

· 综 述 ·

## 芽孢杆菌几种重要抗菌脂肽研究进展

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**摘 要:** 多种芽孢杆菌为益生菌, 能分泌多种天然抗菌活性物质, 其中脂肽是重要的一类。目前已鉴定的脂肽约有 90 多种, 多数为环脂肽。脂肽中表面活性素 (surfactin)、伊枯草菌素 (iturin)、芬原素 (fengycin)、杆菌霉素 (bacillomycin)、多粘菌素 (polymyxins) 等是研究最广泛的脂肽。其中 surfactin、iturin、fengycin 由于其具有表面活性剂特性及抗真菌、抗细菌、抗病毒、抗肿瘤、抗炎等功能, 应用潜力巨大。本文对 surfactin、iturin 及 fengycin 的结构、功能、合成调控及其分离纯化和生产等方面的研究进展进行了评述。合成生物学是提高脂肽产量的重要手段, 未来脂肽可用于种植业、养殖业、食品、医药、石油工业和环保等领域, 因此需要在新型脂肽的发现、高产活性脂肽的生产、脂肽低廉生产技术的研发及安全性的评估等方面加强研究。

**关键词:** 枯草芽孢杆菌; 脂肽; 结构; 功能; 合成调控

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## Advances in several important antimicrobial lipopeptides from *Bacillus* spp.

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**Abstract:** *Bacillus* spp. are probiotics and can secrete a variety of natural antimicrobial active substances, of which lipopeptides are an important class. Up to now, about 90 lipopeptides have been identified, and most of them are cyclic lipopeptides. surfactin, iturin, fengycin, bacillomycin and polymyxins are widely studied, and the first three have huge potential for application due to their properties of surfactants and anti-fungal, anti-bacterial, anti-viral, anti-tumor and anti-inflammatory functions. In this paper, the research progress in the structure, function, synthesis regulation, separation, purification and production of surfactin, iturin and fengycin was reviewed. Synthetic biology is a vital means to increase the yield of lipopeptides, and in the future, lipopeptides can be used in crop cultivation, animal farming, food, medicine and petroleum industries as well as environmental protection. Future research should be strengthened on the discovery of new lipopeptides, synthesis of high-activity lipopeptides, economical production of lipopeptides on a large scale and their safety evaluation.

**Keywords:** *Bacillus subtilis*; lipopeptides; structure; function; biosynthetic regulation

芽孢杆菌属 (*Bacillus* spp.) 细菌广泛地存在于生物体和环境中, 是一类在农业、工业及医药卫生等领域具有重要应用价值的细菌<sup>[1]</sup>, 其中枯草芽孢杆菌 (*Bacillus subtilis*) 是一种公认的益生菌, 已广泛用于植保和动物饲料添加剂<sup>[2-3]</sup>。芽孢杆菌属细菌是防治植物病害的常规农药的最有前途的替代品, 能够促进植物生长<sup>[4]</sup>, 诱导植物自身抗性<sup>[5]</sup>, 抑制不同病害, 以及通过竞争空间和营养成分直接对微生物病原体造成拮抗作用<sup>[6]</sup>。虽然芽孢杆菌的有益保

护作用涉及多种机制, 但抗菌脂肽化合物的产生是影响其生防活性的最重要因素之一, 因此抗菌脂肽的深入研究具有重要的理论意义和应用价值。

多数芽孢杆菌可产生低分子量、两亲性的脂肽。脂肽类化合物的结构和功能多样, 性能稳定, 表面活性优异, 可广泛应用于农业、石油化工、环保、食品工业和生物医药等领域<sup>[7-11]</sup>。表面活性素 (surfactin)、伊枯草菌素 (iturin) 和芬原素 (fengycin) 是脂肽的 3 个重

要家族。这 3 个家族都是环脂肽，都含有一个不同 C 链长度的  $\beta$ -羟基脂肪酸链及由不同氨基酸组成的环状肽。由于 C 链长度不同及氨基酸组成不同，被分成不同的家族，具有不同的功能。前人对抗菌脂肽 surfactin、iturin、fengycin 在结构与功能、合成机理和调控等方面已取得重要研究进展。3 种环脂肽结构明显不同，功能和应用价值有较大差异。研究显示 surfactin 不仅对细菌的运动和生物膜的形成意义非凡<sup>[12]</sup>，它们的抗病毒/抗菌活性也使得表面活性素具有广泛用途<sup>[13]</sup>。fengycin 和 iturin 通常以它们优越的抗真菌能力为人所熟知。三者都是通过非核糖体肽链合成酶合成的，但三者的合成基因及调控机制不同。目前通过合成基因调控元件的改造，能够使 surfactin 的产量大幅提高至 12.8 g/L<sup>[14]</sup>，为功能脂肽的规模生产开辟了新途径。本领域的研究进展虽然已有少量综述<sup>[8]</sup>，但尚缺综合性进展总结和评述。本文将全面总结 surfactin、iturin 和 fengycin 的结构、功能、合成机理和调控、分离纯化和发酵生产以及用途等方面的研究进展，并对未来发展方向做了讨论，有助于更好地理解这三类脂肽产生的分子调控机制、作用机理、相关关系及潜在产品的研发和应用。

