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专论与综述

细菌胞外多糖生物合成转录调控因子研究进展

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摘　要：细菌胞外多糖(Exopolysaccharide，EPS)因其独特的理化特性和生理活性，在食品、制药和化工等领域广泛应用。在食品行业中，黄原胶、结冷胶和热凝胶等细菌 EPS 备受青睐。转录调控因子能在转录水平上调控 *eps* 基因的表达，影响细菌 EPS 的生物合成。目前细菌 EPS 转录调控因子的研究报道较少，且多数已知的 EPS 转录因子调控机制尚未阐明。本文总结了近年来细菌 EPS 调控因子的研究进展，重点介绍其研究方法和调控机制，以期为细菌 EPS 转录调控研究提供借鉴。

关键词：细菌胞外多糖，生物合成，转录调控因子，调控机制

Advances in transcription regulators of bacterial exopolysaccharides biosynthesis

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Abstract: Bacterial exopolysaccharides (EPS) are widely used in food, pharmaceutical, and chemical fields due to their unique physicochemical properties and physiological activities. Bacterial EPS such as xanthan gum, gellan gum, and thermal gel are widely applied and favored in food industry. Transcriptional regulators can regulate the expression of *eps* genes at transcription level to affect bacterial EPS biosynthesis. Moreover, the regulatory mechanism of most reported EPS transcriptional regulators has not been elucidated. This review summarizes the progress of bacterial EPS transcriptional regulators in recent years and focuses on the research methods and regulatory mechanisms, in order to provide reference for transcription regulation research of bacterial EPS.

Keywords: bacterial exopolysaccharides, biosynthesis, transcription regulators, regulation mechanism

细菌胞外多糖(Exopolysaccharide，EPS)是细菌自身合成并分泌到细胞外的糖类化合物，包括粘液多糖和荚膜多糖。根瘤菌、乳酸菌和假单胞菌等细菌是常见的 EPS 来源菌。作为一种备受青睐的天然食品添加剂[1]，细菌 EPS 可作为增稠剂和稳定剂用于改变产品的质构[2-3]。细菌 EPS 也是安

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全的“粘性”发酵剂[4]，能在发酵过程中显著改善乳制品的风味和品质[5]。其中，黄原胶、结冷胶和热凝胶等细菌 EPS 由于产量高、成本低等优点在食品工业中广泛应用。

根据单糖组成，细菌 EPS 可分为同型多糖和异型多糖。同型多糖(如热凝胶、葡聚糖和果聚糖)在胞外直接合成，无须脂载体参与，主要通过对应的糖基转移酶完成 EPS 的合成；而异型多糖(如黄原胶和结冷胶)都是脂载体依赖型胞内合成，需要糖核苷酸前体、酶催化系统、酰基供体、脂载体和糖基受体，其合成包括糖核苷酸前体的胞内合成、重复单元的合成和多糖的延伸、聚合及输出[6]。细菌 EPS 生物合成由 *eps* 基因簇控制，包括调控基因、链长决定基因、重复单元合成基因、聚合和输出基因，而且不同细菌中 *eps* 基因簇的数量和种类有所差异[7-8]。Song 等[9]发现干酪乳杆菌(*Lactobacillus casei*) *eps* 基因簇中 3 个关键基因对 EPS 生物合成至关重要。Xiong 等[10-11]完成了嗜热链球菌(*Streptococcus thermophilus*) S-3 的 EPS 及其前体生物合成基因簇和表型分析。Kong 等[12]利用强组成型启动子同时表达了嗜热链球菌 *eps* 基因簇中 2 个关键基因，显著提高了 EPS 的合成。细菌 *eps* 基因簇的深入研究为探索 EPS 生物合成的转录调控奠定了基础。

转录调控因子是一类具有转录调节活性的蛋白质，参与调控细菌内糖、脂和碳代谢等重要生理活动[13]。调控因子与 *eps* 基因簇中靶基因位点结合，启动 *eps* 基因的转录及控制其转录效率，从而调控细菌 EPS 的生物合成[14]。但目前仅少数细菌 EPS 调控因子被鉴定，且调控机制尚未阐明，尚有大量未知的 EPS 调控因子亟待研究。本文主要从细菌 EPS 调控因子的研究方法和调控机制展开综述，为深入研究细菌 EPS 转录调控提供参考。

