综述

基于群体感应抑制肠道致病菌的研究进展

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摘要 群体感应(quorum sensing, QS)是依赖于自诱导剂的产生、释放和识别的微生物细胞间的通讯过程, 在微生物生长过程中起重要作用。在肠道环境中, 鼠伤寒沙门氏菌、霍乱弧菌、艰难梭菌、大肠杆菌等肠道致病菌感染宿主机体, 影响宿主机体的正常免疫代谢过程, 引发急性肠胃炎、痢疾等疾病。传统治疗方案中, 抗生素可以应对肠道致病菌引起的并发性感染, 但抗生素的滥用会导致细菌出现多重耐药性, 且耐药性会在遗传水平上发生迁移和突变。近年来, 越来越多的研究表明, 可以通过操纵QS系统来调控菌体生物膜形成、毒力因子产生和耐药性抑制等行为。该文首先对几种典型菌体QS系统的工作路径进行了总结; 并综述了几种常见的肠道致病菌的QS治疗策略, 以期为肠道疾病的新型治疗方案和相关的肠道致病菌抑制剂的开发提供一定的参考。

关键词 群体感应; 肠道致病菌; 毒力因子; 生物膜形成

Research Progress of Inhibiting Intestinal Pathogenic Bacteria Based on Quorum Sensing

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Abstract QS (quorum sensing) is a communication process between microbial cells that relies on the production, release and recognition of AIs (autoinducers), and plays an important role in the growth of microbes. In the intestinal environment, enteric pathogenic bacteria such as *Salmonella typhimurium*, *Vibrio cholerae*, *Clostridium difficile*, *Escherichia coli* and other intestinal pathogenic bacteria infect the host body and affect the normal immunometabolic process of the host body, triggering acute gastroenteritis, dysentery and other diseases. Traditional treatment regimens in which antibiotics cope with intercurrent infections caused by enteropathogenic bacteria have developed, but the misuse of antibiotics can lead to multi drug resistance in bacteria, which migrate and mutate at the genetic level. In recent years, more and more studies have shown that the QS system can be manipulated to regulate the formation of bacterial biofilm, the production of virulence factors, and the inhibition of drug resistance. This paper begins with a summary of the working paths of several typical bacterial QS systems; and several QS therapeutic strategies for common enteropathogenic bacteria are reviewed in the hope of providing some references

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for the development of novel treatment options and related enteropathogenic inhibitors for intestinal diseases.

Keywords quorum sensing; enteropathogenic bacteria; virulence factor; biofilm formation

细菌在环境中繁殖的过程除了细菌与环境发生营养物质的物质交换外, 还存在细菌之间的信息传递和交流, 这种信息的交流过程称为群体感应(quorum sensing, QS)[1]。QS是存在于细菌间的通信系统, 通过被称为自诱导剂(autoinducers, AIs)的信号分子与群体感应受体结合, 进而使得细菌可以感应种群密度的信息并相应地在种群范围内调节基因的表达[2]。随着细菌种群密度的增加, AIs在环境中积累至阈值浓度后, 被细菌QS受体蛋白识别并结合, 激活或抑制特定靶基因的表达, 进而调节许多生物过程的发生[3], 如毒力因子分泌[4]、生物膜形成[5]、菌群共生[6]、生物发光[7]、抗生素合成[8]、核酸合成[9]等。

人体肠道菌群由多种多样的微生物组成, 致病菌会分泌毒素来引发疾病, 益生菌会通过益生物质来治疗疾病[10-11]。人体肠道致病菌包括沙门氏菌(*Salmonella*)、李斯特菌(*Listeria*)、志贺氏菌(*Shigella*)、致病性大肠杆菌(*Escherichia coli*)、艰难梭菌(*Clostridium difficile*)等, 会定植于肠道上皮细胞中, 通过QS系统调控毒素分泌和细胞定植, 引起肠道代谢和免疫失衡, 导致人体急性肠胃炎、细菌性腹泻、痢疾等疾病发生。肠道益生菌如双歧杆菌(*Bifidobacterium*)、乳酸菌(*Lactobacillus*)、益生芽孢杆菌(*Probiotic Bacillus*)等细菌会刺激肠道分泌球蛋白(SlgA), 提高宿主免疫力和抗氧化水平, 抑制肠道炎症等[12]。肠道菌群通过QS系统和病原体–宿主通讯机制与宿主进行信息交流, 当细菌菌群间平衡被打破时, 细菌和宿主之间的信息交流受到干扰, 影响人体的正常机体功能, 进而引发人体疾病[13]。

肠道致病菌群在宿主机体感染和危害方面发挥协同促进作用, 多种细菌感染引起的并发症日益成为疾病治疗方面的难题。使用抗生素极大地解决了肠道细菌性感染的问题, 但抗生素的泛滥使用会造成细菌耐药性的问题, 且耐药性会在种内和种间水平发生遗传迁移以及会在环境压力下发生突变[14-15], 以上因素使得抗生素在治疗病原体感染方面受到极大的限制[16-18]。常规抗生素通过抑制细菌的代谢过程和破坏细菌细胞膜的完整性来抑制或杀死细菌, 而微生物可通过多种机制来抵抗抗生素的作用, 如细菌生物膜的形成限制抗生素进入细胞发挥功能。细菌的QS系统调控细菌的毒力因子产生和生物膜形成, 因此抑制细菌的QS系统, 不仅可以有效减弱致病菌的毒性和侵袭能力, 而且会减弱生物膜对抗生素的限制。因此, 基于QS系统在调节细菌毒力和耐药性的重要作用, 开发新的QS抑制剂可为解决细菌的耐药性问题开辟新的思路。

