synthesis. Phosphorylation of Spo0A can be accelerated by RicA-RicF-RicT, and they may contribute to biofilm formation in another way. Grey letters and dashed lines refer pathways suppressed.

组分系统 EpsAB 为起始，参与生物被膜调节[111]。

组氨酸激酶的特定感受域揭示了双组分系统如何识别环境信号。例如，当环境中氧分压下降时，解淀粉芽孢杆菌 (*Bacillus amyloliquefaciens*) SQR9 中细胞氧含量不足，NAD^+ (nicotinamide adenine dinucleotide) 与 NADH (reduced form of nicotinamide-adenine dinucleotide) 的比值下降，启动子 PresA 转录活性升高，使组氨酸激酶 ResDE 表达。ResE 可以与 NAD^+结合并被激活，细胞得以识别 NAD^+/NADH 比值下降，即氧含量不足的信号，开启后续生物被膜形成的调控。SQR9 中一些细胞色素复合物的合成也受 ResDE 调节，该色素还可与 KinB 蛋白相互作用，通过另一途径 KinB-Spo0A 干预细胞呼吸以应对氧气不足的情况[108]。

3.2　胞间信号调节的途径：群体感应

群体感应是细胞群体通过一些化学信号进行交流的过程，用于监测自身群体密度从而协调群体行为[112]。换言之，双组分系统强调细菌个体对信号的识别，群体感应系统则解释细菌个体、不同菌种之间的信号如何在整体环境中传递并发挥作用。一个 QS 系统由信号分子、传感分子和调控蛋白组成。细菌生长时会产生某种信号因子，随着群体内细菌繁殖数量增多，

信号因子总量也会增加；信号因子浓度达到阈值时，会介导细菌发生一系列生理变化，发挥单个细胞不能独立进行的生理功能，如生物被膜的形成，其目的是调节种内、种间各菌达到平衡，有利于其对抗恶劣的环境[113-114]。这种胞间信号因子最早在一种可发光的费氏弧菌(*Vibrio fischeri*) 被发现，一开始被描述为自诱导分子 (autoinducer, AI)，后经鉴定明确其结构与名称，即一类广泛存在于其他细菌并被研究的 N-酰基高丝氨酸内酯 (N-acyl homoserine lactone, AHL)[115]，随后发现的代表性信号因子还有扩散信号因子 (diffusible signaling factor, DSF)、自诱导肽 (autoinducing peptide, AIP) 等。不同细菌产生不同信号因子，其对应的调控路径也有差别，但一些结构高度保守的信号因子可以被广泛共用于种间群体感应，比如与生物被膜形成密切相关的自诱导信号分子 AI-2 (autoinducer-2)[116]。

近年来 AI-2 在益生菌形成生物被膜中的作用受到广泛关注。例如，AI-2 参与解淀粉芽孢杆菌 SQR9 生物被膜的形成，提高其在植物根部的定植能力。刘蕾等[117-119]详细研究了种间 QS 调控路径 LuxS/AI-2 对类植乳杆菌 (*Lactobacillus paraplantarum*) L-ZS9 促生物被膜形成的机制，证明信号分子 AI-2 受到其合成酶基因 *luxS* 的调控。过量表达 *luxS* 增加了 AI-2 的合成与分泌，促进 L-ZS9 生物被膜的形成，该效果受 SugE 蛋白以及核酸内切酶的抑制；*luxS* 过表达还会影响 AraC、PadR 等与生物被膜形成有关的转录调节子表达，进而影响与转运和膜功能相关的蛋白合成。研究表明，*luxS* 缺陷型鼠李糖乳杆菌的生物被膜形成量低于野生菌株[85]，从反面证明 LuxS/AI-2 的重要性；该途径同样存在于其他益生菌如柠檬明串珠菌 (*Leuconostoc citreum*)、嗜酸乳杆菌等[120-121]。LuxS 还被报道调节乳酸杆菌 AI-2 合成增加以抵抗环境中酸的胁迫，这对益生菌在胃肠道中的存活有较大意义[80]。

