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·综 述·

植物提取物及其活性成分抑制细菌生物被膜的研究进展

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摘 要：大量研究报道生物被膜细菌对抗生素的耐药性是浮游菌的 10–1 000 倍，据报道细菌生物被膜是 80%以上细菌感染的罪魁祸首，对医疗保健领域构成了严峻的挑战。植物提取物及其活性成分对细菌生物被膜有明显的抑制作用，包括减少生物被膜量、生物被膜活菌数以及清除已经成熟的生物被膜等。该文对这些有效的植物提取物及其活性成分进行了总结，并分析了其抗细菌生物被膜的作用机制。旨在为防治细菌生物被膜感染的植物类药物的开发提供参考。

关键词：细菌生物被膜；植物提取物；群体感应；作用机制

Using plant extracts and their active ingredients to inhibit bacterial biofilms

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Abstract: Numerous studies have reported that the resistance of biofilm bacteria to antibiotics can be

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up to 10–1 000 fold higher than that of planktonic bacteria. Bacterial biofilms are reported to be responsible for more than 80% of human microbial infection, posing great challenges to the healthcare sector. Many studies have reported that plant extracts and their active ingredients can inhibit the formation and development of bacterial biofilms, including reducing biofilm biomass and the number of viable bacteria in biofilms, as well as eradicating mature biofilms. This review summarized the plant extracts and their active ingredients that are inhibitory to bacterial biofilms, and analyzed the underpinning mechanisms. This review may serve as a reference for the development of plant drugs to prevent and treat biofilm infections.

Keywords: bacterial biofilm; plant extracts; quorum sensing; mechanism

细菌生物被膜 (bacterial biofilms, BF) 是由细菌群落分泌能够包裹自身的胞外基质 (extracellular polymeric substances, EPS) (包括胞外多糖，蛋白质和 eDNA 等物质) 与细菌结合形成的复杂聚合体[1-2]。细菌生物被膜生长可分为粘附阶段、形成阶段、成熟阶段和分散阶段[3] (图 1)。据报道超过 80%的人类细菌感染是由于细菌生物被膜引起[4]。细菌生物被膜具有动态结构、内部细菌代谢率低、存在持留菌以及胞外基质能阻挡抗生素等特点，据报道生物被膜细菌对抗生素的耐药性是浮游菌的 10–1 000 倍[3-5]。因此，传统的抗生素治疗细菌生物被膜感染有一定局限性[6]。

几个世纪以来，在预防和治疗疾病时，植物源药品在一些国家得到了广泛应用[7]。大量的植物源于自然环境中，是易获得的资源，但只有少数植物得到了科学的评价 (300 000 种中有 6%)[8-10]。大量研究表明植物提取物及其活性成分具有抗细菌生物被膜感染的能力，又因其毒副作用相对较小，不易产生耐药性等优点，因此，探寻合适的植物提取物及其活性成分将成为防治细菌生物被膜感染的有效策略[9]。

1 抗细菌生物被膜的植物提取物及其活性成分的筛选

具有抗细菌生物被膜作用的植物来源非常丰富，如药用植物、香料、花卉和蔬菜等[11]。

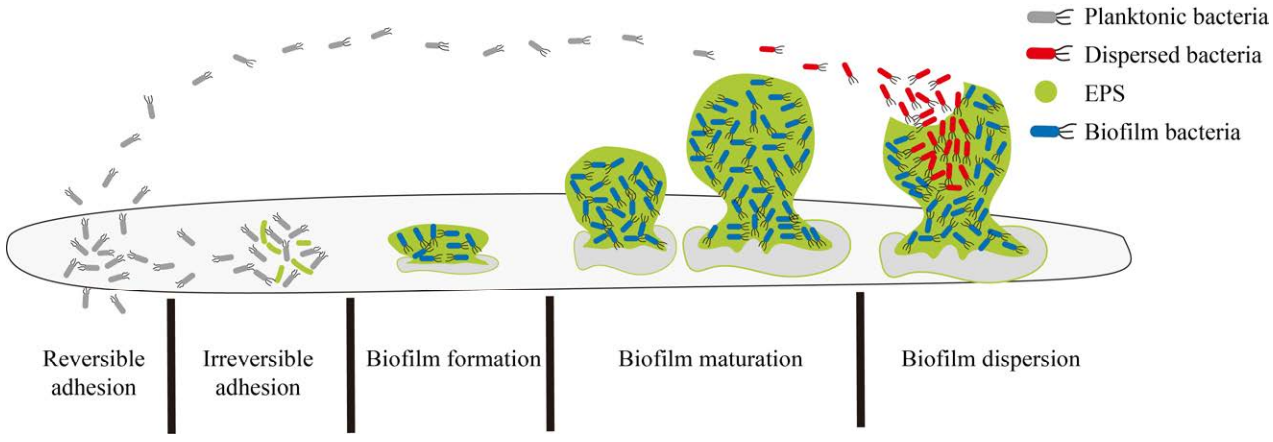

图 1　细菌生物被膜生长的不同阶段

Figure 1　Different stages of bacterial biofilm formation and development.