1 细菌 EPS 生物合成转录调控因子的信号传导调控机制

转录因子调控 EPS 生物合成的过程中，调控蛋白对靶基因的识别和结合是调控机制的核心。调控蛋白含有特殊构象的结构域，能特异性识别并结合靶基因中对应的结合位点(保守短序列片段，Motif)[14]，调控靶基因表达。许多调控蛋白家族有相似的结合结构域，与 EPS 生物合成密切相关(表 1)。通过信号分子传导，调控因子影响 RNA 聚合酶识别特定短序列片段，对下游编码基因的转录表达进行正/负调控。EPS 转录因子调控机制中，存在 3 类经典的信号传导机制：群体感应系统、双组分系统和第二信使分子传导系统。

1.1 群体感应系统

群体感应(Quorum Sensing)是细菌因自身种群密度变化，利用信号分子调控基因表达的机制，参与调控 EPS 生物合成、抗生素合成和生物膜发育等代谢过程。群体感应系统中，细菌合成的信

表 1 细菌中常见的调控因子

Table 1 Common regulatory factors in bacteria

Family	Action	Binding domain conformation	Regulation function	References
LuxR	+	HTH	Quorum sensing, biofilm formation, and metabolism	[15-17]
TetR	−	HTH	Antibiotic synthesis, biofilm formation, and osmotic stress	[18-19]
GntR	−	HTH	General metabolism, CPS synthesis, and virulence	[20-21]
LacI	−	HTH	Carbon source utilization, virulence, cell activity, and carbohydrate metabolism	[22-24]
DeoR	−	HTH	Antibiotic synthesis and carbohydrate metabolism	[25-27]
ArsR	−	HTH	Metal and acid resistance	[28-30]
LysR	+/−	HTH	Carbon and nitrogen metabolism, biofilm formation, and flagellar movement	[31-33]
OmpR	+	Winged helix	Heavy metal, CPS synthesis, and virulence	[34-35]

号分子及其感应机制可分为3类：G^-细菌的酰基高丝氨酸内酯类信号分子(N-Acyl-Homoserine Lactones，AHL)系统、G^+细菌的寡肽类信号分子(Autoinducing Peptides，AIP)系统和细菌通用信号分子AI-2系统。Licciardello等[17]在波纹假单胞菌(*Pseudomonas corrugata*)中发现，信号系统PcoI/AHL和LuxR家族调控因子PcoR组成群体感应系统，调节海藻酸钠生物合成。在苜蓿根瘤菌(*Rhizobium meliloti*)中，信号系统SinI/AHL与调控因子ExpR组成的群体感应系统，调控细菌内多种EPS的生物合成[36]。此外，一些细菌中同时存在多种不同的信号分子调节机制。例如，霍乱弧菌(*Vibrio cholerae*)中存在3种参与调控因子LuxO调控细菌EPS合成和生物膜形成的群体感应系统：CqsA/CAI-1 (AHL类信号分子)、LuxS/AI-2和一种未知的信号系统[37]。

1.2 双组分调控系统

双组分系统是细菌适应体内外环境变化的重要信号传导系统，一般由组氨酸激酶与应答调控蛋白组成，通过双组分蛋白磷酸化传递信号，调控胞内基因表达(图1)[38-39]。Black等[40]在黄色粘球菌(*Myxococcus xanthus*)中发现途径特异性EPS调控因子EpsW和组氨酸激酶DifE，EpsW被DifE激活后调控EPS合成。转录调控因子RcsB与激酶RcsC组成双组分系统，协同LuxR家族调控因子RcsA共同激活大肠杆菌(*Escherichia coli*)荚膜多糖基因簇的表达[41]。淀粉液化芽孢杆菌(*Bacillus amyloliquefaciens*) SQR9中存在双组分调控系统ResDE，ResD被激酶ResE激活后促进生物膜形成，潜在调控EPS[42]。VicRK (调控因子VicR和激酶VicK)、ComDE (调控因子ComE和激酶ComD)和CiaRH (调控因子CiaR和激酶CiaH)等双组分系统在乳酸菌中调控多糖合成等多种代谢过程[43]。此外，细菌中也存在非典型的EPS双组分调控系统，如Minic等[44]发现嗜热链球菌*eps*基因簇上的酪氨酸激酶EpsD可激活调控蛋白EpsE，调控EPS合成。本课题组过表达嗜热链球菌*eps*基因簇上关键基因*epsA*和*epsE*，能显著提高EPS的合成，其中*epsA*编码途径特异性调控因子EpsA[12]。此外，我们利用构建的CRISPR-Cas9基因编辑系统解析了干酪乳杆菌LC2W中EPS合成关键基因[45]，也发现基因簇上存在途径特异性调控因子LC2W_2170[9]。

