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细菌根际黏附的界面作用机制研究

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摘要　界面作用是细菌在环境中黏附、定殖、形成生物膜和发挥生态功能的基础和前提,对植物吸收养分、病原微生物拮抗具有重要意义。微生物-植物根际相互作用研究大多数从生态学和分子生物学角度出发,应用多种组学手段研究根系分泌物对于根际微生物定殖数量、群落组成和生理功能的影响,忽略了细菌定殖过程中物理、化学的界面作用机制及其对黏附的贡献。本文从界面作用机制出发,探讨了不同类型根系分泌物对细菌表面性质、细菌胞外聚合物(EPS)分子组成以及黏附功能的调控作用;梳理了黏附过程中细菌-植物生物大分子相互作用的主要模式及其微观机制;归纳了根际定殖过程可视化研究方法和微生物-植物生物大分子相互作用的分析手段;提出了根际生物分子组成分析、黏附蛋白质功能预测、根际定殖原位观测方法等亟需加强的研究方向。

关键词　界面作用;根际定殖;根系分泌物;胞外多糖;黏附蛋白质

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微生物与植物的相互作用在矿质养分循环、植物养分吸收及耐受生物和非生物胁迫中发挥着至关重要的作用[1-2]。微生物根际黏附是植物-微生物相互作用的前提,可促进微生物利用丰富的根际营养形成稳定的群落结构,进而发挥一系列生态功能[3]。早在20世纪50年代,陈华癸院士就指出根际微生物对于作物生产的重要作用,并探索了根际微生物定殖数量的检测方法[4-5]。近年来的研究发现,趋化、固相表面附着和形成生物膜是根际微生物定殖的3个关键步骤。根际附着是由不同类型生物分子调控的复杂界面过程,是微生物根际成功定殖的前提,同时受环境条件、根系特性、细菌表面性质的共同影响[6-7]。

根系分泌物改变根际周围养分和化学条件,通过趋化诱导、信息传递、改变细菌胞外聚合物(extracellular polymeric substances,EPS)调控微生物根际黏附。微生物在根部的黏附具有时空特异性,黏附和定殖的部位不仅受根系分泌物中养分、信号分子的调控,还受到根表特异性植物大分子的影响(图1)。与亲水表面相比,微生物倾向于附着在疏水性表面,不同的根际环境通过调控细胞表面的疏水性、菌毛和鞭毛基因表达以及细胞EPS的数量和性质影响微生物的附着[6]。同时,根毛结构和细胞壁组成、粗糙度、表面自由能、疏水性等也影响细菌在根表的定殖[8]。固相表面黏附和细胞间自团聚是微生物在根际的主要黏附和定殖途径[9]。

根际定殖是土壤有益微生物发挥作物养分功能和拮抗病原菌感染宿主的基础。相关研究表明病原菌存在与有益菌类似的根际黏附与定殖机制,包括对植物组织的可逆和不可逆附着,生物膜形成等过程,均是以菌毛和EPS等为主参与的黏附行为[10]。近些年关于微生物根际定殖的相关研究集中于利用生态学、分子生物学手段研究根系分泌物对根际微生物数量、组成和功能的影响,对于微生物定殖过程的详细物理化学机制关注较少[3,11-12]。本文通过总结近年来涉及根系分泌物、根系表面大分子与细菌界面作用的相关研究,期望在分子层面深入理解微生物根系定殖的生物化学机制;同时,通过介绍微生物根际黏附过程的研究方法和亟需解决的科学问题,推进微生物根际黏附分子机制的认识,以期为微生物资源发掘和应用提供理论参考。

