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·综 述·

微生物法合成红景天苷

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摘 要: 红景天苷是红景天属植物的主要有效成分之一，具有耐缺氧、抗辐射、抗疲劳、抗肿瘤、降血糖、提高免疫力等多重功效。随着其需求量的日益增加和植物资源的不断减少，微生物法合成红景天苷因具有周期短和易调控等优势而倍受关注。目前微生物法合成红景天苷尚处于基础研发阶段，为了方便相关领域研究者系统了解其研究现状和探讨其未来发展方向，文中对红景天苷生物合成途径、糖基转移酶、野生菌/天然酶资源和工程菌/重组酶体系进行了综述。

关键词: 红景天苷，生物合成途径，野生菌/天然酶，工程菌/重组酶

Microbial synthesis of salidroside

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Abstract: Salidroside, as one of the main active ingredients of *Rhodiala* plant, has the effects of anti-hypoxia, anti-radiation, anti-fatigue, anti-tumor, hypoglycemia and improving immunity. With the increasing demand for salidroside and the decreasing of plant resources, microbial production of salidroside has attracted much attention due to its advantages of short period and easy controlling. At present, microbial production of salidroside is still at the basic research stage. In order to make it easier for researchers to understand the advances of microbial synthesis of salidroside, the biosynthesis pathways, uridine diphosphate glucosyltransferases, wild strain/natural enzymes and engineered strain/recombinant enzymes were reviewed.

Keywords: salidroside, biosynthesis pathways, wild strains or natural enzymes, engineered strains or recombinant enzymes

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红景天苷 (Salidroside) 亦称红景天甙，其化学结构式为酪醇 8-*O*-β-D-葡萄糖苷 ($C_{14}H_{20}O_7$)，是以酪醇 (4-羟基苯乙醇，Tyrosol，$C_8H_{10}O_2$) 为苷元的醇羟基与尿苷二磷酸葡萄糖 (Uridine diphosphate glucose, UDP-glucose, $C_{15}H_{24}N_2O_{17}P_2$) 半缩醛羟基脱水后形成的糖苷 (图 1)[1-2]。作为红景天属药用植物的主要活性成分，红景天苷被证实具有耐缺氧、抗辐射、抗疲劳、抗肿瘤、降血糖、提高免疫力和记忆力等重要生理功效[1,3]。随着人们对红景天苷药理作用认识的不断深入，其需求量与日俱增。

最初人们依靠野生红景天属植物提取红景天苷，深受资源有限和含量低的约束[4]；后来利用组织培养和细胞悬浮培养等技术克服了野生资源的不足[5-6]，可仍然存在生产周期长、产量低等问题；近些年，相关研究者通过不断尝试和比较各种方法，普遍认为微生物法合成红景天苷具有潜在工业化应用价值[7-8]。

1 红景天苷生物合成途径

微生物法合成植物天然产物在实现产业化的进程中面临的挑战之一就是理清其生物合成途径[7]。如图 1 所示，红景天苷生物合成途径的最后一步反应已经被证实为尿苷二磷酸葡萄糖基转移酶 (Uridine diphosphate glucosyltransferase, UGT) 催化底物酪醇与尿苷二磷酸葡萄糖合成红景天苷[6-7]。反应中涉及的尿苷二磷酸葡萄糖 (UDP-glucose) 其生物合成途径研究较成熟，属于初级代谢范畴，微生物合成 UDP-glucose 通常选择如图 2 的代谢

