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红球菌的组学、遗传改造及其应用研究进展

吴金芳¹ 张雨潇² 彭仁¹✉¹江西师范大学生命科学学院 南昌 330022²北京工业大学生命科学与生物工程学院 北京 100124

摘要 红球菌是一类专性好氧的革兰氏阳性菌, 其种类多样、分布广泛。红球菌因其具有多种水解酶、合成酶以及能够降解许多异源化学物质而备受关注。综述红球菌的组学研究、遗传改造以及在生产实践中的应用。人们利用基因组学、转录组学、蛋白质组学和代谢组学等各种组学手段解析了红球菌能够降解污染物质、产生活性物质、积累脂质以及适应胁迫环境的分子机制, 并寻找出相应的基因, 同时对一些有应用前景的菌株进行了各种方式的遗传改造以提高它们的利用价值。由于红球菌在代谢方面具有多能性, 因此它们在环境治理、生物合成与转化以及医药、生物能源等领域都具有广泛的应用价值。在今后红球菌的研究中, 需要不断地引入各种组学新技术来推进红球菌的深入研究, 整合基因组学、转录组学、蛋白质组学和代谢组学数据来挖掘新的基因, 开发基因组编辑等新的遗传工具箱以及解析其响应环境刺激的信号转导通路。(表3 参86)

关键词 红球菌属; 分类; 组学; 遗传改造; 生产应用

Research advance in omics, genetic modification, and application of *Rhodococcus*

WU Jinfang¹, ZHANG Yuxiao² & PENG Ren¹✉¹ College of Life Science, Jiangxi Normal University, Nanchang 330022, China² College of Life Science and Bioengineering, Beijing University of Technology, Beijing 100124, China

Abstract *Rhodococcus* is a group of obligate, aerobic, gram-positive bacteria that has a wide variety of species and is widely distributed. *Rhodococcus* species are frequently studied because they possess multiple hydrolases and synthases and can degrade many xenobiotics. This paper reviews omics research, genetic modification, and practical applications of the genus *Rhodococcus*. The molecular mechanisms of pollutant degradation, active compound production, lipid accumulation, and adaptation to environmental stress are interpreted using genomics, transcriptomics, proteomics, and metabolomics, and the corresponding genes are analyzed. At the same time, some strains have been genetically modified to increase their application value. *Rhodococcus* has various applications in the fields of environmental management, biotransformation, medicine, and biofuel because of its versatility in metabolism. In future research related to *Rhodococcus*, new omics technologies are required to advance in-depth research on *Rhodococcus*. Genomics, transcriptomics, proteomics, and metabolomics data have been integrated to identify new genes. Novel genetic tool kits such as genome editing have been developed, and signal transduction pathways in response to environmental stimuli have been analyzed.

Keywords *Rhodococcus*; classification; omics; genetic modification; application

红球菌最先由Zoof在1891年建立, 并由Tsukamura再次引入^[1]。Goodfellow和Alderson在1992年对其进行修正, 并将其重新定义为红球菌复合体^[2]。红球菌属隶属于放线菌门、诺卡菌科, 一般来说, 红球菌属可以划分4个或6个分支^[3]。

红球菌为专性好氧的革兰氏阳性菌, GC含量高, 运动性不强, 部分菌株耐酸并含多种水解酶^[4]。该属具有独特的被膜, 膜上含有大型支链脂质, 细胞壁中含有长度为34-64个碳

原子的分支菌酸盐。与普通的细菌不同的是红球菌一般没有鞭毛结构, 但少数菌株存在菌毛^[5]。菌株分布于各种不同的环境, 主要集中在土壤、水和海洋环境中^[5]。近年来, 随着相关研究的不断深入, 人们从极地和高山地区在内的多种生境中也分离到了该菌。由于能够降解多种天然和人工合成的有机化合物, 红球菌在系统发育和分解代谢方面具有多样性^[6]。

迄今为止, 关于红球菌的组学研究、遗传改造和应用已经

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✉通信作者 Corresponding author (E-mail: renpeng@jxnu.edu.cn)

取得了一些成果,但在分子信号转导机制方面仍缺乏系统、深入的研究.本文主要总结了近10年国内外对红球菌的组学研究、遗传改造及其在各方面的应用状况,并对未来的相关研究进行了展望.