近年来, 随着各种QS调控系统的机制不断被解析, 对群体感应系统参与肠道失衡的调控机制和路径有了愈加深入的研究。为了让更多的研究者对这一领域有更好地了解, 本文从细菌体内不同类型信号分子介导的QS系统介绍开始, 总结群体感应系统在常见的肠道致病菌的最新应用, 以期对肠道疾病的治疗和基于QS肠道致病菌抑制剂的开发提供一些新的参考。

1 细菌的QS系统

细菌细胞内QS系统多种多样, 目前研究的较为清楚且常见的QS系统按信号分子分类主要有以下几类: *N*-高丝氨酸内酯类(*N*-acyl-homoserine lactone, AHL)[19]、呋喃硼酰二酯类(autoinducer-2, AI-2)[3]、诱导寡肽类(autoinducing peptide, AIP)[20]等。

1.1 AHL介导的QS系统

AHL类QS系统也被称为LuxI/R类QS系统, 主要存在于革兰氏阴性菌中, 是目前被研究得最广泛的QS系统。以LuxI/LuxR为代表的QS系统最初是在海洋微生物费氏弧菌(*Vibrio fischeri*)中被发现并阐明的[7,21]。在AHL型QS系统中, AHLs通常由两个部分构成: 亲水的高丝氨酸内酯部分和疏水的酰基侧链部分。AHL基于酰基侧链的键饱和性可以分为不同的类型, 如费氏弧菌(*Vibrio fischeri*)中的3-oxo-C6-HSL[22]、铜绿假单胞菌(*Pseudomonas aeruginosa*)的3-oxo-C12-HSL和C4-HSL[23]、鲍曼不动杆菌(*Acinetobacter baumannii*)中的OHC12-HSL[24]等。在不同细菌中*luxI*基因高度保守[21], 说明合成的AHL分子结构类似, 但由于侧链酰基载体蛋白(acyl-ACP)的不同, 使得细菌可以从一个AHL合酶中合成不同类型的AHL分子。ORTORI等[25]发现, 假结核耶尔森氏菌(*Yersinia pseudotuberculosis*)中AHL合成酶YtbI和YpsI共同可产生至少24种带有C_4-C_{15}的侧链酰基

的AHL。LuxR蛋白转录调节因子包括自诱导结合域(ABD域, N-端)和结合DNA的Helix-Turn-Helix域(HTH域, C-端)。相比于*luxI*, 不同细菌*luxR*基因的相似性只有18%~25%[25]。在革兰氏阴性菌中, *luxR*基因通常与*luxI*基因相邻。对原核生物基因组进行的生物信息学研究调查发现, 基因组中有部分*luxR*基因是独奏型, 即可响应不相邻的LuxI产生的内源AHL信号, 也可响应外源未知信号分子[26]。

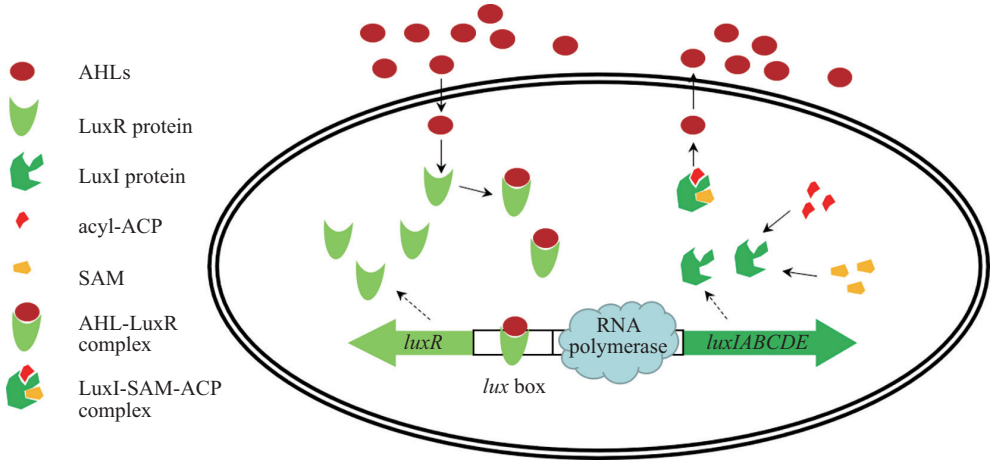
在费氏弧菌中, LuxI催化*S*-腺苷甲硫氨酸(*S*-adenosyl methionine, SAM)和带电荷的acyl-ACP酰化得到acyl-SAM, 随后acyl-SAM内酯化, 脱去甲硫蛋氨酸形成AHL信号分子。AHL结合并激活LuxR, AHL-LuxR复合物会在称为*lux*框的反向重复区域结合20 bp的DNA片段, *lux*框位于调控基因转录起始位点上游约40 bp处。低种群密度下, 荧光素酶操纵子*luxICDABE*转录水平较低; 高种群密度下, AHLs积累至阈值浓度后与LuxR结合, AHL-LuxR复合体在*lux*框处与DNA结合, 并募集RNA聚合酶到启动子区域, 刺激下游基因*luxIABCDE*的表达, 引起费氏弧菌的生物发光过程(图1)[27]。