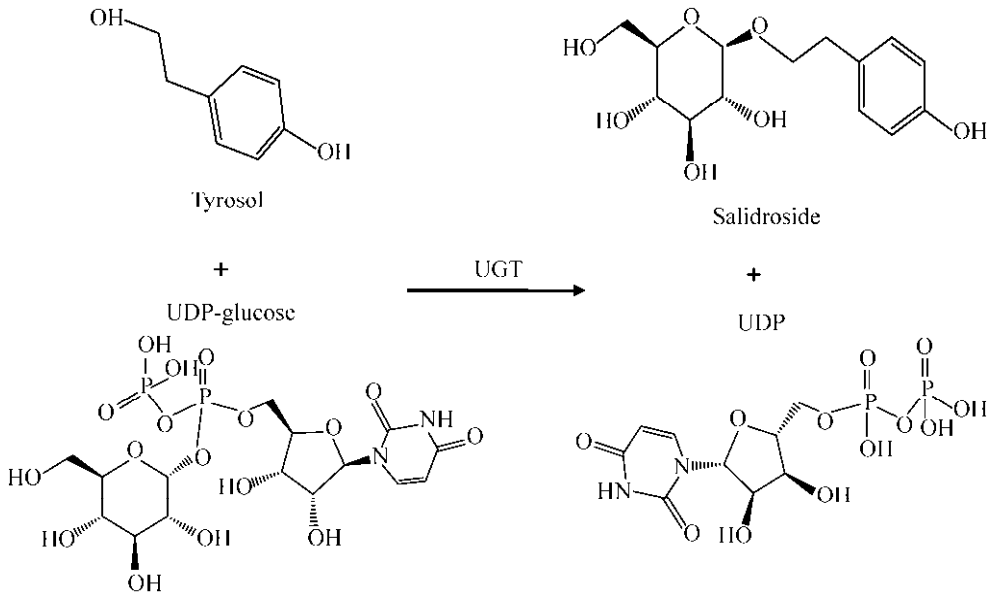

图 1　红景天苷生物合成的最后一步反应[2]

Fig. 1　The last reaction of salidroside biosynthesis[2]. UGT: uridine diphosphate glucosyltransferases; UDP: uridine diphosphate.

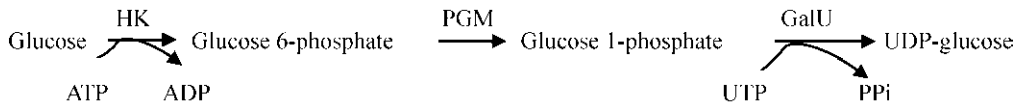

图 2　UDP-葡萄糖的生物合成途径 (参照陈圣等[9])

Fig. 2　UDP-glucose biosynthesis pathway (adapted from Chen et al[9]). ATP: adenosine triphosphate; ADP: adenosine diphosphate; HK: hexokinase; PGM: phosphoglucomutase; UTP: uridine triphosphate; PPi: pyrophosphatic acid; GalU: glucose-1-phosphate uridylytransferase.

途径，通过弱化竞争途径或过表达关键酶 (如 PGM 和 GalU 等) 保障其供应量[9-11]。因此，关于红景天苷生物合成途径的研究，人们一方面致力于途径中催化反应的关键酶 UGT 相关的研究：如本课题组率先获得催化活性较高的植物 UGT73B6[12]、UGT72B14[2]；随后通过调研植物 UGT 超家族晶体结构，介绍了 UGTs 的整体结构特点 (如保守的 PSPG 结构单元) 以及蛋白与底物相互作用的细节 (如口袋结构结合糖基供体)[13]；Fan 等根据植物 UGTs 的 PGSG 结构特征进一步筛选获得高活性微生物 UGT[14]。另一方面，研究者还重点关注了底物酪醇 (Tyrosol) 生成途径，研究表明其来源于莽草酸途径 (Shikimate pathway) 所生成的阿罗酸 (Arogenate)，阿罗酸合成酪醇的生物途径因体系差异而有所不同[15-18]：根据植物芳香族天然产物代谢特点和规律，研究者最初认为酪醇来源于苯丙氨酸解氨 (PAL) 途径 (图 3 中支路①) 和酪氨酸脱羧 (TyrDC) 途径 (图 3 中支路③)[18]，如 Keski-Saaris 等和 Hu 等证明了 PAL 的活性对红景天苷积累有重要影响[19-20]，Landtag 等和 Lan 等研究均表明 TyrDC 的活性对红景天苷的积累很重要[21-22]，本课题组前期工作确认高山红景天 *Rhodiola sachalinensis* 植物中其苷元酪醇的主要合成途径为酪氨酸脱羧途径，其中 TyrDC 为关键酶和限速酶[23]；最近，Michael 等发现红景天植物中含有一种依赖于磷酸吡哆醛的羟基苯乙醛合酶 (HPAAS) 可直接催化酪氨酸获得 4-羟基苯乙醛然后还原生成酪醇 (图 3 中支路④)[24]；另外，酿酒酵母 *Saccharomyces cerevisiae* 以酪氨酸为底物经过转氨脱羧反应合成酪醇机制很早就有报道，符合图 3 中支路⑤TAT 途径[25]，Satoh 等则在大肠杆菌 *Escherichia coli* 中同时构建了图 3 中支路③和⑤实现了酪醇的有效合成[26]；按照图 3 中支路②TAL 途径合成酪醇的案例暂未见报道，