1 红球菌的组学研究和遗传改造

随着组学技术的迅速发展,当人们认识到红球菌在生物降解、生物转化、生物合成等方面具有重要应用价值后,就开始从基因组、转录组、蛋白质组和代谢组学等不同层次着手,分析红球菌具有独特生理生化特征的原因,挖掘出相应的基因,为菌株的遗传改造奠定基础.表1概述了一些红球菌的组学研究情况^[7-21].其中Zhao等人对一株能够进行邻苯二甲酸酯矿化的*Rhodococcus* sp. 2G基因组进行了测序^[7].他们发现337个酯酶、水解酶基因涉及了邻苯二甲酸酯水解成邻苯二甲酸的过程,并且37个转移酶和脱羧酶基因参与了邻苯二甲酸脱羧形成苯甲酸.此外,一个基因族(*xyl*)负责将苯甲酸转变为儿茶酚,而两个儿茶酚分解代谢基因族分别控制儿茶酚的邻位和间位裂解途径.为了揭示*Rhodococcus* sp. CNS16在低温下调控苯胺降解的机制,Wang等采用转录组学方法研究了不同生长温度下*Rhodococcus* sp. CNS16中一些基因的表达水平差异情况^[17].研究表明在低温条件下,去饱和酶上调,这将促进不饱和脂肪酸的合成,并增强该菌株在低温下的适

应性.此外,在低温胁迫下,与能量代谢相关的基因也发生上调.这些存在显著变化的基因能够作为低温条件下调控污染物降解的分子靶标.本课题组筛选到一株耐受多种有机溶剂的*R. ruber* SD3.为了揭示*R. ruber* SD3有机溶剂耐受性的机理,我们采用定量蛋白质组学的方法研究了*R. ruber* SD3分别在甲苯和苯酚胁迫下各种蛋白的表达差异.结果表明*R. ruber* SD3在甲苯和苯酚胁迫下分别存在362个和488个表达显著差异的蛋白,这些蛋白质涉及细胞膜上的转运、生物降解途径和双组分系统^[20].*R. opacus* PD630能够有效累积脂质,代谢组学的研究表明*R. opacus* PD630在正丁醇胁迫下,会出现短肽含量降低、细胞膜磷脂的不饱和度和丙酰CoA含量提高的现象^[21].从红球菌的组学研究来看,基因组学的报道居多.然而解析红球菌独特的生理生化特性,需要整合多组学数据,这样才能全面阐述这些特征存在的分子机制.

对于通过组学研究发现的潜在分子靶标,人们就可以利用各种遗传改造的方式改善菌株的特定功能.在近20年的研究中,基因突变、基因敲除、基因增强以及转基因等各种遗传改造方式在菌种改良、提高菌株生产效率上发挥了很大的作用.在红球菌的遗传改造中,质粒的构建是至关重要.表2概括了红球菌的遗传改造中常用的各种质粒^[22-36].迄今为止,人们根据红球菌的特性进行了有针对性的遗传改造.在木质素的利用方面,*R. opacus* PD630具有较快的生长速度、产脂质多、能耐受和利用多种木质素衍生的芳香族化合物等优势