另外，部分农业废弃物也有抑制细菌生物被膜的能力，如柑橘果皮和稻草等[12-13]。通过检索2011–2021 年的国内外文献，我们从细菌生物被膜的种类、抗细菌生物被膜的研究方法 (包括生物被膜量、生物被膜活菌数或活力、形态学观察等)、作用于细菌生物被膜的发育时期(主要包括形成和成熟阶段) 的角度对具有抑制细菌生物被膜的植物提取物及其活性成分进行了归纳总结，见表 1 和表 2。

目前，通常采用对细菌生物被膜进行染色的方法，对具有抗细菌生物被膜作用的药物进行初步筛选，如结晶紫染色法检测细菌生物被膜量，表 1 和表 2 中大量文献采用了这种方法来进行抗细菌生物被膜作用的植物提取物或活性成分的初步筛选，但是这种方法工作量很大，只适合小规模的初级筛选[48,74]。另外，如表 1 和表 2 所示，文献中也常采用结晶紫染色(crystal violet staining, CV)、生物被膜活菌计数(colony count, CC) 或细菌活力 (如噻唑蓝 MTT 法等) 结合形态学观察 (如激光共聚焦扫描显微镜 CLSM 等) 的方法进行筛选和验证活性成分的抗细菌生物被膜作用效果。另外基于对细菌生物被膜群体感应 (quorum sensing, QS) 等方面作用机制的深入研究 (有关 QS 内容详见本文 2.1 部分)，以此为基础也开发出了一些先进的筛选方法，有研究报道，采用计算机虚拟筛选的方法，可对大量活性成分进行初筛，如丁婷[75]以荧光假单胞菌的 LuxI 型及 LuxR 型蛋白为靶点，两个蛋白受该菌群体感应 QS 系统基因调控，使用分子对接软件对靶蛋白进行预处理，选择食物源组分数据库和中药数据库的活性成分，使用分子对接软件然后分别与这两个蛋白进行对接，筛选出来的活性成分物质，再进行 QS 抑制活性的试验验证，最终筛选到能抑制 QS 系统的活性成分为苯甲醇和 (+)-儿茶素。还有研究使用生物传感器菌株进行筛选，如 Wang 等[76]利用紫色杆菌 (*Chromobacterium violaceum*) CV026 的特性 (该菌在外源性加入 QS 信号分子 C_6-HSL 或 3-oxo-C_{12}-HSL 的 LB 琼脂上会产生紫色色素，当遇到 QS 的抑制剂时，则会抑制紫色色素的产生)，证实千层树叶精油在 CV026 的亚抑菌浓度有抑制 QS 活性的能力。而类似生物传感器菌株还有根癌农杆菌(*Agrobacterium tumefaciens*) NT1 和哈维氏弧菌(*Vibrio harveyi*) BB170 等，如哈维氏弧菌 BB170 可在含有 AI-2 (autoinducer-2) 信号分子 (受 QS 系统调控) 培养基中诱导发光反应，发光强度与 AI-2 浓度呈正比，采用这个原理，陈菲发现，和厚朴酚在亚抑菌浓度下，能够通过影响大肠杆菌 AI-2 信号分子分泌量抑制其生物被膜的形成[73,77]。另外，党敏燕[78]对铜绿假单胞菌生物被膜相关基因启动子库的构建，将铜绿假单胞菌形成生物被膜相关基因启动子与 *lu xCDABE* 发光报道基因进行整合，将启动子-报道基因整合到铜绿假单胞菌的染色体上，通过发光强弱，推断该基因表达情况，从而筛选出潜在的具有抗细菌生物被膜作用的化合物。

2 植物提取物及其活性成分抗细菌生物被膜的作用机制

2.1 作用于 QS 系统

细菌群体感应系统 QS，即细菌通过释放的激素样有机物-自诱导物 (又称信号分子) 来交流，从而改变胞内遗传物质的表达，调节细菌的生长代谢，并导致细菌毒力、耐药性的变化等。当细菌的数量达到一定的水平，信号分子的浓度会发生改变，被其他细菌感知，诱导细菌特异性基因的表达，从而调节细菌的生理活