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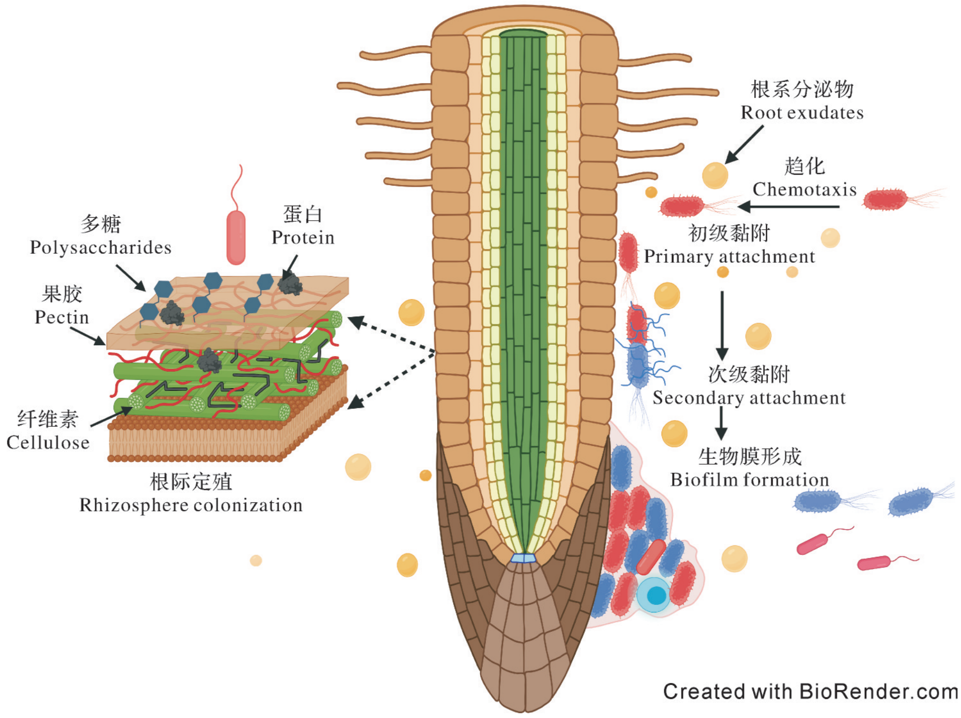

图1 细菌根际黏附和定殖过程示意图

Fig. 1 Diagram of bacterial adhesion and colonization processes in the rhizosphere

1 根系分泌物调控细菌根际黏附与定殖

根系分泌物种类复杂，主要以参与细菌生长代谢、维持细菌基本生命活动的能源物质，如糖类、有机酸类、脂类、氨基酸类等小分子物质为主。根系还会释放黏液物质，主要包括果胶、纤维素、半纤维素和阿拉伯半乳聚糖蛋白、胞外DNA、组蛋白等生物大分子[13]。根系分泌物也包含黄酮类、类黄酮、萜烯类、苯并恶嗪和植物激素等数量较少，但是具有化感、抵抗病原菌等特异功能的次生代谢物[14]。这些不同类型的分泌物对细菌的影响各不相同，可通过调控细菌繁殖、信号传导、表面性质进而影响细菌在根际的黏附与定殖(表1)。例如，根系分泌物通过调控胞外多糖基因表达、促进生物膜形成，改变鞭毛基因表达等影响细菌发生不可逆黏附[15-16]。

葡萄糖浓度的增加可导致 *Bacillus thuringiensis* KPWP1的生物膜表面蛋白质、多糖等物质含量增加，使得其疏水性与黏附性增强[17]。拟南芥根系分泌的蔗糖则通过触发信号级联反应，刺激产生大量的表面蛋白质与超级鞭毛结构，增强固体表面能动性，从而促进细胞在根际的定殖[18]。*L*-苹果酸则可以诱导生物膜形成所需关键操纵子 *yqxM* 的表达，促进 *B. subtilis* FB17 生物膜的形成[19]。此外，Zhang 等[20]研究表明柠檬酸和富马酸促进了 *B. velezensis* SQR9 和 *B. subtilis* N11 生物膜形成，但其分子机制有待深入研究。

与小分子代谢物相比，根系分泌的黏液不仅可以为微生物提供碳源，也可以改变根际的疏水性、渗透性和保水性能，直接影响微生物栖息地的表面性质[21]。例如，玉米气生根黏液中具有独特结构的多糖可通过提供碳源和维持适宜的环境，特异性选择固氮细菌的定殖[22]。作为根际信号分子，芹菜素和芹菜素-O-葡萄糖苷可促进固氮细菌 *Gluconacetobacter diazotrophicus* 形成生物膜，进而增强根际定殖和固氮功能[23]。木麻黄分泌的酚类与类黄酮物质会引起放线菌 *Frankia* sp. strain CcI3 的菌丝卷曲，细菌表面的脂蛋白含量减少，进而促进细菌的黏附[24]。