表1 红球菌的组学研究

Table 1 Omics research of *Rhodococcus*

菌株 Strain	独特生理生化特性 Unique physiological and biochemical characteristics	组学手段 Omics	参考文献 References
<i>Rhodococcus</i> sp. 2G	邻苯二甲酸酯的矿化 Phthalate acid ester-mineralising	基因组学 Genomics	[7]
<i>R. erythropolis</i> B7g	表面活性剂海藻糖四酯的产生 Production of trehalose-containing surfactants	基因组学 Genomics	[8]
<i>Rhodococcus</i> sp. HS-D2	邻苯二甲酸正丁基苯酯的降解 Degradation of the <i>n</i> -butyl benzyl phthalate	基因组学 Genomics	[9]
<i>R. rhodochrous</i> B-276	丙烯的降解 Degradation of propene	基因组学 Genomics	[10]
<i>Rhodococcus</i> sp. H-CA8f	抑菌物质的产生 Production of antimicrobial compounds	基因组学 Genomics	[11]
<i>Rhodococcus</i> sp. Eu-32	噻吩类化合物的脱硫 Dibenzo thiophene desulfurization	基因组学 Genomics	[12]
<i>Rhodococcus</i> sp. JG-3	冷适应 Cold adaptation	基因组学 Genomics	[13]
<i>R. equi</i> B0269	病原体 Pathogen	基因组学 Genomics	[14]
<i>Rhodococcus</i> sp. NJ-530	二甲基巯基丙酸的降解 Degradation of dimethylsulfoniopropionate	基因组学 Genomics	[15]
<i>R. ruber</i> TH3	丙烯酰胺的产生 Production of acrylamide	转录组学 Transcriptomics	[16]
<i>Rhodococcus</i> sp. CNS16	苯胺的降解 Degradation of aniline	转录组学 Transcriptomics	[17]
<i>R. opacus</i> PD630	脂质的累积 Accumulation of lipids	转录组学 Transcriptomics	[18]
<i>Rhodococcus</i> sp. BAP-1	荧蒽的降解 Degradation of fluoranthene	蛋白质组学 Proteomics	[19]
<i>R. ruber</i> SD3	苯酚等有机溶剂的降解 Degradation of phenol and other organic solvents	蛋白质组学 Proteomics	[20]
<i>R. opacus</i> PD630	脂质的累积 Accumulation of lipids	代谢组学 Metabonomics	[21]

表2 红球菌遗传改造的常用质粒

Table 2 Plasmids commonly used in genetic modification of *Rhodococcus*

菌株 Strain	质粒 Plasmid	质粒类型 Plasmid type	参考文献 References
<i>R. opacus</i> PD630	pAL5000, pNG2, pB264	穿梭质粒 Shuttle plasmid	[22-23]
<i>R. opacus</i> PD630	pNC9503, pNC9501		[24]
<i>R. erythropolis</i> CCM2595	pSRK1, pSRK21, pSRK211, pSRKgfp		[25]
<i>R. erythropolis</i> CCM2595	pFAJ2574		[26]
<i>R. erythropolis</i> MAK154	pRET1102, PRET1202, PRET1123		[27]
<i>R. rhodnii</i>	pBP5	自杀质粒 Suicide plasmid	[28]
<i>R. ruber</i> TH	pNV18.1		[29]
<i>R. ruber</i> TH	pPHU281		[30]
<i>Rhodococcus</i> sp. I24	pRhodoCOS, pR4		[31]
<i>R. erythropolis</i> MAK154	PRET1104, PRET1132, PRET1135, PRET1138, PRET1172, PRET1119	表达质粒 Expression plasmid	[32]
<i>R. erythropolis</i>	PRET11100		[33]
<i>R. ruber</i> TH	pPHA, pNVA		[34]
<i>R. jostii</i> RHA1	pTACHis18		[35]
<i>R. erythropolis</i>	pTip-QC1, pNit-QC1		[36]