不同于费氏弧菌等可以自身合成AHLs的细菌, 一些不能自身合成AHL信号分子的细菌如大肠杆菌、沙门氏菌等可以表达SdiA蛋白来识别其他细菌产生的AHL信号分子[28], 从而控制细菌的毒力因子产生和生物膜形成[29]。

1.2 AI-2介导的QS系统

在革兰氏阴性菌胞内除了AHL型信号分子还存在结构为呋喃硼酰二酯的AI-2分子。AI-2信号分子由LuxS合成, 因此AI-2系统又称为LuxS系统。AI-2受体主要有2种类型: 即LuxP和LsrB受体, 最初在哈氏弧菌(*Vibrio harveyi*)和鼠伤寒沙门氏菌(*Salmonella typhimurium*)中得到表征[30], 相比于只在弧菌属中发现的LuxP, LsrB还存在于大肠杆菌[31]、植物共生菌中华根瘤菌(*Sinorhizobium meliloti*)[32]、放线菌(*Aggregatibacter actinomycetemcomitans*)[33]中。LuxS在胞内合成AI-2前体分子DBD[(4,S)-4,5-二羟基-2,3-戊二酮], DBD在胞内聚集形成以动态平衡方式存在的异构体形式。LuxP和LsrB由于和AI-2分子结合部位的氨基酸组成不同, 导致结合底物结构存在差异, LuxP结合S-THMF-硼酸酯[(2S,4S)-2-甲基-2,3,3,4-四羟基四氢呋喃-硼酸酯], LsrB结合R-THMF[(2R,4S)-2-甲基-2,3,3,4-四羟基四氢呋喃][34-35]。在与AI-2信号分子结合后, LuxP调节跨膜传感器组氨酸激酶蛋白的活性, 进而调节磷酸化信号转导级联反应, 而LsrB与LsrACD转运蛋白协同作用, 调节后续*lsr*系统的进行[35]。

以鼠伤寒沙门氏菌中LuxS-LsrB介导的QS系统为例(图2)。LuxS在胞内合成DBD, DBD随着浓度积累异构化成AI-2分子, 亲水的AI-2通过膜蛋白TqsA被输送到细胞膜外[36]。当胞外的AI-2积累到阈值浓度, 它就会被LsrK内在化和磷酸化[37]。然后, 磷酸化



SAM和acyl-ACP在LuxI催化下酰化得到acyl-SAM, acyl-SAM内酯化并脱去甲硫蛋氨酸形成AHLs。AHLs结合并激活LuxR, 随之AHL-LuxR复合物会在*lux*框处结合DNA, RNA聚合酶就被寡集到启动子区域, 并刺激下游基因*luxIABCDE*的表达。虚线箭头: 基因转录翻译为蛋白; 实线箭头: 反应过程流程。

SAM and acyl-ACP are acylated under LuxI catalysis to obtain acyl-SAM, and acyl-SAM lactones and removes methionine to form AHLs. AHLs bind and activate LuxR, then AHL LuxR complex will bind DNA at *lux* frame, RNA polymerase will be oligomerized to the promoter region, and stimulate the expression of downstream gene *luxIABCDE*. Dashed arrows: gene transcription translated as protein; solid arrows: reaction process flow.

图1 AHL介导的QS系统模式(根据参考文献[27]修改)

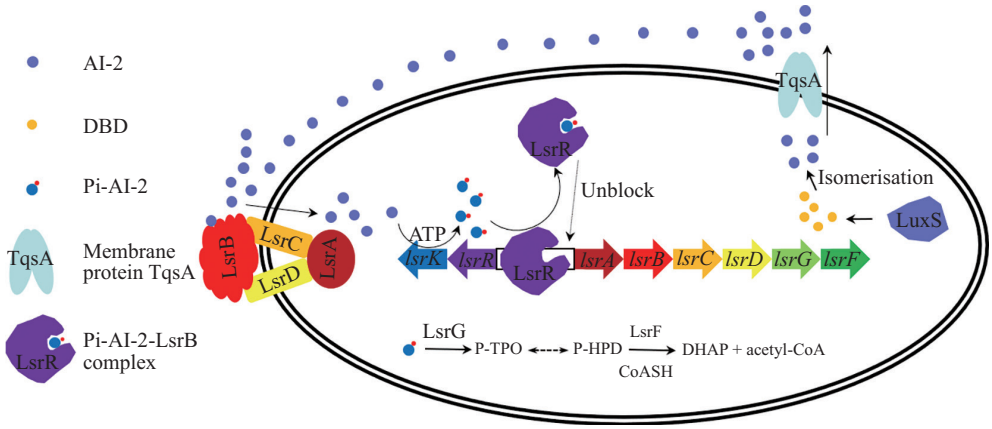
Fig.1 AHL-mediated QS system model (modified from reference [27])