表 1 植物提取物抑制细菌生物被膜汇总

Table 1 Summary of the plant extracts inhibitory to biofilms

Plant	Types of bacterial biofilm	Methods	The period of action on the biofilm	References
Trachyspermum ammi (seeds)	*Streptococcus mutans*	SEM, transcriptional analysis	Biofilm formation	[14]
Quercus cerris (leaves, stems and fruits)	*Staphylococcus aureus*	CV, CLSM	Biofilm formation	[15]
Arctium lappa L.	*Escherichia coli*	SEM, CC	Biofilm formation	[16]
Euphorbia hirta L.	*Pseudomonas aeruginosa* *Enterococcus faecalis*	p-iodonitrotetrazolium violet dyeing	Biofilm formation Biofilm maturation	[17]
Vitis vinifera	*Streptococcus mutans*	CLSM	Biofilm formation	[18]
Eucalyptus globulus, *Eucalyptus urograndis* (essential oil)	*Streptococcus mutans*	Safranine dyeing	Biofilm formation	[19]
Ginkgo biloba	*Escherichia coli* *Staphylococcus aureus*	CV, CLSM, swimming and swarming motility, transcriptional analysis	Biofilm formation	[20]
Rosa rugosa (tea)	*Pseudomonas aeruginosa* *Escherichia coli*	CV, CLSM, swimming and swarming motility	Biofilm formation	[21]
Sclerocarya birrea (stem bark)	*Pseudomonas aeruginosa*	CV, CC, OM, swimming and swarming motility, determination of virulence factors	Biofilm formation	[22]
Streblus asper (leaf)	Oral pathogenic bacteria	CV, quantification of viable biofilm bacteria with real-time PCR	Biofilm formation	[23]
Sanguisorba officinalis L.	*Staphylococcus aureus*	CV, CLSM, transcriptional analysis	Biofilm formation	[24]
Azadirachta indica A. Juss (leaf)	*Staphylococcus aureus*	CV, AFM	Biofilm formation	[25]
Cinnamon bark oil	*Pseudomonas aeruginosa* *Escherichia coli*	CV, CLSM, SEM, determination of virulence factors, swimming and swarming motility, transcriptional analysis	Biofilm formation	[26]
Syzygium aromaticum *Cinnamomum zeylanicum*	*Staphylococcus aureus*	CV, transcriptional analysis	Biofilm formation	[27]
Vaccinium macrocarpon (oligosaccharides)	*Escherichia coli*	CV	Biofilm formation	[28]
Hibiscus sabdariffa L.	Oral pathogenic bacteria	CV	Biofilm formation	[29]
Punica granatum L. (leaf)	*Staphylococcus aureus*	Safranine dyeing	Biofilm formation	[30]
Rhus coriaria L. (leaf)	*Pseudomonas aeruginosa*			
Coriandrum sativum (Essential oil) *Pimpinella anisum* (Essential oil)	*Escherichia coli* *Staphylococcus aureus*	CV, XTT, OM	Biofilm formation Biofilm maturation	[31]
Andrographis paniculata	*Pseudomonas aeruginosa*	CV, CLSM, determination of virulence factors, transcriptional analysis	Biofilm formation	[32]

(待续)

(续表 1)