而受到人们的重视。Henson等人结合适应性进化和多组学的方法研究了*R. opacus*对木质素模型化合物的耐受性。他们证明了*R. opacus* PD630可以通过适应性进化来提高芳香族化合物的消耗量,并且在适应菌株中鉴定出可能影响芳香族化合物耐受和消耗的共同突变位点。他们还发现在混合的木质素模型化合物中,适应性菌株PVHG6比野生菌株有更高的芳香族化合物的消耗速率,并鉴定出针对5种木质素模型化合物的不同漏斗途径以及2个芳香族副产物的去毒途径^[37]。为了进一步*R. opacus* PD630的利用价值,Delorenzo等人*R. opacus* PD630中开发了一个用于基因表达调控和基因组修饰的分子工具箱。他们构建了一个启动子文库来促进异源基因的表达,并利用定量PCR技术测定了4个外源质粒和9个内源质粒的拷贝数。通过优化两种抗生素抗性标记来拓宽选择标记的数目。此外,Delorenzo等人还设计了一个基因组修饰系统,并根据转录组数据、基因组数据和生长速率情况确定了3个中性整合位点,使得基因盒能够整合到这些位点上,同时又不影响菌株的生长。最后,他们利用CRISPR干扰方法在红球菌中开发了首个靶向并且可调控的基因抑制系统^[22]。Vesel等人选择能降解苯酚的*R. erythropolis* CCM2595菌株进行了遗传学研究。他们成功地构建了pSRK21质粒,并将该质粒应用于*R. erythropolis* CCM2595的遗传学操作^[25]。红球菌I24能通过至少两个双加氧酶系统和一个单加氧酶系统的作用将萜烯转化为吡啶醇。Treadway等将红球菌I24中的一些基因通过柯斯质粒移至*R. erythropolis* SQ1中,使得*R. erythropolis* SQ1也具备生产吡啶醇的能力^[31]。Yamamura通过对红球菌中的MAK154菌株进行遗传改良,利用穿梭质粒PRET1102作为表达载体转入至宿主红球菌中,极大地增加了氨基醇脱氢酶(AADH)的表达量,从而提高了全细胞的生物转化效率^[32]。为了提高丙烯酰胺的产量,清华大学化工系通过多种方式对*R. ruber*进行遗传改造。Ma等人敲除了*R. ruber* TH中主要副产物丙烯酸的酰胺酶基因,因此副产物的产生量减少了87%^[34]。他们还采用全局转录工程的方法构建了含有sigA突变体的*R. ruber* TH3/pNV-sigA^M文库,随后在丙烯腈/丙烯酰胺的胁迫条件下筛选到突变株*R. ruber* TH3/ M4N1-59^[38]。此外,他们在*R. ruber* TH3中将*Escherichia coli*的分子伴侣GroEL-ES过表达,然后利用遗传改造后的菌株转化合成丙烯酰胺。新菌株和*R. ruber* TH3相比,生成的丙烯酰胺的浓度由原来的49%提高到64%^[39]。总的来说,尽管与其他模式微生物相比,红球菌的遗传工具为数不多,但是随着红球菌遗传背景的深入研究,将会开发出更多有效的手段来进行红球菌的遗传改造。

2 红球菌的应用研究

红球菌具有广泛的代谢功能,它能够转化脂肪族、芳香烃、含氧化合物、卤代化合物、硝基芳烃、杂环化合物、腈类和各种农药,在环境治理、生物合成与转化以及生物医药、食品、能源领域都具有潜在的应用价值^[40-86](表3)。

2.1 环境治理

造成环境污染的化合物包括有机化合物和无机化合物。从数量上看,人们更加关切各种碳氢化合物及其衍生物造成的污染问题。人为来源特别是石油产品的泄漏、工业废水的排放和运输事故,向环境释放了大量的碳氢化合物。此外,腐烂的植物材料(如木质素)、土壤风化过程和火山喷发产生了一些芳香族化合物。由于这些化合物的分子结构多样,因此降解

难度大。

红球菌中酶的种类和含量比较丰富,表现出对短链烷烃、长链烷烃、卤代芳香族化合物和硝基取代芳香族化合物、杂环化合物和多环化合物具有良好降解能力,并且由于它们的底物适应性强和有机溶剂耐受性强^[6],因而在环境治理中具有良好的应用前景。红球菌的细胞壁中有黏菌酸的脂族链,表面疏水性强,该特性可使细胞黏附在油相和水相之间,从而更容易降解疏水性污染物。当把红球菌放在含水的无机盐培养基和液态碳氢化合物为碳源的培养基中培养时,细胞明显地处于油相和水相之间。同时,红球菌还能通过产生表面活性剂和改变细胞表面组成结构来提高在疏水环境中的适应能力,因此红球菌在石油污染的生物修复领域有着极其重要的应用。Florianio等在生化、遗传和调控等不同层次阐述了*Rhodococcus* sp. strain TFB中四氢化萘的分解代谢情况^[52];Chen等发现*Rhodococcus* sp. LH可以通过氧化反应降解柴油,并对芳香族化合物具有一定的降解能力^[53]。此外,Shintani等发现红球菌中的嗜冷菌在10-15℃时对燃油的降解效率也能达到80%以上^[54],这对寒冷气候条件下的生物修复具有重要的意义。除此之外,红球菌还能分解邻苯二甲酸酯和异戊二烯等有机物,因此对水污染和大气污染治理也能起到显著效果^[1,60]。