的AI-2(Pi-AI-2)可以与转录调节因子LsrR结合, 阻断LsrR对*lsr*操纵子的阻遏作用并激活LsrABCD的转运[38]。LsrB受体和相关的ABC转运蛋白(LsrACD)促进AI-2内在化并进而耗尽细胞外AI-2。在大肠杆菌中, Pi-AI-2被LsrG进一步异构化为动态平衡的P-HPD(3-羟基-2,4-戊二酮-5-磷酸酯)与P-TPO(3,4,4-三羟基-2-戊酮-5-磷酸)。然后, LsrF催化乙酰基从P-HPD到CoA的转移, 产生DHAP(磷酸二羟基丙酮酯)和乙酰基CoA(细胞在糖酵解和柠檬酸循环等代谢途径中使用的关键代谢物)[39-40]。

不同于沙门氏菌中LsrB受体, 霍乱弧菌中的AI-2型QS系统为双组分QS系统。在低细胞密度下, LuxPQ受体发生自磷酸化, 引起LuxO磷酸化, 磷酸化的*LuxO*编码转录调节性小RNA(Qrr sRNAs), 并激活低细胞密度转录因子AphA和抑制高细胞密度转录因子HapR的表达。在高细胞密度下, LuxPQ受体与AI-2结合后由激酶转换为磷酸酶, 导致LuxO去磷酸化, 减少*Qrr* sRNAs基因的表达水平, 从而降低AphA的表达, 激活HapR的表达, 进而调控群体行为[41]。霍乱弧菌中, 胞内转录因子VqmA可以与Tdh合成的DPO(3,5-二甲基-吡嗪-2-醇)结合, 激活sRNA VqmR, VpmR抑制生物膜形成基因(*vpsT*)和毒力因子产生基因(*rtx*)的翻译[42]。

1.3 AIP介导的QS系统

不同于革兰氏阴性菌中的QS信号分子多样化, 革兰氏阳性菌一般采用环状寡肽的分子进行群体交流, 被称为自诱导肽(autoinducing peptide, AIP)。肽类在细菌中的QS一般可分为2种类型(图3)。一是双组分系统。如经典的金黄色葡萄球菌(*Staphylococcus aureus*)的*agr*型QS系统, AgrD合成AIPs的前肽, 被膜蛋白AgrB加工成熟运输至胞外积累, AIPs达到阈值浓度后会被组氨酸激酶受体AgrC识别信号并转导至胞内。运输至胞内的AIPs会激活响应调节因子AgrA, 引起AgrA自磷酸化, 促进*agrABCD*操纵子基因和QS下游基因的表达。AgrC在细胞膜外环也参与影响了细菌的QS行为[43]。双组分系统还存在于肺炎链球菌(*Streptococcus pneumoniae*)的*com*系统和粪肠球菌(*Enterococcus faecalis*)的*fsr*系统中[44]。二是RRNPP系统。由最初在不同物种中发现的受体蛋白而命名, 如Rap(枯草芽孢杆菌, *Bacillus subtilis*)、Rgg(链球菌, *Streptococcus*)、NprR(蜡状芽孢杆菌, *Bacillus cereus*)、PlcR(*B. cereus*)和PrgX(粪肠球菌, *E. faecalis*)。在RRNPP系统中, AIP前体由小的开放阅读框(small open reading frame, sORF)编码, 经过Sec依赖性分泌系统(Rap、PlaR、NprR)和PptAB-Eep转运系统(PrgX、Rgg)加工成熟并分泌。AIP在胞外积累至阈值浓度后, 经寡肽渗透酶复合物(Opp)导入, 被受体蛋白识别激活, 调节细菌的孢子形成和毒力相关基因的转录[45]。