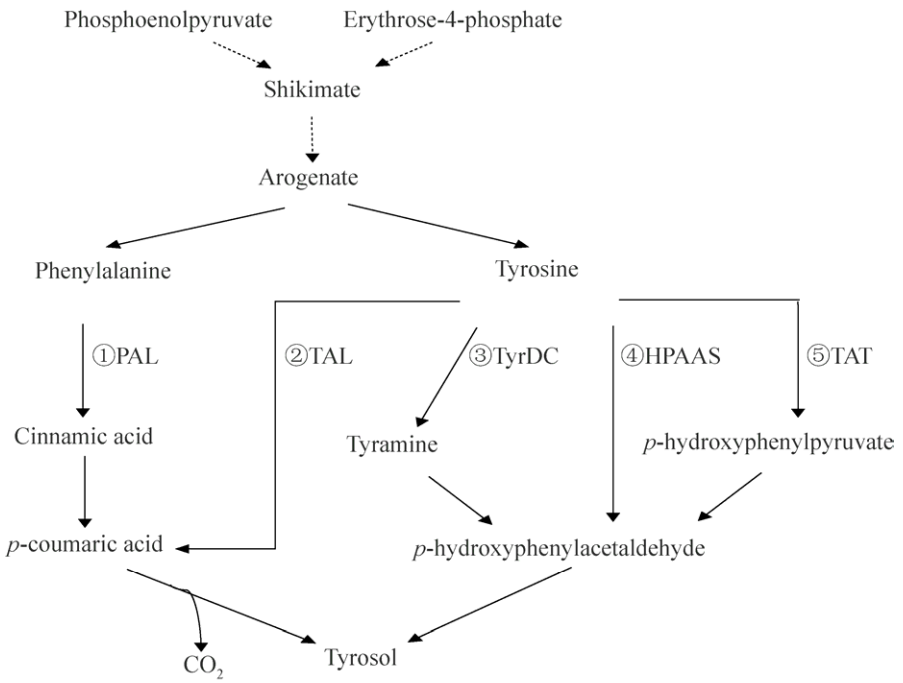

图 3　酪醇可能的生物合成途径

Fig. 3　Proposed pathways of tyrosol biosynthsis. PAL: phenylalanine ammonia lyase; TAL: tyrosine ammonia lyase; TyrDC: tyrosine decarboxylase; HPAAS: hydroxyphenylacetaldehyde synthase; TAT: tyrosine aminotransferase. The dotted arrow represents the multistep reaction. The solid arrow represents one-step reaction.

但利用 TAL 催化活性实现由酪氨酸 (Tyrosine) 合成 4-香豆酸/对羟基肉桂酸/对羟基苯丙烯酸 (*p*-coumaric acid) 已有研究，如 Kim 等在阐述类苯乙醇合成现状时介绍了 TAL 催化合成 4-香豆酸进而生产咖啡酸苯乙酯的途径[8]；Rodriguez 等研究表明在酿酒酵母中超量表达约氏黄杆菌 *Flavobacterium johnsoniaeu* 的 TAL 能够增加 4-香豆酸合成量[27]；Vannelli 等和 Vargas-Tah 等研究均表明利用粘红酵母 *Rhodotorula glutinis* 双功能酶 PAL/TAL 可以实现对羟基肉桂酸的有效合成[28-29]。为此，本课题组在发现粘红酵母代谢产物中有酪醇组分后已经开展了其酪醇合成途径包括图 3 中支路①和②的验证工作，以期更进一步阐述酪醇的生物合成途径，为建立新的微生物法合成红景天苷体系提供基础。

2 微生物法合成红景天苷

2.1 野生菌/天然酶合成红景天苷

利用野生菌或天然酶合成红景天苷的文献报道中涉及的微生物以真菌为主 (表 1)。最初的研究思路是以红景天植物浸出物为培养基成分发酵获得微生物菌体后提取酶液进行体外催化酪醇合成红景天苷：如贾艳萍等利用犁头霉 *Absidia* sp. 的粗提酶液催化 15 g/L 的酪醇合成约 1.5 g/L 的红景天苷[30]；Zhang 等利用黑曲霉 *Aspergillus niger* 提纯酶催化 1.5% 的酪醇合成红景天苷可达 10%[31]；王梦亮等从红景天植物根系土壤中筛选获得微生物菌株米曲霉 *Aspergillus oryzae* 能够利用 5 g/L 的酪醇合成 0.7 g/L 的红景天苷[32]。后来研究者发现可以利用双菌株共培养 (Coculture) 的协同效应或微生物细胞融合 (Cell fusion) 的方式来提高红景天苷的含量：宋伟舟等利用双菌株协同液体发酵红景天使其红景天苷含量提高 86.29%[33]；冯敏等利用细胞融合双亲菌株固体发酵大花红景天粉末，使其红景天苷含量提高 140%[34]。近期有利用植物内生真菌合成红景天苷的报道：如曲霉 *Aspergillus* 和镰刀霉 *Fusarium*[35]，深色有隔内生真菌 *Phialocephala fortinii* 培养 7 d 后可将红景天苷产量提高到 2.339 g/L[36]。