AI-2 是一种体内代谢产物。S-腺苷-L-甲硫氨酸 (S-adenosyl-L-methionine, SAM) 是细胞中 DNA、蛋白质等合成中主要的甲基供体，经转化成为有毒性的 S-腺苷-L-高半胱氨酸 (S-adenosyl-L-homocysteine, SAH)，SAH 可被酶催化生成腺苷和 S-核糖基-高半胱氨酸 (S-ribosyl-homocysteine, SRH) 以解毒；LuxS 催化 SRH 裂解产生 4,5-二羟基-2,3-戊二酮 (4,5-dihydroxy-2,3-pentanedione, DPD) 与高半胱氨酸；DPD 作为 AI-2 的前体，可以自发环化成 *R* 型或 *S* 型的 2,4-二羟基-2-甲基氢-3-呋喃酮 (2,4-dihydroxy-2-methylhydro-3-furanone, DHMF)。不同细菌有着能引起细胞群体感应的不同 AI-2 信号，例如在最先发现 AI-2 的哈维氏弧菌 (*Vibrio harveyi*) 中，其 AI-2 分子为 *S* 型 DHMF 硼酸盐[122-124]。AI-2 产生的途径在细菌中广泛保守，其被检测和信号转导机制却是特异性的，目前已鉴定出的 3 种类型 AI-2 受体 (LuxPQ、LsrB 、RbsB[125]) 均来自于病原菌。尽管 LuxS/AI-2 通路在益生菌生物被膜形成中的作用已被证实，但有关益生菌中 AI-2 的检测与转导机制却没有进一步报道，值得深入研究。

3.3 不同调节机制间的关系

生物被膜形成是一个复杂的调控行为，涉及到细胞多组分、多系统的参与，这意味着不同机制理论之间并非完全割裂，从细菌生命活动这一整体角度去认识调控过程是有必要的。

在益生菌识别、转导信号到合成代谢生物被膜的过程中，涉及的机制、环节、产物相互关联，相互影响。EPS 作为生物被膜的组成成分，可能是信号分子存在的介质，其浓度与细胞密度成正比，因此与 QS 有着密切的联系[3]；

且有报道称含 EPS 的生物被膜层有利于机体组织抵抗病原体及毒素的干扰[126]，故 EPS 与群体感应、生物被膜益生作用的关系值得深入研究。Liu 等[95]结合转录和代谢组学分析确定了参与双歧杆菌 (*Bifidobacterium*) 生物被膜形成的关键基因及产物，明确群体感应、双组分系统、氨基酸代谢在双歧杆菌生物被膜形成过程中的必要性，体现了生物被膜调控过程的复杂性。再如，在枯草芽孢杆菌的生物被膜形成过程中，双组分系统介导的磷酸化 Spo0A 与 SinR 对种间群体信号分子 AI-2 有负调控作用[124]，这种通过双组分系统识别群体感应信号的现象较为常见。

病原菌生物被膜形成机制的研究成果对益生菌生物被膜的研究也有启示。例如，笔者课题组前期对生物被膜模式菌铜绿假单胞菌 (*Pseudomonas aeruginosa*, PA) 进行了研究，发现鼠源抗菌肽 CRAMP 和鸡源抗菌肽 Cath2 具有明显的抗铜绿假单胞菌 PAO1 株生物被膜的作用[127-129]，后续采用 RNA-Seq (ribonucleic acid sequencing) 技术考察了 CRAMP 对该菌成熟生物被膜的影响，通过分析发现 CRAMP 可能作用于其环二鸟苷酸 (cyclic diguanylic, c-di-GMP) 系统，降低了 c-di-GMP 水平，增强了细菌运动性，导致生物被膜分散，并且与其 QS 系统和藻酸盐合成相关[130]。c-di-GMP 同样参与枯草芽孢杆菌多种生理功能的调节，但其对于生物被膜调节作用存在争议[99,131]。另一种第二信使环二腺苷酸 (cyclic diadenylate, c-di-AMP) 被报道调节枯草芽孢杆菌的 Spo0A，控制细菌生长、孢子萌发，然而其对该菌生物被膜的效果同样无统一定论[132-133]。第二信使在病原菌生物被膜调控中的普遍存在，揭示其对益生菌的潜在影响，有一定研究价值。