## 1 抗菌脂肽的结构和功能

脂肽是一类由亲水的环状或短线性寡肽和疏水的长链脂肪酸两部分组成的结构高度多样性的生物表面活性剂，基于氨基酸结构的不同可分为环状脂肽和线性脂肽两类。脂肽结构的多样性除由氨基酸种类不同导致外，脂肪酸链的长度和分支差异也是主要原因。目前，26 个家族中约 90 种脂肽化合物已被鉴定，其中 24 个家族中 86 种脂肽为环脂肽，只有剩下的 2 个家族 4 种脂肽为线性脂

肽<sup>[15]</sup>。产脂肽的微生物主要是芽孢杆菌属细菌，如枯草芽孢杆菌 (*B. subtilis*)、地衣芽孢杆菌 (*Bacillus licheniformis*)、多粘芽孢杆菌 (*Bacillus polymyxa*)、解淀粉芽孢杆菌 (*Bacillus amyloliquefaciens*)、甲基营养型芽孢杆菌 (*Bacillus methylotrophicus*)、萎缩芽孢杆菌 (*Bacillus atrophaeus*)、副 (类) 地衣芽孢杆菌 (*Bacillus paralicheniformis*)、短小芽孢杆菌 (*Bacillus pumilus*)、蜡状芽孢杆菌 (*Bacillus cereus*)、巨大芽孢杆菌 (*Bacillus megaterium*) 等。表面活性素 (surfactin)、伊枯草菌素 (iturin)、芬原素 (fengycin)、杆菌霉素 (bacillomycin)、多粘菌素 (polymyxins) 等是芽孢杆菌中研究最广泛的脂肽<sup>[16]</sup>。单个菌株可以产生至少一种环脂肽，有的菌株可同时产生 surfactin、iturin 和 fengycin<sup>[17-18]</sup>。脂肽具有广谱抑菌活性，在抑制真菌生长方面表现优异<sup>[19]</sup>。

### 1.1 Surfactin

Surfactin 是枯草芽孢杆菌产生的一类最主要的抗菌活性物质。1968 年 Arima 等最初是将其作为一种有效的凝血抑制剂，并命名为 surfactin<sup>[20]</sup>。从生物化学角度来看，surfactin 包含 17% 的脂质和 13% 的肽链。典型的 surfactin 是由 7 个  $\alpha$ -氨基酸残基和一个长度为 C13-C15 的羟基脂肪酸，以内酯键结合形成的环状化合物分子<sup>[21]</sup>。在水溶液中，surfactin 带负电荷的 Glu 和 Asp 形成一个微小的极性结构域，在折叠的骨架中肽环呈“马鞍”状拓扑结构，促成各种各样的生物学特性<sup>[22]</sup>。surfactin 在肽环中典型的氨基酸顺序是：L-Glu<sup>1</sup>-L-Leu<sup>2</sup>-D-Leu<sup>3</sup>-L-Val<sup>4</sup>-L-Asp<sup>5</sup>-D-Leu<sup>6</sup>-L-Leu<sup>7</sup><sup>[23]</sup>，如图 1A 所示。由于肽链上第 2、4、7 位氨基酸不保守，脂肪酸中碳原子数不同及构型的差异导致 surfactin 具有众多同系物和同分异构体。1991 年 Baumgart 等报道了在典型肽链组成的第 7 位 L-Leu 分别

被 L-Val 和 L-Ile 所取代的两种 surfactin 同系物, surfactin A (Val<sup>7</sup>) 和 surfactin B (Ile<sup>7</sup>)<sup>[24]</sup>。1994 年 Peypoux 等又报道了一种在典型肽链组成的第 4 位 L-Val 被 L-Ala 所取代的 surfactin (Ala<sup>4</sup>)<sup>[25]</sup>, 随后的研究更精确地测定了十余种同系物。不同亚型的 surfactin 具有细微的物理化学特性差异。

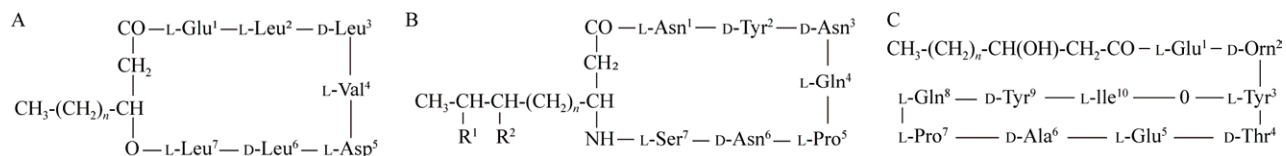


图 1 Surfactin、iturin 和 fengycin 的一级结构<sup>[28-29]</sup>

Figure 1 Primary structures of three lipopeptides. (A) surfactin,  $n=9-11$ . (B) iturin,  $n=14-17$ . (C) fengycin,  $n=11-15$ .  $n$ : the number of CH<sub>2</sub> groups in the peptide chain. L and D are L-amino acids and D-amino acids in the  $\alpha$ -amino acid residues, respectively. Modified from [28-29].