Plant	Types of bacterial biofilm	Methods	The period of action on the biofilm	References
Moringa oleifera	*Staphylococcus aureus*	CV, CLSM	Biofilm formation	[33]
Rosmarinus officinalis L. (Essential oil)	*Staphylococcus epidermidis*	CV, FLM, resazurin dyeing	Biofilm formation Biofilm maturation	[34]
Rosmarinus officinalis *Tetradenia riparia*	*Staphylococcus aureus*	MTT, SEM	Biofilm maturation	[35]
Juglans regia L. (leaf)	*Pseudomonas aeruginosa*	CV	Biofilm formation	[36]
Pogostemon heyneanus (Essential oil) Cinnamomum tamala (Essential oil)	*Staphylococcus aureus*	CV, CC, OM, CLSM, SEM, determination of extracellular polysaccharide	Biofilm formation Biofilm maturation	[37]
Tea tree oil	*Staphylococcus aureus*	CV, CLSM, transcriptional analysis	Biofilm maturation	[38]
Bruguiera cylindrica *Laguncularia racemosa*	*Escherichia coli*	Determination of extracellular polysaccharide and protein	Biofilm formation Biofilm maturation	[39]
Citrus (peel and pulp)	*Escherichia coli* *Pseudomonas aeruginosa* *Staphylococcus aureus*	CV, MTT	Biofilm formation	[13]
Black pomegranate (peel)	*Pseudomonas aeruginosa*	CV	Biofilm formation	[40]
Hypericum perforatum	*Pseudomonas aeruginosa*	CV	Biofilm formation	[41]
Green tea	*Pseudomonas aeruginosa*	CV, XTT, OM, SEM, CLSM, determination of extracellular polysaccharide, determination of virulence factors	Biofilm formation	[42]
Rice straw	*Staphylococcus aureus*	CV, CC, AFM, CLSM, resazurin dyeing	Biofilm maturation	[12]
Cinnamomum zeylanicum (Essential oil) *Origanum majorana* (Essential oil) *Thymus vulgaris* (Essential oil)	Single or double species biofilm system	CV, SEM	Biofilm formation	[43]
Peppermint (Essential oil)	*Staphylococcus aureus*	CV, CC, ESEM	Biofilm formation	[44]
Clove (Essential oil)	*Escherichia coli*	CC, CV, CLSM, SEM, resazurin dyeing, determination of extracellular polysaccharide	Biofilm maturation	[45]
Cardamom (Essential oil)	*Staphylococcus aureus*	CC, CV, CLSM, SEM, resazurin dyeing, transcriptional analysis	Biofilm maturation	[46]
Dandelion	*Streptococcus suis*	CV, SEM	Biofilm formation	[47]
Honeysuckle, houttuynia, scutellaria baicalensis, etc.	*Streptococcus suis*	CV	Biofilm formation	[48]
Cuphea carthagenensis (Jacq.) J.F.Macbr. (leaf)	*Pseudomonas aeruginosa*	CV, OM, SEM, determination of extracellular polysaccharide, determination of virulence factors	Biofilm formation	[49]
Acca sellowiana (Fruit)	*Pseudomonas aeruginosa* *Staphylococcus aureus*	CV, TEM, CLSM	Biofilm formation Biofilm maturation	[50]

表 2 植物活性成分抑制细菌生物被膜汇总

Table 2 Summary of the plant active ingredients inhibitory to biofilms

Plant active ingredients	Types of bacterial biofilm	Methods	The period of action on the biofilm	References
Grapefruit bioactive limonoids	*Escherichia coli*	CV, transcriptional analysis	Biofilm formation	[51]
Curcumin	*Pseudomonas aeruginosa* *Escherichia coli*	CV, OM, CLSM, determination of extracellular polysaccharide, determination of virulence factors	Biofilm formation	[52]
Luteolin	*Escherichia coli*	CV	Biofilm formation	[53]
Eugenol	*Streptococcus mutans*	CLSM, transcriptional analysis	Biofilm maturation	[54]
Coumarins	*Escherichia coli*	CLSM, transcriptional analysis	Biofilm formation	[55]
Cranberry proanthocyanidins	*Pseudomonas aeruginosa*	CV, resazurin dyeing, CLSM	Biofilm formation Biofilm maturation	[56]
Ribose combined with xylitol	*Streptococcus mutans*	Bacterial live/death counting kit, transcriptional analysis	Biofilm formation	[57]
Soy isoflavones	*Escherichia coli*	CV	Biofilm formation	[58]
Epigallocatechin gallate	*Enterococcus faecalis*	CC, CLSM	Biofilm maturation	[59]
Berberine hydrochloride	*Enterococcus faecalis*	CV, CLSM, transcriptional analysis	Biofilm formation Biofilm maturation	[60]
Eugenol Carvacrol Thymol	Oral pathogenic bacteria	XTT, SEM	Biofilm formation	[61]
Carvacrol Citral	*Escherichia coli* *Staphylococcus aureus*	CV, CC	Biofilm maturation	[62]
Rutin	Single or double species biofilm system	CV, SEM, determination of extracellular polysaccharide	Biofilm formation	[63]
Gallic acid	*Staphylococcus aureus*	CV, FLM, ESEM, transcriptional analysis, determination of extracellular polysaccharide	Biofilm maturation	[64]
Betulin Betulinic acid	*Pseudomonas aeruginosa*	CV, TTC, OM, CLSM, determination of virulence factors	Biofilm formation	[65]
Octyl gallate	*Streptococcus mutans*	CV, transcriptional analysis	Biofilm formation	[66]
Myrtenol	*Staphylococcus aureus*	CV, CC, OM, CLSM, resazurin dyeing, determination of virulence factors, transcriptional analysis	Biofilm formation Biofilm maturation	[67]
Quercetin	*Enterococcus faecalis*	CV, SEM, CLSM	Biofilm formation	[68]
Cinnamaldehyde	*Staphylococcus epidermidis*	CV, CC, CLSM	Biofilm formation Biofilm maturation	[69]
Punica granatum sarcotesta lectin	*Staphylococcus aureus*	CV	Biofilm formation	[70]