2 根表生物大分子参与细菌黏附

除了受到小分子根系分泌物的影响，细菌在根际黏附还受到根系表面功能大分子的影响(表1)。根表生物大分子指存在于植物根系表面、溶解扩散能力较低、可直接参与微生物黏附过程的植物大分子物质。植物细胞壁主要由纤维素、半纤维素、果胶等多糖，蛋白质、木质素和脂质等物质组成。细菌通过鞭毛、菌毛、固着器等附属结构与根表细胞接触，

表1　根系分泌物和根系表面功能大分子调控细菌定殖的分子机制

Table 1　Molecular mechanisms of root exudates and surface macromolecules in regulating bacterial colonization

作物 Crop	主要分泌物 Exudates	菌株 Strains	过程机制 Process mechanism	参考文献 References
番茄 Tomato	*L*-苹果酸 *L*-Malic acid	*B. subtilis*	促进生物膜形成 Promote biofilm formation	[25]
黄瓜 Cucumber	精胺与鸟苷 Spermine and guanosine	*B. velezensis* SQR9	触发Spo0A蛋白磷酸化，激活生物膜形成 Triggering Spo0A protein phosphorylation and activating biofilm formatio	[26]
玉米 Maize	葡萄糖、苹果酸、丙二酸 Glucose, malic acid and malonic acid	*P. putida* KT2440	趋化作用，促进生物膜形成 Chemotaxis and promote biofilm formation	[27]
玉米 Maize	有机酸与氨基酸 Organic acids and amino acids	*B. velezensis* S3-1	趋化性和集群运动能力，促进成膜 Chemotaxis and swarming motility promote film formation	[28]
番茄 Tomato	果胶 Pectin	*B. velezensis* SQR9	增加表面活性素、促进固体表面运动 Increase surfactant and induce solid surface motility	[18]
番茄 Tomato	半乳糖醛酸聚糖 Galacturonan	*B. velezensis*	促进*epsB*和*tasA*基因表达 Promote *epsB* and *tasA* gene expression	[29]
拟南芥 Arabidopsis	纤维二糖、果胶 Cellobiose, pectin	*B. subtilis* 3610	促进EPS团聚 Promote EPS aggregation	[30]

然后通过分泌黏附素、胞外多糖、黏附蛋白等进一步发生不可逆黏附[31]。前期研究发现*B. velezensis*在番茄根际形成生物膜依赖于植物多糖的刺激，这些植物多糖既作为信号触发生物膜基因表达，又作为碳源用于生物膜基质的合成[29]。果胶作为一种复合多糖，主要由半乳糖醛酸、支链阿拉伯糖和1,4-β-半乳糖组成，占细胞壁的40%。*B. velezensis*可通过感知果胶来刺激表面活性素的生成[32]。表面活性素能改变细菌疏水性、电荷性质，在与植物根系相互作用的最初几个小时内，表面活性素被认为是细菌调控其黏附行为的主要途径[33-34]。果胶可诱导*B. amyloliquefaciens*产生强烈的趋化反应，同时促进表面活性素和生物膜形成相关基因*eps*和*tasA*的表达，进而促进生物膜的形成[35]。此外，*B. subtilis*定殖时可以用根系表面葡聚糖作为碳源合成EPS，也可以通过控制主调控子Spo0A磷酸化状态的激酶，激活EPS基因的转录来促进生物膜形成[30]。