Surfactin 具有独特的两亲结构, 是优秀的生物表面活性剂, 能够降低表面和界面张力, 具有良好的起泡、增溶、润湿、去垢和乳化特性, 在工业中具有广泛的应用价值<sup>[30]</sup>。其中对原油良好的乳化作用, 可应用于石油污染处理和高效、可持续石油回收, 强化石油开采, 降解柴油<sup>[31]</sup>。surfactin 还能有效减小海水和油之间的张力, 改变环境的润湿指数, 因此具有截留油的能力<sup>[32]</sup>。

Surfactin 具有较强抗菌作用。Krishnan 等在体外发现 surfactin 对串珠镰刀菌 (*Fusarium moniliforme*) 抑菌效果比多菌灵更显著<sup>[33]</sup>。Park 等发现贝莱斯芽孢杆菌 (*Bacillus velezensis*) GH1-13 分泌的 surfactin 对多种植物病原真菌有较强的拮抗作用, 明显抑制菌丝生长<sup>[34]</sup>。

除此之外 surfactin 还表现出抗病毒、抗肿瘤和抗支原体活性, 具有非常强的溶血功能<sup>[35-37]</sup>。Qi 等报道 *Bacillus amyloliquefaciens* WH1 产生一种表面活性素, 命名为 WH1 fungin<sup>[38]</sup>。Gao 等发现在以小鼠为模型的实验中 WH1 fungin

通常, 脂肽的杀菌活性随着脂肪酸链长度 (通常为 C10-C12) 的增加而增强, 脂肪酸链含有较多碳原子 (14 或 16) 的脂肽具有更强抗细菌活性或抗真菌活性<sup>[26]</sup>。surfactin 碳链的长度影响其灭活病毒的能力, 随着脂肪酸链碳原子数目增多, 疏水性增加, 灭活病毒的能力增强<sup>[27]</sup>。

能够改善 I 型糖尿病<sup>[39]</sup>。

生物膜是一种结构复杂的多细胞微生物群落, 细胞外多聚物能够保护细胞, 并牢固黏在物质表面。surfactin 在去除病原体形成的生物膜方面表现出优异的性能。许多研究证明, surfactin 能够降低细菌粘附作用<sup>[40]</sup>。低浓度的 surfactin 与单一细菌或混合病原体短间接接触就能破坏其生物膜。因此, 在去除病原微生物在外科手术用具上形成的生物膜, 保护生物材料方面, surfactin 抗细菌黏着性和抗细菌形成生物膜特性具有潜在的医学应用价值。

值得注意的是, 有研究发现 surfactin 还具有潜在蚊虫杀蛹作用。Geetha 等分离的一株芽孢杆菌分泌的 surfactin 对埃及伊蚊 (*Aedes aegypti*)、埃及按蚊 (*Anopheles stephensi*) 和埃及库蚊 (*Culex quinquefasciatus*) 具有灭蚊活性<sup>[41]</sup>, 可用于防治病媒传播疾病等公共防控项目。

## 1.2 Iturin

Iturin 是一大类强烈抑制真菌生长的脂肽类化合物。在结构上与 surfactin 较为类似, 同

样包含一个由 7 个  $\alpha$ -氨基酸残基组成的肽链, 是一种环脂七肽。七肽的第 7 位氨基酸是 Ser, 由 14–17 个 C 原子的脂肪酸链的  $\beta$ -氨基酸残基连接 (图 1B)。七肽的氨基酸序列为 (L-)Asn-(D-)Tyr-(D-)Asn-(L-)Gln-(L-)Pro-(D-)Asn-(L-)Ser。该家族主要包括的亚型有: iturin A、C、D、E, 杆菌抗霉素 (bacillomycin) D、F、L、Lc 和抗霉枯草菌素 (mycosubtilin)、杆菌肽素 (bacillopeptin) 等<sup>[42]</sup>。其中最具有代表性的 iturin A 由一个含有 23–17 个碳原子的  $\beta$ -氨基脂肪酸和 7 个氨基酸残基组成的环状结构, 其环状结构由 7 位的 Ser 的羧基和  $\beta$ -氨基脂肪酸的氨基缩合形成<sup>[43]</sup>。Ser6-Asn7 互换便成为 mycosubtilin, 它的抗菌活性比 iturin 更高。而 bacillomycin 的基本结构和 iturin A 类似, 只是在 1、4、5、6 和 7 位上的氨基酸有所不同, 分为 bacillomycin D、F、L、Lc。

Iturin 对黄瓜 (*Cucumis sativus*) 细菌性叶斑病和软腐病、小麦 (*Triticum aestivum*) 赤霉病有着很好的防控作用<sup>[44]</sup>。此外 iturin 有很强的溶血作用, 但其生理活性和 surfactin 不同, 对真菌 (包括酵母菌) 具有强烈的拮抗作用, 但是对细菌拮抗作用很小<sup>[45]</sup>。研究表明, 伊枯草菌素对红色毛癣菌具有较强抑制作用<sup>[46]</sup>。Iturins 抗菌谱广, 稳定性高, 毒性低, 致敏性低, 应用前景广。

### 1.3 Fengycin

Fengycin 由  $\beta$ -羟基脂肪酸和 10 个氨基酸残基的小肽组成, 经典结构如图 1C 所示。脂肪酸链往往由 14–18 个碳原子组成, 肽链包含 4 个 D-氨基酸和 6 个 L-氨基酸, 肽链中氨基酸组成顺序为 L-Glu<sup>1</sup>-D-Orn<sup>2</sup>-L-Tyr<sup>3</sup>-D-Thr<sup>4</sup>-L-Glu<sup>5</sup>-D-Ala<sup>6</sup> (Val)<sup>6</sup>-L-Pro<sup>7</sup>-L-Gln<sup>8</sup>-D-Tyr<sup>9</sup>-L-Ile<sup>10</sup>, 肽链的第 10 位上的 Ile 羧基和第 3 位的 Tyr 上羟基缩合形成环状结构<sup>[47]</sup>。