(待续)

(续表 1)

Plant active ingredients	Types of bacterial biofilm	Methods	The period of action on the biofilm	References
Glycyrrhetinic acid	*Pseudomonas aeruginosa*	MTT, determination of virulence factor TEM, SEM	Biofilm formation Biofilm maturation	[71]
Total alkaloids of Sophora alopecuroides and matrine	*Staphylococcus aureus*	XTT	Biofilm formation Biofilm maturation	[72]
Honokiol	*Escherichia coli*	CV, TTC, XTT, transcriptional analysis, determination of AI-2 signaling molecules by biosensor	Biofilm formation Biofilm maturation	[73]

AFM: atomic force microscope; CC: colony count; CLSM: confocal laser scanning microscope; CV: crystal violet staining; ESEM: environmental scanning electron microscope; OM: optical microscope; SEM: scanning electron microscope; TEM: transmission electron microscope; TTC: determination of cell metabolic viability; XTT: determination of cell metabolic viability; MTT: determination of cell metabolic viability.

动[77]。对于大多数菌种来说，QS 系统对细菌生物被膜形成发挥着至关重要的作用[79]。常见的信号分子包括革兰氏阴性菌的高丝氨酸内酯类信号分子 (N-acylhomoserine lactones, AHLs)，革兰氏阳性菌的寡肽和氨基酸，种间和种内的特异性通讯信号分子 AI-2 以及一些特定细菌产生的信号分子，包括大肠杆菌的信号分子 AI-3 (autoinducer-3) 和铜绿假单胞菌中喹诺酮信号分子等[11,80]。据报道，植物提取物及其活性成分可作为群体感应抑制剂 (quorum sensing inhibitor, QSI)，图 2 展示了可能干扰 QS 系统调控过程的不同途径[77]，主要包括竞争信号分子受体、信号分子受体活性降低、降解信号分子、信号分子合成受阻以及信号分子结合受体的过程受阻。

Jia 等[72]的研究表明，AI-2 的活性与表皮葡萄球菌生物被膜形成呈正相关，发现苦豆子总碱和苦参碱能显著抑制表皮葡萄球菌野生株 S13 的生物被膜细菌的 AI-2 活性。Vikram 等[51]发现，试验所选用的几种柠檬苦素类化合物对大肠杆菌的 AHLs 和 AI-2 介导的信号转导均有不同程度的抑制作用，而且抑制作用强度与药物浓度呈正相关。陈一强[81]发现，绿原酸减少了铜绿假单胞菌 PAO1 的 AHLs 类信号分子 C_4-HSL 和 3-oxo-C_{12}-HSL 的合成释放，抑制了 PAO1 藻酸盐的合成和生物被膜的形成。陈思敏[82]发现，穿心莲内酯能下调铜绿假单胞菌 QS 系统中 *lasR* 和 *rhlR* 基因表达，干预了铜绿假单胞菌生物被膜的形成。

因此，以 QS 系统为靶标，药物对 QS 系统相关基因或者信号分子进行干预，可能是阻止细菌生物被膜感染的一种有效方法。

2.2 作用于 c-di-GMP 系统

环二鸟苷酸 (bis-(3′-5′) cyclic diguanylicacid, c-di-GMP) 是细菌中广泛存在的胞内第二信使，在细菌中，二鸟苷酸环化酶 (diguanylate cyclases, DGCs) 催化合成 c-di-GMP 分子，磷酸二酯酶 (phosphodiesterases, PDEs) 来降解 c-di-GMP 分子[83]。c-di-GMP 参与调节细菌的多种生理功能，包括细菌游动性、毒力和生物被膜形成[84]。以铜绿假单胞菌为例，高浓度的 c-di-GMP 可以促进胞外多糖的合成从而促进细菌生物被膜的形成，而低浓度的 c-di-GMP 则使细菌趋向于浮游状态存在[84-85]。