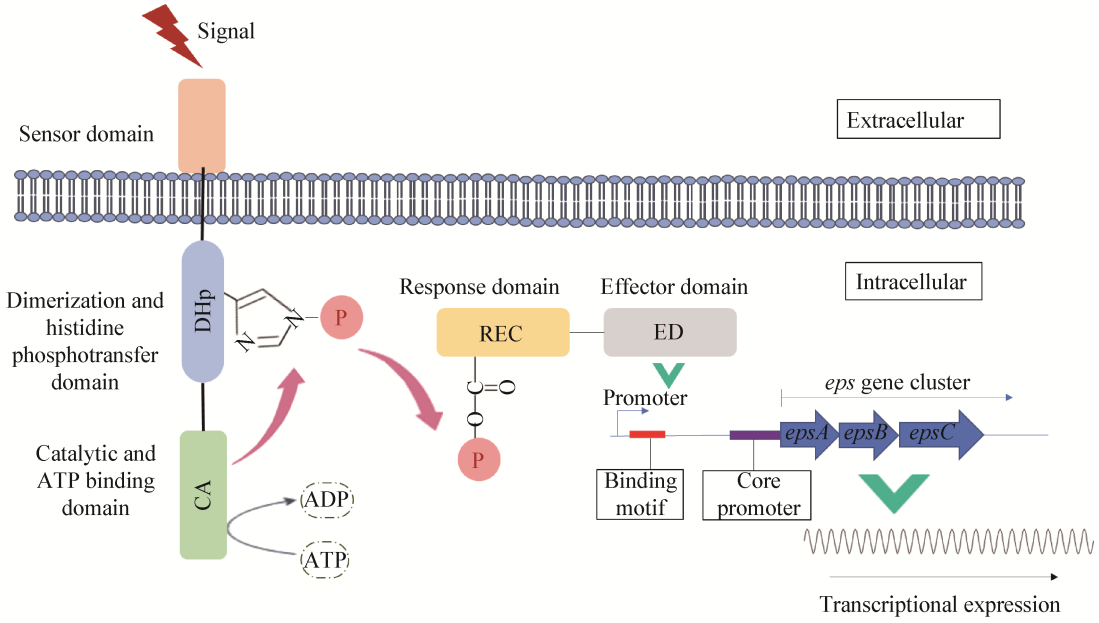

图1 经典双组分调控体系

Figure 1 Classical two-component regulation system

1.3 第二信号分子传导系统

第二信使分子与细胞表面的受体结合后，通过受体信号的转导形成信号通路，调控胞外蛋白和 EPS 分泌。其中，环二鸟苷酸(c-di-GMP)和环磷酸二腺苷(c-di-AMP)在细菌 EPS 调控中最为常见。Schäper 等[46]在草木樨中华根瘤菌(*Sinorhizobium meliloti*)中发现，AraC 家族调控因子 CuxR 的二聚体在 c-di-GMP 的作用下调控细菌 EPS。恶臭假单胞菌(*P. putida*) KT2440 中，c-di-GMP 抑制调控因子 FleQ 与 EPS 合成基因 *bcs* 启动子的结合[47]。铜绿假单胞菌(*P. aeruginosa*)中，c-di-GMP 抑制 FleQ 负调控 EPS 基因簇上 *pel* 在内的多个基因[48]。Fazli 等[49]发现 c-di-GMP 在新生伯克霍尔德菌(*Burkholderia cepacia*)中通过转录调控因子级联调节 EPS 合成：c-di-GMP 首先激活 BerB-RpoN 体系控制 *berA* 编码调控因子 BerA，BerA 再与 c-di-GMP 结合，促进 EPS 合成基因 *bep* 转录表达。与已鉴定出数百种结合蛋白的 c-di-GMP 不同，迄今为止在细菌中只发现了少数与 c-di-AMP 作用的 EPS 调控因子。在变形链球菌中，c-di-AMP 与受体蛋白 CabPA 相互作用，影响转录因子 VicR 调控 EPS 合成中关键基因 *gtfB* (编码葡萄糖基转移酶)的表达[50]。同样在变形链球菌(*S. mutans*)中，Cheng 等[51]和 Rismondo 等[52]发现调控因子 CdaR 调控单二磷酸环化酶 CdaA 的合成，并通过 c-di-AMP 信号网络调控氧化反应和 EPS 生物合成。金黄色葡萄球菌 KdpDE 双组分系统中的组氨酸激酶 KdpD 也是 c-di-AMP 受体蛋白，参与调控荚膜多糖生物合成与生物膜形成[53]。