Fengycin 主要有两种类型 (fengycin A 和 fengycin B), 当肽链的第 6 位上是 Ala 时, 属于 fengycin A, 而当肽链的第 6 位上是 Val 时, 属于 fengycin B<sup>[48]</sup>, 分子量在 1 500 Da 左右。制磷脂菌素 (plipstatin) 是由 *B. cereus* BMG302-fF67 和 *B. subtilis* 168 产生的一种和 fengycin 非常类似的菌脂肽, 其结构及性质和 fengycin 大致相同, 目前多数文献将其归类于 fengycin 系列<sup>[49]</sup>。

Fengycin 具有良好的抑真菌活性, 尤其对丝状真菌更为明显。对细菌和酵母无作用<sup>[50]</sup>, 能抑制芳香酶和磷脂酶 A2 的活性。此外, fengycin 还具有溶解红细胞作用<sup>[51]</sup>。Piewngam 等证明枯草芽孢杆菌产生的 fengycin 能抑制金黄色葡萄球菌 (*Staphylococcus aureus*) 在小鼠体内定殖<sup>[52]</sup>。

### 1.4 Surfactin、iturin 及 fengycin 结构和功能的比较

芽孢杆菌产生的 surfactin、iturin 及 fengycin 都是环脂肽类小分子活性物质, 都含有亲水性肽链和亲脂族烃链, 具有双亲性, 都具有多种生物活性, 但三者一级结构明显不同, 功能也有显著差异, 因此其应用领域也有不同 (表 1)。脂肽具有多种生物活性 (如抗菌、抗病毒、溶血和杀虫等), 脂肽一个很重要的特性是使生物膜穿孔, 即通过直接作用于细菌和真菌的生物膜, 使膜形成孔隙并导致渗透性不平衡, 从而使细胞死亡。这种特性可以帮助脂肽的生物活性得以实现<sup>[53]</sup>。脂肽或分泌脂肽的菌剂已被尝试作为抑制各种植物病原体的多功能武器, 对包括细菌、真菌和卵菌在内的多种植物病原体均有拮抗作用。Iturin 和 fengycin 具有抗真菌活性, 而 surfactin 具有更广泛的强效抗菌活性, 也被用作幼虫杀虫剂<sup>[54-55]</sup>。

表 1 Surfactin、iturin 及 fengycin 结构和功能的比较

Table 1 Structures and functions of surfactin, iturin and fengycin

Items	Surfactin	Iturin	Fengycin	References
Molecular weight	About 1 036 Da	About 1 057 Da	About 1 463 Da	[21,43,47]
The length of fatty acid chain	$\beta$ -hydroxy fatty acid chain, C13–C16	$\beta$ -hydroxy fatty acid chain, C14–C17	$\beta$ -hydroxy fatty acid chain, C14–C18	[21,42,47]
The length of cyclic peptide chain	Seven $\alpha$ -amino acids	Seven $\alpha$ -amino acids	Ten $\alpha$ -amino acids	[21,42,47]
	L-Glu <sup>1</sup> -L-Leu <sup>2</sup> -D-Leu <sup>3</sup> -L-Val <sup>4</sup> -L-Asp <sup>5</sup> -D-Leu <sup>6</sup> -L-Leu <sup>7</sup>	L-Asn <sup>1</sup> -D-Tyr <sup>2</sup> -D-Asn <sup>3</sup> -L-Gln <sup>4</sup> -L-Pro <sup>5</sup> -D-Asn <sup>6</sup> -L-Ser <sup>7</sup>	L-Glu <sup>1</sup> -D-Orn <sup>2</sup> -L-Tyr <sup>3</sup> -D-Thr <sup>4</sup> -L-Glu <sup>5</sup> -D-Ala <sup>6</sup> (Val) <sup>6</sup> -L-Pro <sup>7</sup> -L-Gln <sup>8</sup> -D-Lyr <sup>9</sup> -L-Ile <sup>10</sup>	[21,42,47]
Subtypes	Surfactin A, Surfactin B Esperin, Halobacillin Pumilacidin	Iturin A, Iturin C, Iturin D Iturin E, Bacillomycin D Bacillomycin F, Bacillomycin L, Bacillomycin Lc, Mycosubtilin, Bacillopeptin	Fengycin A Fengycin B Plipastatin A Plipastatin B	[53]
Main activity	Strong surfactant, antifungal, antibacterial, antiviral, anti-tumor, anti-mycoplasma, biofilm removal, strong hemolysis and pore formation	Surfactant and antifungal	Surfactant and antifungal	[16,53]
Applications	Biopesticides (plant disease control, such as sclerotinia, stem rot, and soybean root and leaf diseases); Medicine (antiviral, anti-fungal, anti-bacterial, anti-cancer, anti-thrombotic); Cosmetics (emulsifiers, antibacterial agents); Food safety (biological surfactants and emulsifiers); and oil extraction, etc.	Biopesticides (plant disease control, such as sclerotinia, stem rot, soybean blight, and powdery mildew of melon)	Biopesticides (plant disease control, such as soybean blight, sclerotinia, stem rot, apple gray mold, and melon powdery mildew etc.)	[16,54-55]