硫的释放是造成环境污染的另一个主要来源,因此脱硫是治理环境的一个重要技术。1989年Kilbane提出了一种对含硫烃进行专一性脱硫,并保持含杂环硫化化合物的芳香结构不变的“4S”途径,该途径被认为是最有工业前景的脱硫途径之一^[66]。迄今为止,国内外学者对红球菌等微生物的脱硫作用进行了深入的研究。Gray等在1996年从*Rhodococcus* sp. strain IGTS8中分离出参与二苯并噻吩(DBT)转化为2-羟基联苯(HBP)的DszA、DszB、DszC以及NADH-FMN酶^[67]。Yu等分离到一株红球菌*R. erythropolis* XP,能够有效地用于苯并蒽并噻吩的脱硫^[68]。

2.2 生物转化

在工业生产上,红球菌是一类非常具有商业价值的微生物,许多菌株被用于大型的生物催化工艺中,其中丙烯酰胺的合成就是范例^[69],在世界各地的多个生产基地采用红球菌催化制备丙烯酰胺,并且已经达到了数吨的规模。在丙烯酰胺合成中,腈水合酶是一个关键酶,它可以催化脂肪族腈类或芳香族腈类向相应的酰胺类物质转化。

除此之外,由于红球菌属中的酶种类丰富,代谢途径复杂多样,人们在探索中发现了红球菌的其他转化潜能。例如Yamamura构建了红球菌表达质粒,并在*R. erythropolis*中表达NADP⁺依赖性的氨基醇脱氢酶,制备双手性氨基醇^[32];祝玉洪等用不透明红球菌DSM 43250转化合成 α -酮异己酸,经过培养条件优化后, α -酮异己酸的产量达到30.97mg/L^[71]。Li等从土壤中筛选获得一株红球菌*Rhodococcus* sp. ECU0066,它能够高对映选择性地使苯甲硫醚氧化成(S)-亚砷,并且对映体过量值达到99%^[72]。

2.3 其他应用

红球菌中存在能有效降解芳香型真菌毒素的菌株,因此它在真菌毒素生物解毒过程具有很大应用潜力。目前,人们对比较常见的黄曲霉毒素的降解进行了一定的研究。Risa等人调查了42种红球菌对黄曲霉毒素的降解活性,发现其中18种红球菌对黄曲霉的降解率达到了90%以上^[73],该研究为红球菌在毒素降解中的运用建立了理论依据。