4 益生菌生物被膜的安全性

从应用角度，益生菌的筛选一般涉及评价益生作用、黏附力、抗不利条件的能力，当确认它的功能和特性后，便需要对其进行安全性评估，常见的指标有耐药性、代谢物、溶血活性、对宿主的毒副作用等。目前针对益生菌安全性的研究还不够，益生菌适合什么年龄、什么身体状况的人使用，如何选择合适的菌株，这些问题有待解决[15]。而益生菌生物被膜作为一个新的分支，其安全性问题同样不可忽视。除要考虑益生菌的常见副作用，还应关注生物被膜可能带来的相关问题。如前文提及的，当寻找促进益生菌生物被膜形成的物质时，应一并考虑它对致病菌生物被膜是否有同样效果。再如，当益生菌摄入体内，以生物被膜形式定植肠道，是否会对原本的菌群产生影响。现有的研究大都认为，益生菌对肠道微生物群落影响不大，或总体上能够维持动态稳定[134-135]，而益生菌生物被膜是否对肠道菌群有影响，需要进一步研究考察。有报道指出，新生儿坏死性小肠结肠炎 (necrotizing enterocolitis, NEC) 会使肠道屏障受损，使用益生菌可能会有菌血症的风险，Al-Hadidi 等[136]的研究中，与对照组、浮游态益生菌相比，生物被膜状态的罗伊氏乳杆菌显著降低疾病模型动物的肠道通透性，肠道屏障功能得到改善，从另一层面表明生物被膜状态的益生菌可能更具安全性。

5 展望

益生菌帮助宿主预防和控制疾病的作用被广泛认可，尤其是对胃肠道疾病的改善，使得益生菌产品越来越受到重视。这对口服益生菌制剂在胃肠道的抗逆能力、定植能力提出了更高要求。作为临床慢性感染与耐药性产生的常

见原因，细菌生物被膜是医学研究的重点对象，如今它与益生菌相结合的研究也正显示出良好前景。相比于浮游态，生物被膜形式的益生菌在胃肠道对不利条件如低 pH 值、机械力甚至抗生素的抗性有所提高，定植率增加，并联合机体自身肠道黏膜免疫系统，对防治某些病原、抑制疾病也显示出巨大的优势与潜力。除筛选益生菌生物被膜优势菌株与促被膜形成的活性物质外，近年来，有关益生菌生物被膜相关制剂改良的研究也逐渐成为热点，包括各种多糖类包埋材料的选择、双层包被工艺、预制生物被膜、生物被膜自涂技术等，在提高菌种抗逆性、抗医用生物材料感染等方面均有不错的效果。

相比于致病菌生物被膜，目前对益生菌在该领域的研究相对较少，且多数停留在促进其生物被膜形成的活性物筛选阶段，缺乏广度与深度。机制研究是生物被膜研究的理论基础，对其认识至今还未透彻，且现有的研究结果复杂繁多，因此需要从全局角度出发把握整体，再细分领域深入探索，或许有助于理解这一细菌生理调节过程。群体感应系统与生物被膜形成密切相关，其分子机制研究在益生菌领域还需完善。体外实验可设置固定的理化参数，但难以完全模拟体内机体一些特征，如胃肠黏膜的存在、流动的营养环境等。另外，不同的实验设计可能导致完全相反的研究结论，如何验证益生菌是否在体内成功以生物被膜形式定植并评价其效果是研究时需要重点关注的问题。有关体内微生物环境、益生菌及其生物被膜三者关系的研究值得引起重视，这对于肠道乃至机体健康具有重大意义。另外，益生菌生物被膜的安全性问题也需要系统评价。

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