在不同类型生物大分子表面，几丁质和纤维素最容易被细菌定殖，其次是木质素和木质纤维素，而角蛋白相对不易定殖[36]。*Ruminococcus albus*通过产生纤维小体和纤维素结合蛋白黏附在纤维素表面[37]。除了植物多糖，根系表面蛋白质也参与了微生物的黏附与定殖。阿拉伯半乳聚糖蛋白(arabinogalactan-proteins, AGPs)是广泛存在于各种植物细胞和分泌物中的植物大分子，在根和细菌识别和定殖过程中起着关键作用[38]。Gaspar等[39]选择性去除或减少AGPs含量，导致*Agrobacterium tumefaciens*黏附效率的降低，表明AGPs参与了细菌的初始黏附。植物大分子的养分供应和诱导作用对细菌的根际定殖具有重要贡献，根系表面生物大分子-细菌互作的分子机制有待深入挖掘。

3　细菌根际黏附的功能蛋白质

细菌根际附着包括可逆的初级黏附和不可逆的特异性结合阶段[3,11]。在不可逆黏附过程中，黏附功能蛋白质起到关键作用。通过比较黏附素基因缺失的突变菌株，发现细胞壁水解酶LytB、鞭毛蛋白质FliD是调控*B. velezensis* SQR9在根系表面黏附的关键生物分子，而只有鞭毛蛋白质FliD影响了细菌在玻璃和聚苯乙烯等非生物表面的黏附，表明细菌特异性黏附可通过不同生物分子识别不同的固相表面[40]。作为*B. subtilis*生物膜主要的蛋白质组分，淀粉样蛋白质TasA可通过增强细菌聚集、EPS结构稳定性从而形成稳定的生物膜三维结构[41]。qPCR结果显示，缺失EPS和TasA的*B. subtilis*在番茄根系的定殖能力下降[42]。此外，*B. subtilis*也可以通过胞外多糖和TasA黏附蛋白二者协同在黑曲霉表面附着并形成生物膜[43]。*B. subtilis* EPS中与保水性有关的高分子聚合物γ-聚谷氨酸(γ-PGA)，通过形成稳定的三维聚合结构支持了生物膜的机械稳定性，也可以直接介导细胞在固相表面的黏附。

黏附蛋白具有丰富的官能团和结构域，通过静电相互作用、范德华力、疏水作用等机制参与根际黏附。这种特异性黏附行为在很大程度上取决于蛋白

质分子链的构象、取代基和环境条件[44]。在胞内信号的作用下,跨膜黏附蛋白构象发生变化,能更有效地与细胞外基质中的配体结合,随后通过增加黏附蛋白分子的密度进一步增强黏附。整合素可以通过与二价阳离子结合,促使跨膜黏附蛋白与配体形成更强的键桥结构,提高黏附的稳定性[45]。细菌黏附过程是一个复杂的多步骤过程,涉及不同类型的黏附蛋白及其与根际固相表面的相互作用,解析这些界面反应机制对于理解细菌在根际环境中的定殖和生存具有重要意义。

表2 芽孢杆菌参与界面黏附的生物分子及其作用机制

Table 2 The biomolecules and mechanisms for the adhesion of *Bacillus*

菌株 Strains	黏附分子 Adhesion molecules	黏附机制 Adhesion mechanism	参考文献 References
B. subtilis	*ydaJ-N*操纵子调控的胞外多糖 Exopolysaccharides regulated by the *ydaJ-N* operon	增加EPS,促进团聚 Increase EPS and promote agglomeration	[46]
B. subtilis	TasA,支撑蛋白TapA TasA, supporting protein TapA	TapA促进TasA形成丝状体,促进生物膜结构稳定 TapA promotes TasA to form filaments and stability of biofilm structure	[41]
B. subtilis	胞外基质蛋白TasA Extracellular matrix protein TasA	诱导杆菌烯,抑制表面活性剂 Induces bacillaene and inhibits surfactin	[47]
B. amyloliquefaciens FZB42	ClpA-D	影响疏水性 Affects hydrophobicity	[48]
B. velezensis SQR9	细胞壁磷壁酸 Teichoic acids	表面活性剂 Surfactin	[49]
B. velezensis SQR9	肽聚糖水解酶LytB、鞭毛蛋白FliD Peptidoglycan hydrolase LytB, flagellin FliD	蛋白质参与多种黏附作用 Proteins involved in a variety of adhesion	[40]