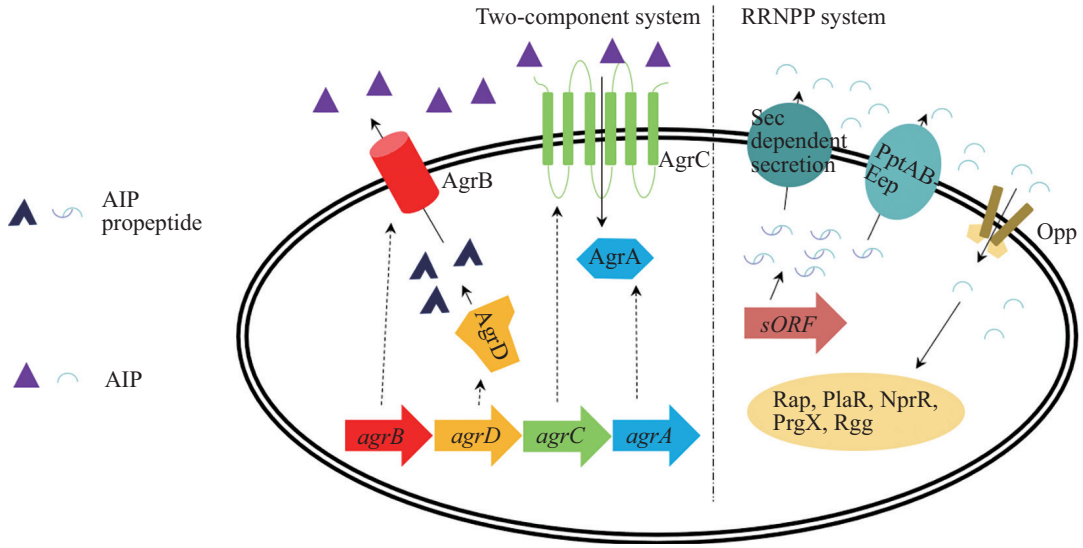


LuxS合成DBD并异构化成AI-2, AI-2被TqsA运输至胞外。AI-2在胞外积累达到阈值浓度后, LsrK磷酸化AI-2为Pi-AI-2, Pi-AI-2结合LsrR, 解除LsrR对lsr操纵子的阻遏并促进LsrABCD对AI-2的转运。在大肠杆菌中, LsrG进一步催化Pi-AI-2异构化为P-HPD和P-TPO, LsrF催化P-HPD产生DHAP和乙酰CoA。虚线箭头: 基因转录翻译为蛋白; 实线箭头: 反应过程流程。

LuxS synthesizes DBD and isomerizes to AI-2, which is transported by TqsA to the extracellular. After AI-2 accumulates in the extracellular area to a threshold concentration, LsrK phosphorylates AI-2 to Pi-AI-2, and Pi-AI-2 binds to LsrR, releases LsrR's repression of the lsr operon and promotes the transport of AI-2 by LsrABCD. In *E. coli*, LsrG further catalyzes the isomerization of Pi-AI-2 into P-HPD and P-TPO, and LsrF catalyzes P-HPD to produce DHAP and acetyl CoA. Dashed arrows: gene transcription translated as protein; solid arrows: reaction process flow.

图2 AI-2介导的QS系统模式(根据参考文献[39]修改)

Fig.2 AI-2 mediated QS system model (modified from reference [39])



双组分系统中, AgrD合成AIP前体并被AgrB加工运输至胞外积累, 胞外的AIP被AgrC识别并转导, AgrA被激活引起自磷酸化, 促进*agrABCD*操纵子和QS下游基因的表达; RRNPP系统中, sORF编码AIP前体, 并通过Sec依赖性分泌系统(Rap、PlaR、NprR)和PptAB-Eep转运系统(PrgX、Rgg)加工成熟并分泌, AIP在胞外积累至阈值浓度后, 经Opp转导被受体蛋白(Rap、PlaR、NprR、PrgX、Rgg)识别激活, 调控下游基因表达。虚线箭头: 基因转录翻译为蛋白; 实线箭头: 反应过程流程。

In the two-component system, AgrD synthesizes AIP precursor and is processed and transported by AgrB to accumulate outside the cell. The extracellular AIP is recognized and transduced by AgrC. AgrA is activated to cause autophosphorylation and promote the expression of *agrABCD* operon and QS downstream genes. In the RRNPP system, sORF encodes the precursor of AIP, which is processed and secreted through the Sec-dependent secretion system (Rap, PlaR, NprR) and the PptAB-Eep transport system (PrgX, Rgg). After AIP accumulates to the threshold concentration outside the cell, it is recognized and activated by receptor proteins (Rap, PlaR, NprR, PrgX, Rgg) through Opp transduction to regulate the expression of downstream genes. Dashed arrows: gene transcription translated as protein; solid arrows: reaction process flow.

图3 AIP介导的QS系统模式(根据参考文献[45]修改)

Fig.3 AIP mediated QS system model (modified from reference [45])

在表皮葡萄球菌(*Staphylococcus epidermidis*)中, 发现了3种不同的*agr*型QS系统(I~III), 其中AIP信号不同, AgrBCD受体也具有可变性。有趣的是, 这些AIP信号还能抑制其他类型的受体, 如AIP-II和AIP-III各自抑制AgrC-I, 而AIP-I抑制AgrC-II和AgrC-III[46]。

1.4 其他类型的QS系统

微生物中除了上述较为清晰明了的QS调控系统之外, 还有一些较为罕见的QS信号分子, 它们介导不同的QS系统。如肠出血性大肠杆菌O157:H7 (enterohemorrhagic *Escherichia coli*, EHEC)可以将肾上腺素衍生物作为信号分子, 调控自身毒力因子产生和生物被膜形成[47]。铜绿假单胞菌(*Pseudomonas aeruginosa*)中除了AHL型信号分子外, 还存在喹诺酮类(pseudomonas quinolone signal, PQS)类信号分子, 参与铜绿假单胞菌毒力因子的形成[48]。青枯雷尔氏菌中还存在3-羟基肉豆蔻酸酯(3-hydroxymyristate, 3-OH MAME)和3-羟基棕榈酸酯(3-hydroxypalmitate, 3-OH PAME)等脂肪酸衍生物作为QS信号分子的*phc*型QS系统[49]。ZHAO等[50]在蜡状芽孢杆菌(*Bacillus cereus*)中, 鉴定出9种结构为顺式-2-不饱和脂肪酸(C8-C15)的DSFs(diffusion signal factors)和6个二酮哌嗪(diketopiperazines, DKPs)类信号分子, 其可以调控细菌生物膜的形成。