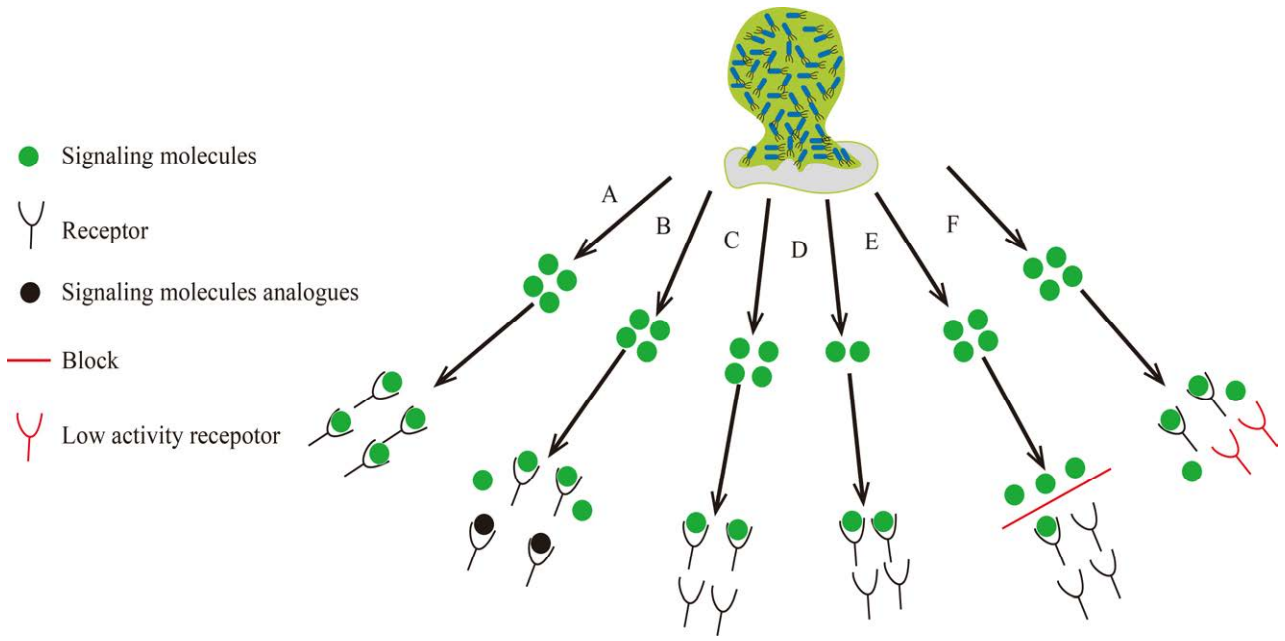

图 2　干扰 QS 系统调控过程的不同途径

Figure 2　Different ways of interfering with the regulation of QS system. A: normal; B: competitive receptor; C: degradation of signaling molecules; D: signaling molecules synthesis blocked; E: binding of the signal molecule to the receptor is blocked; F: reduced receptor activity.

药物作用于不同时期的细菌生物被膜，可能会导致 c-di-GMP 浓度产生不同的变化。有关植物提取物及其活性成分抗细菌生物被膜机制的研究主要集中在 QS 系统，而 c-di-GMP 作为细菌生物被膜形成的关键调控因子，目前正受到广泛关注。崔收庆[86]发现，在 0.6 g/L 的抑菌浓度下使用秸秆酚酸中的活性成分香豆酸作用于金黄色葡萄球菌生物被膜，导致生物被膜量的增加，而生物被膜内的细菌的 c-di-GMP 含量升高，该现象可能是生物被膜细菌应对不利环境使 c-di-GMP 含量升高所致。Topa 等[87]发现，铜绿假单胞菌与肉桂醛在亚抑菌浓度 3 mmol/L 共培养 5 h 后，c-di-GMP 浓度水平下调了 66.2%，抑制了生物被膜的形成。Kim 等[88]发现，1%的生姜提取物作用于铜绿假单胞菌浮游菌后，显著降低了其 c-di-GMP 的浓度水平，抑制了生物被膜的形成。有研究报道了部分植物活性成分可以清除已经成熟的细菌生物被膜，Albano 等[69]通过结晶紫染色的方法发现，肉桂醛在 4–6 倍 MIC 的浓度下可以清除表皮葡萄球菌的生物被膜。而这种清除作用是以生物被膜量的减少率为依据的，具体机制有待不断探索。根据细菌生物被膜分散的机理，分为主动分散和被动分散，主动分散通常是指细菌生物被膜感知环境条件的改变，如缺氧、营养消耗、应激反应、一氧化氮 (NO) 水平等，生物被膜细菌通过降低 c-di-GMP 水平，增加生物被膜细菌的运动性，降低粘性等，使细菌从固着状态向浮游状态进行转变。而被动分散往往是指受外界诱因导致细菌生物被膜提前分散，如诱导产生胞外基质降解酶或通过物理方式直接破坏细菌生物被膜结构[89-90]。主动分散和被动分散都会导致细菌生物被膜结构发生改变，减少生物被膜量和生物被膜活菌数，然而