表3 红球菌属应用概况

Table 3 Application of *Rhodococcus*

应用方向 Application direction	参与菌株 Participating strain	主要作用 Main effect	参考文献 References
环境治理 Environmental management	<i>R. corynebacteroides</i>	降解2-乙基己酸丁酯 Biodegradation of butyl 2-ethylhexanoate	[40]
	<i>R. erythropolis</i> D310-1	降解氯噻磺隆 Biodegradation of chlorimuron-ethyl	[41]
	<i>R. globerulus</i> P6	降解多氯联苯 Biodegradation of polychlorinated biphenyls	[42]
	<i>R. koreensis</i>	降解2,4-二硝基酚 Biodegradation of 2,4-dinitrophenol	[43]
	<i>R. maanshanensis</i> DSM 44675	降解吩嗪-1-羧酸 Biodegradation of phenazine-1-carboxylic acid	[44]
	<i>R. opacus</i> R7	降解多环芳香化合物 Biodegradation of polycyclic aromatic compounds	[45-48]
	<i>R. percolatus</i>	降解2,4,6-三氯苯酚 Biodegradation of 2,4,6-trichlorophenol	[49]
	<i>R. pyridinivorans</i> GF3	降解蒽醌化合物 Biodegradation of anthraquinone compounds	[50]
	<i>R. rhodochrous</i> PY11	降解2-羟基吡啶 Biodegradation of 2-hydroxypyridine	[51]
	<i>Rhodococcus</i> sp. TFB	降解四氢化萘 Biodegradation of tetralin	[52]
	<i>Rhodococcus</i> sp. LH	降解柴油、芳香烃 Biodegradation of diesel oil and aromatic compounds	[53]
	<i>R. erythropolis</i> A/C	低温高效降解石油 Oil-degrading under low temperature condition	[54]
	<i>R. ruber</i> DP-2	降解邻苯二甲酸酯和苯酚 Degradation of di- <i>n</i> -butyl phthalate and phenol	[55]
	<i>Rhodococcus</i> sp.Y2-2	低温降解油料 Consuming kerosene, gasoline, and diesel at low temperature	[56]
	<i>R. erythropolis</i> ATCC 4277	石油脱硫、脱氮 Desulfurization and denitrogenation of heavy gas oil	[57]
	<i>R. erythropolis</i> PR4	高效降解柴油、烃类化合物 Degrade diesel oil, normal-, iso- and cycloparaffins and aromatic compounds	[58]
	<i>R. erythropolis</i> M-25	原油降解 Degradation of crude oil	[59]
	<i>Rhodococcus</i> sp. 2G	减轻邻苯二甲酸二异辛酯累积 Mitigate di (2-ethylhexyl) phthalate contamination	[60]
	<i>R. erythropolis</i> D310-1	降解磺酰类除草剂 Degradation of sulfonylurea herbicide	[61]
	<i>Rhodococcus</i> sp. P14	降解类固醇 Bioremediation of steroids	[62]
	<i>Rhodococcus</i> sp. YYL	高效降解四氢呋喃 Efficient tetrahydrofuran degradation	[63]
	<i>R. ruber</i> C208	降解聚乙烯 Polyethylene biodegradation	[64]
	<i>R. aetherivorans</i> BCP1	亚硒酸盐转化 Selenite bioconversion	[65]
	<i>R. erythropolis</i> XP	苯并蒽并噻吩的脱硫 Desulfurization of benzonaphthothiophene	[68]
生物转化 Biotransformation	<i>R. ruber</i> TH3	转化合成丙烯酰胺 Acrylamide bioproduction	[69]
	<i>Rhodococcus</i> sp. Y22	生物转化尼古丁 Biotransformation of nicotine	[70]
	<i>R. opacus</i> DSM 43250	转化合成 α -酮异己酸 Biosynthesis of α -ketoisocaproate	[71]
	<i>Rhodococcus</i> sp. ECU00 66	将苯甲硫醚氧化成(S)-亚砷 Bioconversion of phenylmethyl sulfide to (S)- sulfoxide	[72]
其他应用 Other applications	<i>R. erythropolis</i> NI1	高效降解黄曲霉毒素B1 Efficient degradation of Aflatoxin B1	[73]
	<i>R. rhodochrous</i> NI2	高效降解黄曲霉毒素B1 Efficient degradation of Aflatoxin B1	[73]
	<i>R. actinobacteria</i>	产海藻糖脂类表面活性剂 Production of trehalolipid biosurfactants	[74]
	<i>R. erythropolis</i> HX-2	产胞外多糖 Production of exopolysaccharide	[75]
	<i>R. erythropolis</i> CCRC10909	产生物絮凝剂 Production of biofloculant	[76]
	<i>Rhodococcus</i> sp. B7740	产类胡萝卜素 Production of carotenoids	[77]
	<i>R. ruber</i> YM-2	产红色素 Production of red pigment	[78]
	<i>R. opacus</i> DSM1069	为生物燃料生产积累脂质 Accumulate lipids for biofuel production	[79]
	<i>R. opacus</i> PD630	分解木质素、工业废物 Degradation of lignin and other industrial wastes	[80]
	<i>R. opacus</i> FMME1-41	产谷氨酸氧化酶 Production of glutamic acid oxidase	[81]
	<i>R. equi</i> 4-2	产胆固醇氧化酶 Production of cholesterol oxidase	[82]
	<i>R. ruber</i> SD3	产二鸟苷酸环化酶 Production of diguanylate cyclase	[84]
	<i>R. equi</i>	研发免疫性药物 Development of immune drugs	[85-86]

另外,红球菌能够用于生物表面活性剂的制备。生物表面活性剂因其结构多样、毒性低和生物降解特性好而被广泛应用。Retamal-Morales等人在*R. erythropolis* B7g的基因组序列中鉴定出与表面活性剂海藻糖四酯产生相关的基因,并对这些基因进行了功能验证^[8]。Kuyukina等阐述了红球菌中多种海藻糖脂类化合物的结构,这些多样的结构使得红球菌有望成为新颖生物活性分子的制造者。其中海藻糖单霉菌酸酯、海藻糖双霉菌酸酯具有较强的免疫调节和抗肿瘤活性,与分枝杆菌产生的衍生物相比,其细胞毒性较低,更适合于临床应用^[74]。Hu等人利用*R. erythropolis* HX-2生产胞外多糖,经过发酵参数优化后,胞外多糖的产量达到8.957 g/L^[75]。红球菌还可用于制备生物絮凝剂,*R. erythropolis* CCRC10909合成的絮凝剂中,80%以上分泌到胞外,约20%停留在细胞中^[76]。