2 基于QS系统抑制肠道致病菌的应用

耐药菌株的出现以及多种细菌感染引起的并发症日益成为人类健康的挑战[51]。QS系统通过靶向毒力来控制细菌感染, 干扰QS系统成为治疗肠道致病菌感染引起的疾病的研究策略之一[52]。下面我们基于QS的应对策略, 对抑制常见的肠道致病菌如鼠伤寒沙门氏菌、霍乱弧菌、艰难梭菌、致病性大肠杆菌等引起的感染进行综述(表1)。

2.1 基于QS系统抑制鼠伤寒沙门氏菌

鼠伤寒沙门氏菌属于革兰氏阴性菌, 易在宿主肠道定植黏附导致腹泻和胃肠炎等。沙门氏菌通过

QS系统和宿主交流并调节致病性, 包括生物膜形成, 孢子形成、毒力因子形成和运动性等[53]。BIRHANU等[54]联合使用邻苯三酚(pyrogallol, PG)和马波沙星(marbofloxacin, MAR)来抑制鼠伤寒沙门氏菌, qPCR显示PG和MAR的结合使用下调*hilA*(中枢调节因子)、*invF*(毒力岛SPI-1转录激活因子)、*sipB*(黏附蛋白)、*arcA*(外排泵)基因的表达量分别为59.3%、78.1%、46.7%、63.8%, 通过下调群体感应、毒力转运、外排泵基因来抑制沙门氏菌的入侵和细胞内存活。MECHESSO等[55]研究发现, 人参皂苷Rg3显著降低沙门氏菌运动、黏附、侵袭和存活能力, 50 μmol/L的Rg3下调*ompD*(黏附蛋白)、*prgK*(SPI-1分泌蛋白)、*sigB*(入侵通道蛋白)、AHL型QS基因(*sdiA*和*srgE*)基因的表达量分别为55.3%、91.1%、78.8%和75.0%, 进而影响沙门氏菌在宿主细胞中的黏附侵袭和繁殖能力。

细菌可通过QS调控外排泵表达和生物膜形成来增加其对药物的多重耐药性(multi-drug resistance, MDR)。SHEN等[56]发现, 0.01%的ε-聚赖氨酸作用于鼠伤寒沙门氏菌会减少约80%生物膜形成, 并使得*csgDBAC*(curli菌毛合成酶)、*lsrKCD*(QS操纵子)、*fliBCDST*(鞭毛蛋白合成酶)、*cheAWRBZY*(趋化性蛋白)等基因的表达水平下降2.0~8.3倍, *cpxAP*(诱导细胞膜错误折叠蛋白质的形成)基因的表达水平提高约3.3~6.3倍, 表明ε-聚赖氨酸通过下调菌毛、QS、鞭毛和趋化性相关的基因表达, 上调包膜应激反应基因表达来抑制生物膜形成, 进而抑制沙门氏菌的增殖。SEO等[57]研究乳酸球菌K10和HW01产生的细菌素对沙门氏菌的抑制作用, 发现两种细菌素在2 mg/mL的浓度下会下降约60%的生物膜形成能力, 且会抑制不锈钢和鸡肉上沙门氏菌的生物膜形成和浮游细胞的生长, 进而抑制沙门氏菌的黏附能力。

2.2 基于QS系统抑制霍乱弧菌

革兰氏阴性菌霍乱弧菌(*Vibrio cholerae*)易在人体肠道中引起腹泻型霍乱, 利用QS来调节毒力因子合成、生物膜形成、VI型分泌系统, 以维持霍乱弧菌在环境压力中的适应性。霍乱弧菌使用4种组氨酸激酶LuxPQ、CqsS、CqsR、VpsS作为QS受体。WATVE等[58]鉴定出乙醇胺与CqsR受体的配体结合d-CACHE域特异性相互作用, 其通过与CqsR结合影响霍乱弧菌的QS基因表达和宿主定植, 但其抑制作用会被其他3种QS作用抵消, 使得霍乱弧菌可以定植于宿主肠道中。MALKA等[59]发现, 酵母产生的乙酸色酯(tryptophol acetate)可抑制霍乱弧菌生物膜的形成, 并且200 μmol/L的乙酸色酯可下调*hapR*(生物膜转录因子)、*hapA*(腹泻调控蛋白)、*ctxA*(霍乱毒素亚基A)等QS相关基因表达, 上调*vpsT*(毒力基因转录调控因子)、*aphA*(毒力基因调控因子)、*tcpHP*(毒力基因)、*toxT*(毒力基因转录激活因子)等毒力基因的表达。BHATTACHARYA等[60]测试了甘草次酸(glycyrrhetinic acid, GRA), 熊果酸(ursolic acid, UA)和桦木酸(betulinic acid, BA)等3种三萜类化合物对霍乱弧菌的作用, 发现200 μg/mL的GRA、UA、BA分别将生物膜的完整性降低至33.82%、21.92%、13.98%, 并调控EPS相关酶的活性。GRA和UA可以增强头孢菌素类β-内酰胺类抗生素作用, BA可以增强氟喹诺酮(环丙沙星)类抗生素作用。化合物与VpsT、LuxP、LuxQ、HapR等QS相关蛋白进行分子对接。结果表明, GRA、UA和BA主要与QS受体蛋白疏水沟结合, 产生极性疏水相互作用, 进而导致三萜化合物对霍乱弧菌的生物膜形成和毒力基因表达的调控。