4 细菌根际黏附界面过程和机制研究方法

随着技术的不断进步,越来越多的方法用来可视化细菌的黏附定殖过程(图2)。扫描电子显微镜和冷冻电子显微镜可以提供细菌黏附原子分子层面的信息,观察发现葡萄糖浓度的增加使*B. thuringiensis* KPWP1产生了更多的EPS,同时促进细胞之间的紧密团聚[17]。微流控技术结合激光扫描共聚焦显微镜(confocal laser scanning microscopy,CLSM)和绿色荧光蛋白(green fluorescent protein,GFP)标记可以实现细菌的原位实时观测,直接获取*B. subtilis*在拟南芥根系表面定殖的数量和位置[50]。微流控装置具备透光、内部结构可调的优势,为根系生长和原位观察根际微生物定殖过程提供了条件,通过独立的腔室设计,可以同时监测多个腔室中根系表面微生物定殖的动态过程。此外,全内反射荧光显微镜也被用于研究细菌定殖的微观过程,观察发现链球菌*Streptococcus salivarius* HB7在疏水性表面上的垂直运动振幅减小,更有利于其黏附[51]。

更多界面相关研究方法从作用能、化学键、分子构型等微观层面解析细菌的根际黏附机制。耗散型石英晶体微天平(quartz crystal microbalance,QCM-D)通过监测*B. subtilis*扩展蛋白BsEXLX1在木质素上的实时吸附-解吸,发现BsEXLX1可以破坏纤维素与葡聚糖之间的氢键,从而更好地与纤维素结合[52]。等温滴定量热(isothermal titration calorimetry,ITC)和表面等离振子共振(surface plasmon resonance,SPR)可以评估生物分子之间作用的能量特征,研究显示主链中疏水性和芳香族部分含量较高的糖类大分子与凝集素伴刀豆球蛋白A具有较高的亲和力[53]。原子力显微镜(atomic force microscope,AFM)可以用来解析生物分子结构,并直接探测生物分子间的作用力,利用单细胞力谱发现*Staphylococcus aureus*通过表面纤连蛋白结合蛋白A引起细胞团聚,从而促进黏附[54]。分子动力学(molecular dynamics,MD)模拟可提供相互作用的分子构型信息,结合电镜观察,展示了细菌Tas A蛋白如何从单体组装成富含β折叠的纤维,以及这些纤维如何在生物膜中组装成束[55]。这些方法极大拓展了我们对细菌界面黏附分子机制的认识。