## 2 抗菌脂肽的合成机理及调控

抗菌脂肽的合成不同于传统的以 mRNA 为模板、tRNA 为载体转录翻译合成蛋白质的模式，是通过非核糖体肽链合成酶 (nonribosomal peptide synthetase, NRPS) 合成的<sup>[56-57]</sup>。NRPS 是一种能识别、激活、修饰和连接氨基酸中间体和产物肽的多模块酶，是目前所发现的最大酶系。它由起始、伸长和终止 3 个主要模块形成脂肽合成的组装线，每一个模块由不同的结构域构成(图 2A)。它的底物可以是标准的氨基

酸或甲基化、羟基化、氯化或其他修饰的变体，但也可能不同于氨基酸如  $\beta$ -或环丙基-氨基酸或  $\alpha$ -羟基酸。NRPS 首先通过腺苷酰化结构域 (adenylation domain, A domain) 识别、结合底物，并将由 ATP 衍生的 AMP 添加到底物上使其激活；激活的底物与 4'-磷酸泛酰巯基乙胺中游离巯基结合，且 4'-磷酸泛酰巯基乙胺绑定在紧邻 A domain 下游的肽酰基载体蛋白结构域 (peptide carrier protein, PCP-domain, 也常称之为 T domain) 上；在缩合结构域 (condensation domain, C domain) 的作用下与前一个模块的

肽链相连接, 该过程按照相邻合成酶各组成模块的顺序依次向前直到多肽合成结束。非核糖体肽合成酶亚基的最后一个模板中通常包含一个差向异构酶结构 (epimerization domain, E domain), 其作用是将被激活的 L-氨基酸转化为 D-氨基酸, 某些合成酶模块也能直接活化 D 构象的氨基酸<sup>[58-59]</sup>。综上所述, 全酶可以由多个模块按照特定的空间顺序排列而成, 模块的数量、种类和排列顺序决定肽链的长短、氨基酸的种类和顺序。非核糖体肽合成机理如图 2 所示。

2019 年 Reimer 等在非核糖体肽合成酶的功能方面取得重要进展, 该团队采用 X 射线晶体技术, 为 NRPSs 拍摄了超高分辨率的 3D 图

像, 第一次看到 NRPSs 的各个模块, 对单个模块与更大的组装线之间的关系进行细致的观察。这项研究发现, 除了模块必须协调才能将中间体从一个工作站传递到下一个工作站外, 其他所有模块之间都缺乏同步性, 各个模块不是以直线或其他有组织的方式排列, 而是可以在许多不同的相对位置排列, NRPSs 具有高度的灵活性。该研究透彻解析了脂肽化合物合成的分子机制, 人们可尝试在基因水平上重新设计非核糖体肽合成酶每个模块或结构域的排列方式, 通过混合和匹配模块来设计 NRPSs, 从而得到具有新的氨基酸组成的脂肽分子, 为获得新的抗菌药物提供基础<sup>[60]</sup>。

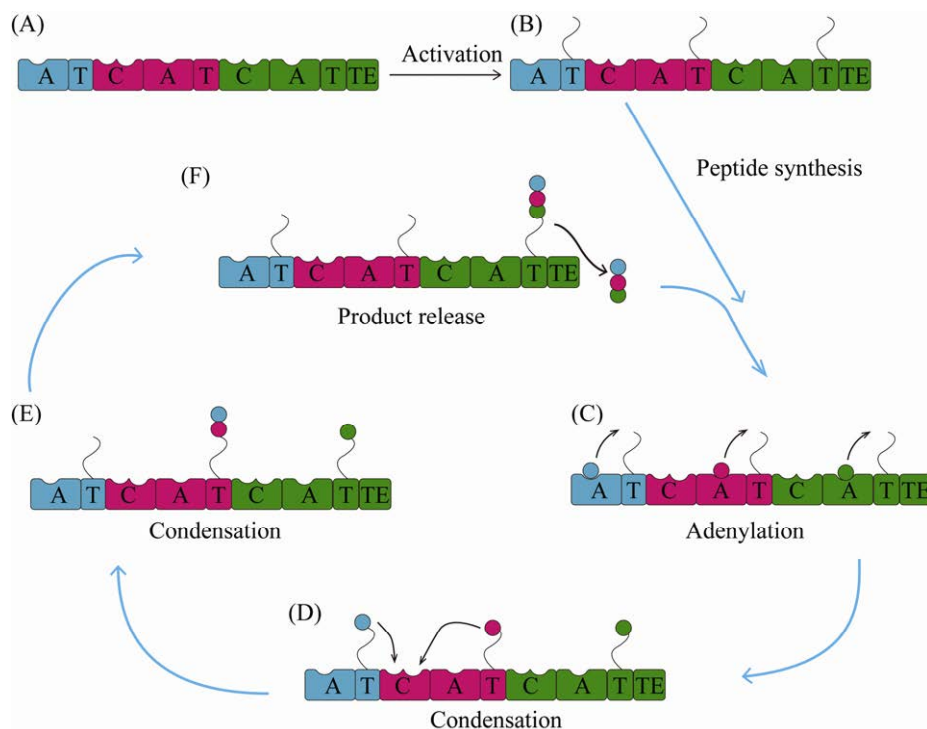


图 2 非核糖体肽合成 (NRPS) 的多模块模型<sup>[61]</sup>

Figure 2 Multi-module model of non-ribosomal peptide synthesis (NRPS) (Modified from [61]). A schematic arrangement of domains within a hypothetical three module NRPS that contains an initiation (blue), an elongation (red) and a termination (green) module. A domain: adenylation domain; T domain: aminoacyl carrier domain; C domain: condensation domain; TE: thioester domain; It is in turn responsible for the activation and transfer of amino acid substrates, the formation of peptide bonds, the termination of extension and the release of products on the domain.