2.3 基于QS系统抑制艰难梭菌

艰难梭菌(*Clostridium difficile*)属于革兰氏阳性菌, 产芽孢, 专性厌氧菌, 易引起抗生素相关性腹泻和假膜性结肠炎。AHMED等[20]在艰难梭菌中发现*agr1*系统由*agrB1*和*agrD1*组成, 当*agrB1*基因缺失时, AgrD1在胞内积累, *tcdABR*(艰难梭菌毒素合成蛋白)基因转录水平增加, 而*agrD1*单基因或*agrB1*/*agrD1*的组合基因缺失则对*tcdA*、*tcdB*、*tcdR*的表达水平影响较小。当AgrB1和AgrD1均被破坏时, 艰难梭菌的孢子形成和运动能力降低。YANG等[61]使用短双歧杆菌YH68(*Bifidobacterium breve*)无细胞上清液(YH68-CFCS)抑制艰难梭菌ATCC 9689(CD), CD暴露于低剂量(50 mL, CDL)和高剂量(90 mL, CDH) YH68-CFCS中的细菌生长抑制和细胞膜完整性呈现剂量依赖关系。在CDH细胞中, QS基因的表达水平被抑制, 毒力合成和孢子形成的基因被增强。在CDL细胞中, 鞭毛组装和生物膜形成的基因的表达被抑制, 耐药性相关基因上调。YONG等[62]研究发现, 发酵乳杆菌Lim2(*Lactobacillus fermentum*)的细胞提取物不会对艰难梭菌生长产生影响, 但影响胞内AI-2分子水平, Lim2细胞提取物会分别引起*luxS*(QS信号分子合成酶)、毒力基因(*tcdA*、*tcdB*、*tcdE*)表

表1 QS系统在抑制肠道致病菌中的应用

Table 1 Application of QS system in inhibiting intestinal pathogenic bacteria

细菌 Bacteria	菌株 Strains	化合物 Compound	QS类型 QS type	抑菌机制 Antibacterial mechanism	抑菌浓度 Inhibitory concentration	参考文献 References
Salmonella typhimurium	KU325552 KU325565 ATCC14028	Pyrogallol (PG) Marbofloxacin (MAR)	AI-1 (SdiA)	The combined effect of PG and MAR inhibits the expression of *hilA*, *invF*, *sipB*, and *arcA* genes	MIC_{PG}=128 μg/mL MIC_{MAR}=128 μg/mL	[54]
	LVPP-STI15 ATCC14028	Ginsenoside Rg3	AI-1 (SdiA)	Rg3 reduces the expression of *ompD*, *prgK*, and *sigB* genes, and QS genes *sdiA* and *srgE*	Rg3 doesn't affect the survival of *Salmonella*, but affects movement, adhesion and invasion through QS	[55]
	NBRC12529	ε-Polylysine	AI-2 (*lsr* operon)	ε-polylysine down-regulates *csgDBAC*, *lsrKCD*, *fliBCDST*, *cheAWRBZY* genes, up-regulates *cpxAP* gene expression, and inhibits biofilm formation	0.01% ε-polylysine inhibits 80% of biofilms and reduces viable bacteria by 10^5 times	[56]
	KCTC1925	Bacteriocin	AI-2 (*lsr* operon)	Bacteriocins inhibit biofilm formation,planktonic cell growth, and adhesion	2 mg/mL bacteriocin reduces biofilm by about 60%	[57]
Vibrio cholerae	*V. cholerae* (derived from C6706*str*2)	Ethanolamine	AI-2 (CqsR)	Ethanolamine binds to the d-CACHE domain of CqsR and inhibits host colonization of *V. cholerae*	_	[58]
	MM920 VC1	Tryptophol acetate	AI-1 (HapR)	Tryptophol acetate down-regulates QS genes *hapR*,*hapA*, *ctxA*, and up-regulates virulence genes *vpsT*, *aphA*, *tcpHP*, *toxT*	$MIC_{Tryptophol\ acetate}$=200 μmol/L $MIC_{50,Tryptophol\ acetate}$=22.8±3.7 μmol/L	[59]
	C6709	Glycyrrhetinic acid (GRA) Ursolic acid (UA) Betulinic acid (BA)	AI-1 (VpsT, LuxP, LuxQ, HapR)	Triterpenoids bind to QS proteins such as VpsT, LuxP, LuxQ, HapR, and regulate biofilm and virulence genes to affect the integrity of biofilms	MBC_{GRA}>1 000 μg/mL MBC_{UA}>500 μg/mL MBC_{BA}>500 μg/mL	[60]
Clostridium difficile	ATCC 9689	*B. breve* YH68-CFCS	Agr	YH68-CFCS regulates QS, biofilm formation, drug resistance, virulence factors and other related genes, and inhibits the growth and biofilm formation of *C. difficile*	10^9 CFU/mL YH68-CFCS inhibits the growth of *C. difficile* by 65.24%	[61]
	O27	*L. fermentum* Lim2 cell extract	AI-2 (LuxS)	Lim2 cell extract inhibits the *luxS* and *tcdABE* genes, up-regulates the *tcdC* gene, and inhibits the expression of QS and virulence genes	100 mg/mL cell extract inhibits AI-2 activity 654 times	[62]
Escherichia coli	EMC17	Vitamin C (VitC)	AI-2 (LuxS)	VitC down-regulates the expression of luxS, fimA, csgA, malA, bssSR gene, and inhibits the ability of biofilm formation	MIC_{VitC}=125 mmol/L $MIC_{50,VitC}$=30 mmol/L	[63]
	COP512	HQNO Pyocyanin	AI-2 (LuxS)	*P. aeruginosa* inhibits the growth of *E. coli* by secreting HQNO and pyocyanin, and down-regulates oxidative phosphorylation, citric acid cycle, flagella assembly, carbon metabolism and other related genes		[64]