这种方式也存在一定局限性，分散出去的细菌较通常所知的浮游细菌具有更强的毒力和粘附性，从而可能带来更严重的细菌感染[90]。但是，分散细菌由于失去了胞外基质的保护作用，如果能通过联合抗菌药物对分散细菌进行抑杀，也将是控制生物被膜细菌感染值得探索的策略。

2.3 其他机制

一些研究报道了植物提取物或植物活性成分能够通过对细菌粘附，细菌菌毛和细菌生物被膜胞外基质相关基因造成影响，从而使细菌生物被膜形成的条件不充分，抑制了细菌生物被膜形成。如地榆的醇提液和没食子酸能对金黄色葡萄球菌的细胞间多糖黏附素 (polysaccharide intercellular adhesion, PIA) 的调控系统基因造成影响，PIA 是金黄色葡萄球菌生物被膜胞外基质的主要成分，该调控系统包含 4 个功能基因 (*icaA*、*icaB*、*icaC* 和 *icaD*) 和 1 个调节基因 (*icaR*)，调节基因 *icaR* 是一种阻遏基因，当 *icaR* 被敲除时，能够显著增加 *ica* 操纵子的表达和 PIA 生成。地榆的醇提液在 1 mg/mL 和 3 mg/mL 时，显著下调 *icaA*、*icaB*、*icaC* 和 *icaD* 基因表达，上调 *icaR* 基因表达，抑制了生物被膜的形成，而没食子酸在 2 mg/mL 时，显著上调 *icaR* 基因表达，下调了 *icaA*、*icaD* 基因表达，抑制了生物被膜的形成[24,64,91]。因此，通过对细菌生物被膜胞外基质造成影响，使其结构发生破坏，使药物更容易通过此道屏障，也是治疗细菌生物被膜感染的方法之一。

另有研究表明，通过作用于初始的细菌粘附和运动能力，可能是导致细菌生物被膜形成受阻的原因之一。Lee 等[55]使用浓度为 50 μg/mL 香豆素干预浮游状态的大肠杆菌，发现显著下调了大肠杆菌卷曲菌毛 (curli) 系统中的基因 *csgA*、*csgB* 和运动性基因 *flhD*、*motB* 的表达，抑制细菌菌毛产生，群集运动和生物被膜形成，而 curli 系统主要调控细菌粘附，使细菌更容易粘附于非生物的表面，导致生物被膜的形成[92]。另外，银杏酸在 5 μg/mL 时，有抑制大肠杆菌生物被膜的作用，而根据转录分析显示，curli 系统相关基因的表达受到了抑制[20]。木犀草素能抑制尿路致病性大肠杆菌 *fimH* 基因的表达，可能同样也会导致细菌粘附性降低，使细菌定植受阻[53]。Chen 等[60]发现盐酸小檗碱在 80 μg/mL 的浓度下能抑制粪肠球菌 *sortase A* 和 *esp* 基因的表达，这两个基因与细菌粘附有关，这可能是粪肠球菌生物被膜的早期形成受到影响的原因之一。

3 植物活性成分应用于控制细菌生物被膜感染的新策略

3.1 联合用药

由于传统抗生素难以渗透进入细菌生物被膜，且易产生耐药性、易残留等问题，单独使用难以在控制细菌生物被膜感染方面有所作用，而将低毒低残留的植物活性成分与抗生素或抗菌肽联合应用，将在减少用药量，降低毒副作用，提高药物渗透进入细菌生物被膜的能力等多方面发挥综合效应，这将为临床控制细菌生物被膜感染提供新的防治策略。Dey 等[93]发现，柚皮苷分别与环丙沙星，四环素联用，能增强两种抗生素抑制铜绿假单胞菌生物被膜感染的能力。我们课题组前期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生物被膜的作用[94-95]，将这种具有抗生物被膜作用的抗菌肽称为抗生物被膜肽[96]。但该类抗菌肽仍存在诸多问题，如高剂量呈现细胞毒性、易被蛋白酶降解以及高成本等问题[97]。通过联合用药可以在一定程度上缓