目前已有通过合成模块的删除、截短改良抗菌脂肽的范例。Gao 等删除 fengycin 合成酶复合体单个模块使整个酶复合体失活,但删除第 7 个模块的 A/T domain 产生了截短的线性脂肽 (C16-C17 $\beta$ -OHFA-Glu-Orn-Tyr-Thr-Glu-Ala/Val),删除 A6 domain 发生模块跳跃现象,产生 3 个新型的 fengycin 亚型,五肽 (C16-17 $\beta$ -OHFA-Glu-Orn-Tyr-Thr-Glu)、六肽 (C16-17 $\beta$ -OHFA-Glu-Orn-Tyr-Thr-Glu-Ile) 和八肽 (C16-17 $\beta$ -OHFA-Glu-Orn-Tyr-Thr-Glu-Gln-Tyr-Ile)<sup>[62]</sup>,但对新型脂肽的生物活性并没有相关研究。

### 2.1 Surfactin 的生物合成及其调控

Surfactin 合成酶基因 *srfAA*、*srfAB*、*srfAC*、*srfAD* 共同构成 *srfA* 操纵子,基因总长达 27 kb,由 *srfAA* 和 *srfAB* 编码蛋白负责组装前 6 位氨基酸,*srfAC* 编码蛋白组装第 7 位氨基酸。*srfAC* 编码的第一个硫酯结构域 TE 负责终止肽链延伸并释放多肽产物,*srfAD* 编码的第二个硫酯酰基转移结构域 (thioesterase/acyltransferase domain, TE/AT domain) 则具有激活并启动 surfactin 肽链合成反应的功能<sup>[63]</sup>。此外,在 *srfA* 操纵子的下游,含有一个 surfactin 合成必需的磷酸泛酰巯基乙胺基转移酶 (PPTases) 基因 *sfp*,行使激活 surfactin 合成酶的功能<sup>[64]</sup>。枯草芽孢杆菌产生的脂肽类抗生素生物合成的基因调控研究最为详细的是 surfactin。Surfactin 合成的基因调控是由复杂的群感效应系统 ComQXPA 诱导的多重级联反应过程。芽孢杆菌信息素 ComX 感受细胞密度,随着细胞密度增加而增加,当细胞密度达到一定阈值,信息素 ComX 与膜蛋白组氨酸激酶 ComP 结合,诱导 ComP 的自磷酸化。磷酸化的 ComP 进而将磷酸基团转移至调控蛋白 ComA。磷酸化的 ComA 与 surfactin 合成酶基因 *srfA* 的启动子特

定区域结合,激活 RNA 聚合酶,使 *srfA* 开始转录<sup>[65-67]</sup>。由 *rap* 基因编码的天冬氨酰基磷酸酶与下游 *phr* 基因编码的感受态刺激因子 CSF 结合后,丧失磷酸酶活性,以致丧失对磷酸化 ComA 的脱磷酸化作用,使 ComA 处于活化状态,利于 *srfA* 基因的转录和 surfactin 的合成<sup>[68]</sup>。负调控因子还有 Rap (天冬氨酸磷酸酶)、CodY (GTP 和支链氨基酸依赖的转录调节因子)、PerR (Fur 家族转录因子)、SinI (转录调控因子)、Spx (调节蛋白)、PhoP (双元件响应调节器)。其中 CodY 通过直接作用 *srfA* 启动子抑制其表达,Spx 通过阻断在启动子区域 ComA 与 RNA 聚合酶作用从而抑制 surfactin 合成<sup>[69]</sup>,SinI 通过正向调节生物膜的形成直接调控 surfactin 合成<sup>[70]</sup>。全局调控因子 DegU 对 surfactin 合成起正调控作用。敲除 *degU* 基因,导致 *srfA* 基因的转录显著下降。此外,由基因 *swrC* (即 *yreP*) 和 *liaIHGFSR* 操纵子编码的蛋白负责 surfactin 转运和细胞对 surfactin 耐受性<sup>[71]</sup>。

多种枯草芽孢杆菌可生产 surfactin,但产量较低 (<1 g/L)。Jiao 等利用启动子改造,使重组菌株 THY-7/Pg3 surfactin 产量达到了 9.74 g/L<sup>[72]</sup>。Wu 等采用系统代谢工程方法构建 surfactin 的高产菌,最终 surfactin 产量增加至 12.8 g/L,产率为 65.0 mmol/mol 蔗糖 (理论产率的 42%)<sup>[14]</sup>。这些研究为 surfactin 的规模生产提供了新的技术途径<sup>[73]</sup>。