2.2 工程菌/重组酶合成红景天苷

2.2.1 糖基化酪醇合成红景天苷

随着基因工程技术的发展、红景天苷生物合成途径的不断明晰和代谢途径中限速酶的逐步明确，利用微生物工程菌或重组酶合成红景天苷的研究取得了突破性进展，特别是以利用模式微生物 (如大肠杆菌和酿酒酵母) 为宿主的研究发展

表 1 野生菌/天然酶合成红景天苷

Table 1 Salidroside production by wild microorganism or natural enzymes

Microorganisms	Substrate	Time	Yield	Highlights	Reference
Absidia sp.	15 g/L tyrosol	6 h	~1.5 g/L	Crude enzyme, enzymic synthesis	[30]
Aspergillus niger	1.5% tyrosol	6 h	10%	Purified enzyme, enzymic synthesis	[31]
Aspergillus oryzae	5 g/L tyrosol	48 h	0.7 g/L	Whole cell catalyst	[32]
Aspergillus niger-Aspergillus niger	*Rhodiala crenulata*	2–4 d	0.630 4%	Two strains, coculture	[33]
Aspergillus niger	*Rhodiala crenulata*	3–4 d	1.019%	Cell fusion, solid fermentation	[34]
Aspergillus, Fusarium	PDB culture medium	8 d	No data	Endophytic fungus of *Rhodiola*	[35]
Phialocephala fortinii	Czapek-Dox culture medium	7 d	2.339 g/L	Endophytic fungus of *Rhodiola*	[36]

PDB: Potato dextrose broth.

迅速 (表 2)。2011 年 Yu 等报道了 3 个高山红景天 UGTs 在大肠杆菌中实现了重组表达，重组酶体外酶促反应均获得了红景天苷产物[2]。2016 年，Xue 等利用密码子优化 (Codon optimization) 的办法实现了 UGT72B14 在大肠杆菌的高效表达，并利用分批-补料 (Fed-batch) 的策略得到微生物红景天苷产量为 6.7 mg/L[37]。2017 年，Fan 等通过基因挖掘进一步获得高活性的地衣芽胞杆菌 $UGT_{BL}1$ 并构建了重组大肠杆菌，工程菌全细胞催化 24 h 后可将红景天苷的产量提高到 1.04 g/L[14]。

2.2.2 从头合成红景天苷

为了进一步增强微生物合成红景天苷的应用可行性，人们充分利用基因工程、代谢工程和发酵工程技术发展了红景天苷以葡萄糖为底物的从头合成 (*de novo*) 技术。2014 年，Bai 等通过引入酿酒酵母 ARO10 和高山红景天 UGT73B6 等关键酶，采用菌体生长和产物合成在不同培养基分段发酵重组大肠杆菌的策略，以葡萄糖为底物可得红景天苷产量为 56.9 mg/L[38]。2017 年，Chung 等通过引入香芹 AAS 和拟南芥 UGT85A1，以葡萄糖为底物同样采用分段培养重组大肠杆菌可使红景天苷的产量提高到 288 mg/L[39]。2018 年，Liu 等通过引入酿酒酵母 ARO10、毕赤酵母 KDC4 和拟南芥 UGT85A1 等关键基因构建两株大肠杆

表 2 工程菌/重组酶合成红景天苷

Table 2 Salidroside production by recombinant microorganism or enzymes

Microorganisms	Genes introduced	Substrate and concentration	Time	Yield	Highlights	Reference
Escherichia coli	*RsUGT73B6*, *RsUGT72B14*, *RsUGT74R1*	Tyrosol, 250 μmol/L	30 min	No data	Enzymatic reaction	[2]
Escherichia coli	*RsUGT72B14*	Tyrosol, 50 mg/L	9 h	6.7 mg/L	Fed-batch cultivation, codon optimization	[37]
Escherichia coli	*Bl*$UGT_{BL}1$	Tyrosol, 1 g/L	24 h	1.04 g/L	Microbial UGT used	[14]
Escherichia coli	*ScARO10*, *RsUGT73B6*	Glucose, 2%	48 h	56.9 mg/L	Growth in LB, synthesis in M9Y	[38]
Escherichia coli	*PcAAS*, *AtUGT85A1*	Glucose, 2%	48 h	288 mg/L	Growth in LB, synthesis in M9	[39]
Escherichia coli-*Escherichia coli*	*PpKDC4*, *AtUGT85A1*, *ScARO10*	Glucose, 8 g/L$^+$ Xylose, 2g/L$^+$	129 h	6.03 g/L	Two strains, coculture, fed-batch fermentation	[40]
Saccharomyces cerevisiae	*Rr4HPAAS*, *RrT8GT*	Glucose, 4%	48 h	1.5 mg/L	Pathway elucidation, codon optimization	[24]
Saccharomyces cerevisiae	*PcAAS*, *AtUGT85A1*	Glucose, 20 g/L$^+$	168 h	732.5 mg/L	Plasmid-free strain, fed-batch fermentation	[41]