解上述问题。但是，目前联合用药针对生物被膜细菌的协同作用判定的研究还处于初级阶段，有文献报道[98]采用时间杀菌动力学研究或棋盘法来判定协同关系，但该方法费时费力，方法准确度也有待进一步考证。我们曾先采用联合用药的生物被膜活菌数比单用药物低 2 个 $\log_{10}$ CFU/well 以上作为协同作用关系的判定依据，然后通过生物被膜量的抑制率，计算 bliss 协同系数，最后筛选出所有具有协同作用的浓度中的最佳协同配伍浓度[97]，但该方法也同样需要进一步验证。随着该研究领域的不断深入，此方法有望形成生物被膜研究中联合用药协同作用关系判定的标准之一。

3.2 偶联其他物质

部分研究通过将药物与其他物质进行偶联，可以增加其抗菌能力、抗细菌生物被膜的活性和应用范围。Zhang 等[99]发现，通过共价结合将菊粉 (植物多糖) 与壳聚糖进行偶联，得到的偶联物菊粉-壳聚糖，具有抑制和清除细菌生物被膜的能力，而且对哺乳细胞具有较低的毒性。Ju 等[100]将 LK_{13} 肽 (一种阳离子抗菌肽) 与聚乙二醇 (PEG) 和壳聚糖 (CS) 进行偶联，得到 CS-PEG-LK_{13} 偶联物，该偶联物充分发挥了壳聚糖和 LK_{13} 的优势，更利于 LK_{13} 在细菌生物被膜内部进行穿梭，作用于生物被膜内部细菌，明显提高了对生物被膜内部细菌的杀伤作用。因此利用植物活性物的化学结构特点进行药物之间的偶联，尽量避免药物本身的缺陷，更好地发挥其优势作用，将有望成为防治细菌生物被膜感染的有效途径。

3.3 纳米制剂

纳米封装技术可更有效地在特定生物环境中递送生物活性化合物和药剂[101]。如前所述，目前由于大多数药物难以进入高度结构化的成熟生物被膜根部，发挥的作用有限。但随着纳米技术的应用，将植物活性成分制成纳米制剂，这有利于在细菌生物被膜的胞外多糖形成的“水道”中自由穿梭，从而使其进入细菌生物被膜根部发挥药效，如 Shariati 等[102]将姜黄素制成纳米姜黄素后，较姜黄素具有更好的抑制铜绿假单胞菌生物被膜的能力。Leung 等[101]报道了纳米颗粒封装的黄芩提取物 (Nano-SB) 与纳米氯己定 (Nano-CHX) 对常见口腔细菌生物被膜具有协同作用。研究表明[103]，纳米大黄素能抑制金黄色葡萄球菌、铜绿假单胞菌和鲍曼不动杆菌生物被膜的形成、清除其成熟生物被膜和降低其生物被膜相关的毒力因子基因的表达。但是，该技术最终应用于临床控制生物被膜感染，还需要解决诸多问题，如纳米制剂制备过程复杂、产率低、成本高、生物相容性差以及潜在的机体毒性和环境危害等。

4 总结与展望

人类使用植物及其提取物来治疗疾病有着悠久的历史，也为防治细菌生物被膜引起的相关感染提供了新的策略，但也存在诸多问题需要克服[35,104]。目前，大多数关于植物抗细菌生物被膜的研究主要集中在对细菌生物被膜形成阶段的影响，而对已经高度结构化的成熟生物被膜有效的植物还鲜有报道。另外，植物提取物在控制细菌生物被膜感染中，往往是多种成分的共同作用，难以阐释具体的作用机制，同样也存在机体安全性等诸多问题。若对单一成分进行研究，尤其当该成分在植物中含量较低时，也会增加提取成本，这将不利于其推广和应用[43,105]。另外，目前抗细菌生物被膜研究大多集中在体外试验，需要进一步开展体内药效学评价。但是，随着研究细菌生物被膜的技术和方法逐渐更新和完善，植物提取物及其活性成分的抗生物被膜研究将迎来更多突破性进展。如采用激

光共聚焦显微技术对细菌生物被膜的形态结构进行表征，生物发光技术对体内生物被膜感染的观察，以及多组学联合分析植物活性成分抗细菌生物被膜的作用机制，并寻求新的作用靶点，为进一步筛选活性成分奠定基础[106-107]。综上所述，植物提取物及其活性成分在抗细菌生物被膜方面展现了广阔的应用前景。我们相信，未来在新的研究方法和技术的推动下，具有高效、安全和稳定的抗细菌生物被膜植物药物的开发将为防治细菌生物被膜感染带来突破性的进展。

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