### 2.2 Iturin 的生物合成及其调控

Iturin 家族的脂肽类抗菌素受不同基因的调控。其中 bacillomycin D 由 *bam/bmy* 基因簇负责合成,mycosubtilin 由 *myc* 基因簇负责合成,iturin A 由 *itu* 基因簇编码负责合成。*itu* 基因簇包括 4 个开放阅读框 (ORFs),分别为



*ituA*、*ituB*、*ituC*、和 *ituD*<sup>[74-75]</sup>。其中 *bam/bmy* 基因簇分别发现存在于 *B. subtilis* AU195 和 *B. amyloliquefaciens* FZB42 中, 该基因簇编码一个多功能复合酶, 包括一个脂肪酸合成酶、一个氨基转移酶和一个肽合成酶。第一个开放阅读框架 *bmyD*、*ituD* 和 *fenF* 编码丙二酰辅酶 A 转移酶, 第二个开放阅读框 *bmyA*、*ituA* 和 *mycA* 分别编码乙酰辅酶 A 连接酶、酰基载体蛋白 (CAP)、 $\beta$ -酮乙基合成酶和转氨酶结构域。Iturin 家族中 mycosubtilin 的基因调控首次在合成 mycosubtilin 的菌株 *B. subtilis* ATCC 6633 得到阐明, *myc* 操纵子的表达依赖于 ComA, 与群体感应有关。SigH 因子和 Spo0H 也影响 *myc* 操纵子表达, 培养基中添加 PhrC 蛋白可以弥补缺失 *spo0H* 缺失突变体产生的现象。AbrB 可以抑制 *myc* 表达, 敲除 *abrB* 基因可以增强 *myc* 表达<sup>[76]</sup>。类似于 *fen* 操纵子, 基因 *degQ* 也是 *bmy* 操纵子的一个正向调控因子。类似于 *srfA* 操纵子, 全局调控因子 DegU 和 ComA 对 *bmy* 操纵子的全转录激活必不可少, DegU 直接作用于 *bmy* 操纵子启动子上游的两个位点, 对 *bmy* 表达起重要作用。而且 DegU 和跨膜蛋白 YczE 对 bacillomycin 转录后调控起重要作用。与 fengycin 和 surfactin 等脂肽一样, 类似于 Sfp 蛋白的 Lpa-14 对 iturin 的合成必不可少<sup>[77]</sup>。将强启动子 C2up 插入 *itu* 操纵子的上游, 可提高 iturins 产量<sup>[78]</sup>。

### 2.3 Fengycin 的生物合成及其调控

Fengycin 生物合成基因簇包含 5 个开放阅读框 ORFs, 分别编码 fengycin 合成酶的 5 个亚基, *fen* 操纵子包含 *fenC* (编码 287 kDa 蛋白)、*fenD* (编码 290 kDa 蛋白)、*fenE* (编码 286 kDa 蛋白)、*fenA* (编码 406 kDa 蛋白) 和 *fenB* (编码 146 kDa 蛋白), 它们线性排列共享一个启动

子<sup>[79]</sup>。同样地, 基因 *sfp* 对 fengycin 的合成必不可少, *B. subtilis* 168 虽然含有完整的 *fen* 操纵子, 但由于 *sfp* 中间含有一个终止密码子, 导致 Sfp 蛋白不完整, 不能合成 fengycin<sup>[14]</sup>。但仅转化 *sfp* 基因的 *B. subtilis* 168 菌株 fengycin 产量仍然很低, 原因是其 *degQ* 的启动子区 -10 位置上的一个碱基 T 突变成了 C, 因此该菌株不能表达 DegQ<sup>[80]</sup>。DegQ 是一种胞外蛋白酶增强子, 编码一个含有 46 个氨基酸的多肽, 是广泛存在于芽孢杆菌中的多效性因子, 控制多种水解酶的产生。*degQ* 基因的敲除会导致 fengycin 同系物合成水平明显下降<sup>[81]</sup>。研究还发现, 通过对 *B. subtilis* F29-3 中 *fen* 操纵子的转录分析, RNA 聚合酶可以结合在 *fen* 操纵子启动子上游富含 A 和 T 的被称为 UP 元件区域, 从而增强 *fen* 操纵子的转录<sup>[82]</sup>。多核苷酸磷酸化酶对 fengycins 合成有重要影响, *pnpA* 基因失活时 fengycins 产量大幅下降<sup>[83]</sup>。Spo0A 蛋白和 ComA 蛋白通过与 *fen* 启动子结合等机制促进 fengycins 的产生<sup>[84]</sup>。近年来, fengycins 的生产工艺也有了较大改进, 如人工全固相合成脱羟基 fengycins 衍生物技术和细胞固定化生产 fengycins 技术<sup>[85-86]</sup>, 为规模生产 fengycins 奠定了基础。

## 3 脂肽发酵生产和分离纯化

尽管抗菌活性脂肽具有多种应用潜力, 但是其实际应用因野生型菌株产量低而受到较大限制。采用固定化细胞发酵<sup>[87]</sup>、泡沫回流发酵、无泡发酵<sup>[88]</sup>、固态发酵<sup>[89]</sup>、调节供氧水平和补充金属元素等手段对生产工艺和培养基成分进行优化, 或采用分批发酵或补料分批发酵方式已成功使枯草芽孢杆菌合成抗菌脂肽物质的产量得到大幅度的提升。