2 细菌 EPS 生物合成转录调控因子的研究方法

在基因调控过程中，转录调控因子作用的本质是蛋白质-DNA 相互作用。研究调控因子常见的方法及其优缺点见表 2。目前染色质免疫沉淀、DNA 亲和层析技术和凝胶阻滞实验在细菌 EPS 调控因子的研究中应用最为广泛。

2.1 染色质免疫沉淀

染色质免疫沉淀技术 (Chromatin Immunoprecipitation Assay，ChIP)在体内研究调控因子-靶 DNA 相互作用，与微阵列芯片(ChIP-on-chip)或高通量测序(ChIP-seq)结合，能准确分析细菌内调控因子的靶基因位点，构建基因表达调控网络。Partridge 等[54]利用 ChIP-on-chip 分析得到大肠杆菌调控因子 NsrR 的结合位点(AANATGCATTT)，该位点存在于细胞膜发育基因 *mqsR-ygiT* 的启动子区域，潜在调控 EPS 合成。ChIP-seq 识别伤寒沙门氏菌(*Salmonella typhi*)与渗透压响应调控因子 OmpR 相互作用的靶点，其中包括 *gltA* (柠檬酸合酶基因)、*sdhC* (琥珀酸脱氢酶基因)和 *tviA* (Vi 多糖生物合成蛋白基因)等多个与 EPS 生物合成相关基因[55]。在假单胞菌中，ChIP-seq 证明全局调节因子 AlgR 不仅直接调控海藻酸钠合成和毒力因子表达，还能与 c-di-GMP 相互作用间接调控 EPS 生物合成[56]。

2.2 DNA 亲和层析技术

DNA 亲和层析能分离与特定 DNA 序列作用的蛋白质，利用细菌 *eps* 基因启动子片段可寻找未知的 EPS 调控因子[57]。Wu 等[58]在肺炎链球菌(*S. pneumoniae*) D39 中用 5′生物素化的荚膜多糖基因簇启动子 *cpsp* 亲和层析，筛选得到 6 个候选调控因子，其中 CpsR 通过结合 *cps* 基因簇阻遏荚膜多糖的生物合成。在金黄色葡萄球菌(*Staphylococcus aureus*)中，生物素化的 *psm* (酚溶性模块蛋白基因，参与细胞膜形成)操纵子亲和层析筛选出调控蛋白 MgrA，MgrA 通过抑制 *psm* 表达阻遏生物膜形成，潜在调控 EPS 合成[59]。在铜绿假单胞菌中，细胞分裂基因 *ftsZ* 启动子区域亲和层析得到调控因子 LexA，并通过 DNase I 足迹法分析出其 DNA 结合位点(LexA Box)，该位点存在于多个 EPS 合成基因中[60]。

表 2　细菌 EPS 调控因子研究方法的优缺点

Table 2　Advantages and disadvantages of research methods for bacterial EPS regulators