5 问题和展望

虽然越来越多的研究开始关注细菌EPS、植物根系分泌物的组成,但关于其分子组成、结构和黏附功能作用之间的关系还了解较少。识别微生物定殖

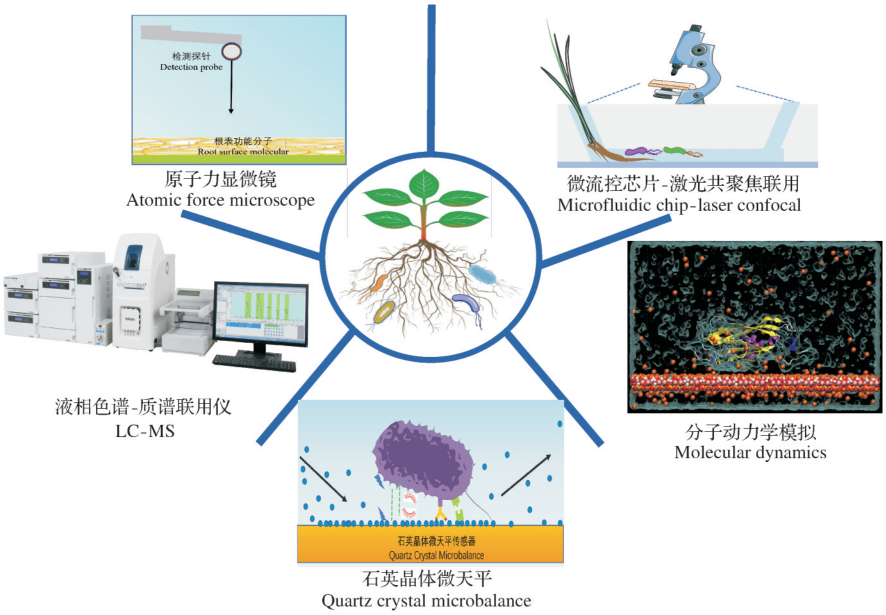

图2　细菌根际黏附过程及机制研究方法

Fig. 2　Research methods of bacterial rhizosphere adhesion mechanisms

的功能分子，解析其结构组成及根际定殖的分子机制，对于功能微生物基因资源的挖掘利用、生物有机肥的创制有重要意义。受限于对细菌功能分子的认识不足和根际环境的复杂性，目前关于根际定殖的相关研究以分子生物学和生物信息学手段为主，界面作用机制研究较少。针对上述问题，应加强以下几方面研究：

1)加强细菌EPS和植物分泌物中多糖分子组成的研究。由于多糖分子在单糖组成、糖苷键连接、分支和聚合度上的高度多样性，导致细菌胞外多糖纯化、分离、定量和鉴定过程十分复杂[22]。同时，目前多糖物质的结构分辨率不高，结构识别与鉴定相比蛋白质研究更加迟缓，对其三维结构组成的鉴定充满了挑战。通过采用液相色谱-质谱联用技术(LC-MS)逻辑衍生序列串联质谱法(LODES/MSn)、化学解聚、寡糖测序和糖苷键定量相结合的新策略，逐步实现了对玉米、地衣素多糖组成和一级结构的表征[22, 56]。方法学上的发展与应用增强了我们对多糖结构及其功能之间的理解，为进一步阐释多糖分子参与的细菌根际定殖机制打下了基础。

2)深入阐明EPS中黏附功能蛋白质的作用机制。EPS蛋白质组分发挥着胞外酶和结构蛋白的功能[57]。目前有关蛋白质结构的研究方法主要以X射线、核磁共振、冷冻电子显微镜为主[58]，计算方法可以利用Jpred4和AlphaFold预测其二级和三级结构，并与蛋白数据库和蛋白结构域数据库对比预测其功能[59]。此外，分子动力学(MD)模拟能够在原子水平下解析蛋白质分子与根系不同类型植物分子的动态作用机制，为微生物根际定殖提供新思路。

3)建立细菌根系定殖的原位研究新方法。微流控芯片是细菌根系定殖原位研究的重要观察装置，已应用于拟南芥和水稻等根系细菌定殖过程的研究，结合激光共聚焦CLSM可实时探测微生物定殖的数量和空间结构。未来亟需建立普适的微流控观测平台，实时观察和分析细菌在不同植物根系表面的定殖和相互作用。同时，结合根际代谢物实时采样和流动分析系统，有望实现对根际微生物黏附及其与植物相互作用更加深入的理解。

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Mechanisms of interfacial interactions for bacterial adhesion in rhizosphere

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Abstract Interfacial reaction is the foundation and prerequisite for bacteria to adhere, colonize, form biofilms, and perform ecological functions in the environment. It is of great significance for plants to absorb nutrients and antagonize pathogenic microorganisms. Most studies on the microbe-plant rhizosphere interactions are conducted from the perspectives of ecology and molecular biology, using multi-omics methods to study the effects of root exudates on the quantity of colonization, the composition of community, and the physiological functions of rhizosphere microorganisms. However, the physical and chemical interfacial mechanisms involved in bacterial colonization and their contributions to adhesion have been ignored. This article reviewed the regulatory effects of different types of root exudates on the properties of bacterial surface, the molecular composition of extracellular polymeric substances (EPS), and the function of adhesion in term of the mechanisms of interfacial interactions. The main modes and micro mechanisms of the interaction between bacteria and plant bio-macromolecules during the process of adhesion were summarized. The visualization methods for studying the process of rhizosphere colonization and methods for analyzing the interaction between microorganisms and plant bio-macromolecules were discussed. The directions of studies that urgently need to be strengthened including analyzing the biomolecular composition of rhizosphere, predicting the function of adhesion protein, and the *in situ* methods for observing the colonization of rhizosphere were proposed.

Keywords interfacial reaction; rhizosphere colonization; root exudates; extracellular polysaccharides; adhesion proteins

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