MIC: 最小抑菌浓度; MIC_{50}: 半抑菌浓度; MBC: 最小杀菌浓度。

MIC: minimum inhibitory concentration; MIC_{50}: half inhibitory concentration; MBC: minimum bactericide concentration.

达水平降低19%、24%、39%、19%, *tcdC*(负调控因子)基因表达水平上升15%。结果表明, Lim2细胞提取物可通过抑制QS相关基因和毒力基因表达来降低艰难梭菌的感染。

2.4 基于QS系统抑制致病性大肠杆菌

人体肠道中含有丰富的大肠杆菌菌群, 大部分大肠杆菌不致病, 但某些特殊血清型的大肠杆菌, 如致病性大肠杆菌(enteropathogenic *Escherichia coli*, EPEC)、肠产毒性大肠杆菌(enterotoxigenic *Escherichia coli*, ETEC)、肠侵袭性大肠杆菌(enterinvasive *Escherichia coli*, EIEC)、肠出血性大肠杆菌(EHEC)、肠黏附性大肠杆菌(enteroadhesive *Escherichia coli*, EAEC), 可通过大肠杆菌中QS系统调控细菌的生物膜形成能力和毒力因子合成, 进而促进病原体克服宿主免疫系统、抗微生物应激和传播毒力因子, 引起感染性腹泻和急性肠炎。SHIVAPRASAD等[63]发现, 维生素C(Vitamin C, VitC)以剂量依赖形式抑制大肠杆菌。VitC损害了群体感应(QS)和胞外多糖(exopoly saccharides, EPS)的产生以及由于活性氧(reactive oxygen species, ROS)的产生而破坏细胞膜导致细菌细胞内生物分子(还原糖和蛋白质)的泄漏。此外, VitC处理导致*luxS*(QS信号分子合酶)、*fimA*(菌毛蛋白)、*csgA*(curli菌毛蛋白)、*malA*(糖水解酶)、*bssSR*(信号分泌蛋白)基因表达水平分别下降27、24、15、2、6倍, 引起生物膜形成能力下降, 并破坏细胞的动态平衡和生存能力, 减弱大肠杆菌的侵袭和感染能力。YUAN等[64]研究了铜绿假单胞菌和大肠杆菌的共培养, 铜绿假单胞菌通过合成4-羟基-2-庚基喹啉(4-hydroxy-2-heptylquinoline noxide, HQNO)和花青素等胞外毒素抑制大肠杆菌的生长。大肠杆菌胞外产物又可增强假单胞菌中QS系统PQS的功能来进一步抑制大肠杆菌的生长。

3 结论与展望

肠道菌群和人体健康有着密切交流, 并且肠道致病菌与肠道疾病的发生和发展息息相关。传统的抗生素治疗疾病是通过抑制致病菌的代谢或破坏致病菌生物膜的完整性来抑制其存活能力, 而这会导致细菌出现耐药性以及耐药性在遗传水平上迁移。因此开发新的抑菌方向或者开发新的抗菌药物尤为重要。近年来, 越来越多的研究发现, 细菌QS系统在调节细菌毒力和细菌的耐药性方面发挥着重要作用。群体感应使细菌不局限于同种个体和环境之间的交流, 还影响不同菌种之间、细菌与宿主之间的信息交换。因此开发新的阻断QS系统的方法不仅可以抑制细菌的独立基因的表达, 从而减弱细菌的侵袭能力, 还可以有效抑制生物膜形成引起的耐药性, 抑制QS调控毒力因子的产生, 减少致病菌对宿主机体的感染。本文首先总结了目前肠道致病菌中常见的QS调控路径, 然后对具体的多种基于QS干扰或者抑制肠道致病菌毒力策略进行了综述, 以期有助于突破当今抗生素在多重耐药性细菌治疗方面的局限, 发掘基于QS调控的肠道致病菌抑制剂, 从而高效特异地抑制肠道致病菌致病性和耐药性, 解决人体健康安全问题。

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