研究方法 Methods	优点 Advantages	缺点 Disadvantages
凝胶阻滞实验 EMSA	体外快速研究 DNA 与蛋白质相互作用，特异性强 Rapid detection *in vitro*, strong specificity	不能真实反映体内情况，不能确定靶序列，结论单一 Cannot reflect the situation *in vivo* and identify the target motif
DNA 微阵列技术 DNA microarray	有效确定下游靶基因 Effective determination of downstream target genes	不能直观体现作用机制，昂贵且分析要求高 Cannot directly reflect the mechanism of action, expensive and high analysis requirements
酵母单杂交技术 Yeast one hybrid	确定 DNA-蛋白质相互作用，提供蛋白前体的折叠和修饰 Identification of DNA-protein interaction with folding and modification of protein precursors	非酵母体系中准确率低 Low accuracy in the non-yeast system
染色质免疫沉淀技术 ChIP	显示 DNA-蛋白质在体内动态作用情况，确定靶基因结合位点 Determination of dynamic action between DNA and protein *in vivo* and identification of the binding sites of target genes	难以获得特异性蛋白质抗体；调控蛋白的基因限制在特定来源 Difficult to obtain specific protein antibody and limited sources of specific gene for regulator
DNA 亲和层析技术 DNA affinity chromatography	活性物质纯度高，性质稳定，步骤简单有效 High purity of active substances, property stability, and simple operation	配基要求高，不可避免非特异性结合 High requirements for ligand and inevitable non-specific binding
RNA-seq	全基因组水平的基因表达差异研究，定量准确、可重复性高、检测范围广 Gene expression differences at the genome level, quantitative accuracy, high repeatability, and wide-range detection	存在核糖体 RNA 影响，不能直观体现相互作用 Ribosomal RNA interference and cannot directly reflect the interaction
噬菌体展示技术 Phage display	大量快速检测、蛋白结构和活性稳定 Fast and simultaneous test, stability of protein structure and activity	噬菌体文库的容量和遗传多样性有限制 Limited capacity and genetic diversity of phage library
荧光素酶实验 Luciferase assay	靶动子和调控因子作用过程光信号强、信噪比高，同时分析多个信号转导通路 Strong light signal and SNR for promoter-regulator interaction, multiple analysis of signal transduction pathways	仅适用于转录激活检测，无法检测转录抑制 Only suitable for transcriptional activation detection, inapplicable for transcriptional inhibition
生物膜干涉技术 Biolayer interferometry	利用光干涉原理检测小分子间相互作用，灵敏度高，样本容量大 Detection of molecular interaction based on light interference, large sample size	样本纯度要求严格，易受非特异性结合影响 Limited sample purity, and easily affected by non-specific binding
扫描探针显微技术 Scanning probe microscope	原子级分辨率检测调控因子与靶基因作用，准确率高 Detection of DNA-regulator by atomic resolution, high accuracy	检测环境和样本纯度要求高，不适用于大样本筛选 High requirements on test environment and sample purity, and unsuitable for large sample screening
凝胶阻滞实验 EMSA	体外快速研究 DNA 与蛋白质相互作用，特异性强 Rapid detection *in vitro*, strong specificity	不能真实反映体内情况，不能确定靶序列，结论单一 Cannot reflect the situation *in vivo* and identify the target motif

2.3　凝胶阻滞实验

凝胶阻滞实验(Electrophoretic Mobility Shift Assay，EMSA)可以直观显示调控因子和 *eps* 基因的相互作用，灵敏度高且特异性强。Zhou 等[61]通过 EMSA 证明调控因子 OpaR 和 AphA 与副溶血弧菌(*V. parahaemolyticus*)荚膜多糖基因 *cpsQ* 启动子

区域有特异性结合。利用 EMSA 证明全局调控因子 CcpA 能与肺炎链球菌 *cps* 启动子区域特异性结合，参与调控细菌 EPS[62]。转录抑制因子 NigR 经 EMSA 表明可特异性结合变形链球菌中糖转运和代谢相关基因，潜在调控细菌 EPS[63]。

3 问题和展望

目前细菌 EPS 转录调控研究仍有较大的局限性，已报道的调控因子多集中在肺炎链球菌、金黄色葡萄球菌和铜绿假单胞菌等毒力强的致病菌中[33,64-66]，而在乳酸菌、地衣芽孢杆菌(*B. licheniformis*)和枯草芽孢杆菌(*B. subtilis*)等益生菌中鲜有报道[67-69]，而且鉴定的多为荚膜多糖调控因子，粘液多糖调控因子的报道较少。此外，细菌中还存在大量 EPS 潜在或未知的调控因子亟待研究。例如，在乳杆菌中发现的蔗糖代谢调节因子 ScrR[70]和中枢糖酵解基因调节因子 CggR[71]可能与 EPS 生物合成相关，但并未实验证实。我们在干酪乳杆菌和嗜热链球菌 EPS 转录调控研究中，利用其 *eps* 启动子区域 DNA 亲和层析发现多个不同类型的潜在调控因子，包含未知调控因子和尚未证明与 EPS 合成相关的调控因子，因此还需利用 EMSA 和 DNAase I Footprinting 等体内和体外技术方法进一步证实和研究其调控机制。将荧光素酶实验、生物膜干涉技术和扫描探针显微技术等分子生物学方法应用于 EPS 调控因子研究，有望鉴定出更多新颖的细菌 EPS 调控因子，为深入解析 EPS 生物合成调控网络奠定基础。

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