Rs: *Rhodiola sachalinensis*; UGT: Uridine diphosphate dependent glycosyltransferase; *Pc*: *Petroselinum crispum*; AAS: Aromatic aldehyde synthase; *At*: *Arabidopsis thaliana*; *Bl*: *Bacillus licheniformis*; *Pp*: *Pichia pastoris*; *Sc*: *Saccharomyces cerevisiae*; ARO10: Pyruvate decarboxylase; KDC: Decarboxylase; *Rr*: *Rhodiola rosea*; 4HPAAS: 4-hydroxyphenylacetaldehyde synthase; T8GT: Tyrosol:UDP-glucose 8-O-glucosyltransferase; "+": Substrate was fed during fermentation, but the feeding titer was not reported.

菌，采用两株菌的共培养方式和分批-补料的调控策略，以葡萄糖和木糖为底物发酵 129 h 可以将红景天苷的产量进一步提高到 6.03 g/L[40]。

随着大肠杆菌合成红景天苷的技术不断成熟，人们又开始致力于酿酒酵母合成红景天苷体系的研发。Torrens-Spence 等通过引入红景天 4HPAAS 和 T8GT，采用密码子优化策略在酿酒酵母中构建了红景天苷合成途径，获得产量为 1.5 mg/L[24]。Jiang 等利用基因整合技术在酿酒酵母中引入香芹 AAS 和拟南芥 UGT85A1，通过分批-补料的发酵调控策略发酵 168 h 同样可实现红景天苷的产量为 732.5 mg/L[41] 。

3 展望

红景天苷作为红景天的有效成分，其抗缺氧、抗疲劳、抗衰老、防辐射、增强心血管系统功能及对肿瘤的抑制等功效越来越受到关注。国内外相关知识产权也由初期集中在植物红景天苷提取方法及综合应用[42-43]，逐渐延伸至红景天苷相关产品制备方法及针对性应用效果[44-46]，并进一步扩展至生物酶催化法[47-49]及微生物发酵法[50-51]合成红景天苷的技术研发。我国植物红景天苷提取及制剂开发技术已经迈向产业发展阶段 (如公安部昆明警犬基地公布了利用红景天苷制备工作犬用抗高原反应药物组合物的方法与应用，四川康美保宁制药有限公司公布了一种乙醇快速提取红景天的方法，西安惠博生物科技有限公司公布了固定化酶催化制备红景天苷的方法等)[52-54]，而微生物法合成红景天苷目前还处于基础研发阶段，虽然国内在“高活性酶和潜力宿主菌的筛选”、“关键基因和代谢途径改造”、“培养方式和调控模式设计”等方面的研究处于领先地位[36,40-41]，但需要继续攻克高效合成及高效利用等瓶颈技术。

一方面要充分发掘利用更多生物资源如植物基因资源构建类似于图 3 中支路②或④的高效途径；或者同时启动多条途径如图 3 中支路①和②提高工作效率；或者借鉴 UDP-葡萄糖原位再生体系增强糖苷合成思路提高红景天苷合成效率[55]。

另一方面要充分利用现代生物学技术如 Liu 等利用基因组分析和合成生物学手段构建了酵母高效合成灯盏花素[56]，同理可以充分利用基因编辑技术、合成生物学和生物信息学手段增强红景天苷微生物细胞工厂运行效率；或者利用代谢组学和微生物发酵联产技术实现红景天苷和其他活性成分 (如络缇及其衍生物[6]、香豆素[16]、羟基酪醇[39]或淫羊藿次苷 D2[24,38,51]等) 联产以提高红景天苷生产和应用效率。

综上，微生物法合成红景天苷已有良好基础，相信通过相关领域研究者深度挖掘丰富的生物资源和充分利用现代生物学技术，能够早日实现产业化。

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