北极海洋红球菌B7740能够产类胡萝卜素,在优化的提取条件下,类胡萝卜素的产率为675 $\mu\text{g/g}$ 。经质谱鉴定,提取物中

含有2种甲基萘醌类胡萝卜素^[77]。红色素是*R. ruber*的代谢产物,*R. ruber* YM-2经过复合诱变后,红色素粗品产量达到1.12 g/L^[78]。

Le等人利用*R. opacus* DSM1069作为生产菌株,能够产11种脂质,其中脂肪酸甲酯的终浓度大约为15 mg/L^[79]。Chatterjee等用*R. opacus* PD630分解木质素和其他可持续利用的、非食品的工业废物,并将其用于微生物生产,实现了废弃料的循环利用^[80]。这些研究为生物质的利用奠定了理论基础。

R. equi 4-2能够产胆固醇氧化酶,用于降低食品原料中的胆固醇,并且降解率可以达到40%^[82]。Robert Van der Geize等对*Rhodococcus* sp. strain RHA1的胆固醇代谢进行了深入研究,他们结合转录组、荧光定量PCR等技术,鉴定出一套完整的与微生物胆固醇降解相关的基因簇^[83]。本课题组将*R. ruber* SD3中的二鸟苷酸环化酶进行异源表达,其中粗酶比活

力达到1.1 U/mg, 能够用于环二鸟苷酸的制备^[84]。人们还根据*R. equi*的致病性研究出了一些免疫性药物, 并且在抗逆转录病毒和人类免疫缺陷病毒治疗中也具有一定的疗效^[85-86]。

从红球菌的应用情况来看, 尽管红球菌种类较多, 但已经利用的红球菌主要包括*R. erythropolis*、*R. opacus*、*R. ruber*和*R. equi*。从红球菌的应用范围来看, 以前红球菌主要用于环境治理方面, 现已拓展到生物能源、医药健康等领域。当今环境、能源、健康已成为人类生存和发展的主题, 在此背景下, 红球菌将发挥重要的作用。

3 问题与展望

从近年来有关红球菌的出版物数量来看, 该属是微生物学的一个热点研究领域。红球菌的遗传多样性和功能多样性, 使其有望成为重要的底盘微生物从而发挥出更大的作用。然而对于红球菌的研究, 有些方面仍然没有涉及。例如红球菌在生物修复过程中, 如何感知那些难以降解的化合物, 如何作出相应的响应? 红球菌是否普遍存在一个信号转导机制来启动生物修复? 这些科学问题可能成为红球菌中一个新的研究方向。

从上述对红球菌的研究成果来看, 目前尽管对红球菌在组学、遗传改造、应用等方面开展了许多工作, 但在以下一些方面还需进一步深入: (1) 从组学研究上看, 近年来在红球菌组学领域已取得许多成就, 但组学结果的可靠性、准确性和有效性依赖于组学技术的进步, 因此组学技术的更新迭代必定能够推动红球菌研究的不断深入; (2) 从遗传操作层面上看, 虽然已经发现了红球菌的一些功能基因, 但是相对整个功能基因库而言数量偏少。在关键基因的功能鉴定和理解菌株的次生代谢机制方面, 仍然面临着很大的挑战。此外, 目前红球菌遗传改造工具和手段较少, 限制了它们的遗传改良以及在环境修复和废弃物利用方面的作用, 因此寻找更多、更有效的针对红球菌基因组编辑等遗传工具箱, 将有助于发挥它们的功能多样性; (3) 从应用层面上看, 无论是生物修复还是生物制备过程, 红球菌都要接触各种化学物质。其中一些化学物质对微生物具有一定的毒性, 影响微生物的生长和代谢。然而, 红球菌具有独特的生理生化特性, 能够克服这些化学物质带来的不利影响。因此深入探讨这微生物如何感应这些化学物质并且传导这些环境刺激的信号转导机制, 对于阐明红球菌独特的生理生化特性具有重要的科学价值, 同时对于生物制备过程和环境污染物的生物修复等方面都具有重要的实践意义。

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