2006年 Yeh 等报道以 ppch 作为固定载体经过 72 h 的发酵, *B. subtilis* ATCC 21332 菌株 surfactin 的产量达 6.45 g/L, 增加 2.0–4.3 倍<sup>[90]</sup>; 在 LB 为培养基发酵液中添加 0.35% 二价铁离子后, *B. subtilis* ATCC 21332 菌株 fengycin 产量由对照组的 310 mg/L 提高到 680 mg/L, 提高了近 1.1 倍<sup>[86]</sup>。

在脂肽研究中, 脂肽物质的分离、提纯和鉴定是相当重要的一部分, 菌体发酵液中含有多种未知的蛋白质等大分子物质和小分子活性物质, 而且大多数微生物可产生两种以上的抑菌脂肽物质。在此混合体系中, 抗菌脂肽物质的

分离纯化程度需视研究工作的类型而定, 至今还没有单独或一种现成的方法可以把任何一种脂肽物质从复杂的混合物中提取并纯化出来, 往往通过几种方法联合使用。

目前, 对于分析级的抗菌脂肽的分离纯化往往联合有机溶剂萃取, 酸沉淀, 硫酸铵沉淀、透析等两种以上的方法得到粗提物, 再经过离子交换层析、固相萃取 (SPE)、聚苯乙烯树脂等方法得到进一步纯化的抗菌脂肽 (表 2)。不同技术有优缺点不同 (表 2), 对于制备级的抗菌脂肽的分离纯化常采用膜超滤技术、吸附法、泡沫分离法。

表 2 分离和纯化脂肽类表面活性剂方法和技术

Table 2 Methods and techniques for separation and purification of lipopeptides surfactants

Extraction/purification methods	Advantages	Disadvantages	References
Precipitation by acid treatment	It is an effective method for recovery of crude lipopeptides and can be applied on large scale. It cost low and operation process is simple	The product is acidic	[91-94]
Precipitation by ammonium sulfate	The operation process is simple	Dialysis is required for reducing salt pollution	[95-96]
Extraction by organic solvent	It is an effective method for recovery of crude lipopeptides and can be applied on large scale. The organic solvent for the lipopeptide extraction can be reused	It is expensive and toxic	[30,97-99]
Separation by collection of foam	The product possessed high purity	It required complicated equipment	[100-102]
Membrane ultrafiltration	The process is fast and the product possessed high purity	It is expensive and must be operated under high pressure	[103-104]
Ion exchange chromatography	The process is fast and the product possess high purity. Resin can be reused	Different effective resin materials need to be selected	[105-107]
Adsorption resin	The product possessed high purity and high quality. Resin can be reused. The process is fast	Desorption is need with organic solvent	[108]
High performance liquid chromatography (HPLC)	The product possessed high purity. It can be used for fractionation of different lipopeptides, the identification of lipopeptide isomers, the qualitative and quantitative analysis of lipopeptides	Retention rate is low	[108-109]
Thin layer chromatography (TLC)	It can be used for identification of cyclic lipopeptides	The retention rate is very low and is generally used to identify lipopeptides	[110-112]
Gel filtration	Matrix can be reused	The retention rate is very low	[98,113-114]

## 4 脂肽研究的挑战与未来

尽管芽孢杆菌产生的脂肽研究已取得了较大进展,并且具有应用价值,但脂肽作为商品在市场上的应用还很初步。迄今为止,仅有少部分国外商家能够小规模生产出商业化 surfactin、iturin 和 fengycin 等产品,如 Sigma 和 OKA 公司生产的脂肽产品。而国内还没有此类商业化产品,即便是国外生产的标准品,同一分子量的脂肽仍包含多种同分异构体。然而能够产生脂肽的菌剂已在国内外广泛应用,如用于饲料添加剂的枯草芽孢杆菌、用于医药的地衣芽孢杆菌等。

鉴于脂肽潜在的应用价值及目前研究存在的局限性,未来需要在下述方面加强研究。1) 筛选天然高产菌株。2) 脂肽的安全性尚缺乏系统的研究。安全性研究是食品、药品及农业领域应用的先决条件,目前虽然有一些脂肽应用研究,但涉及其安全性研究有限。3) 高产廉价脂肽的生产技术。目前的脂肽生产主要是利用天然菌株或工程菌株探讨其生产工艺,产量不高。目前只有少量脂肽的合成生物学研究,以提高脂肽的产量。例如, Jiao 等<sup>[72]</sup>、Wu 等<sup>[14]</sup>将 surfactin 的产量分别提升至 9.74 g/L 和 12.8 g/L。但还缺乏完善的规模生产、分离纯化工艺流程。需要开发更高效的生产工艺及构建更高产脂肽的工程菌。利用廉价的农业废弃物作为生产原料以降低生产成本也是未来研究的方向。4) 高活性脂肽研究。高活性脂肽是未来研究的重要目标,可通过合成生物学增加或减少脂肪酸链的长度或改变肽链中氨基酸成分获得活性改变的脂肽,目前缺乏更高活性的脂肽化合物。5) 具有新功能脂肽的获得。微生物可产生多种天然脂肽,不同微生物分泌的脂肽种类也不同,有些脂肽含量很低,但可

能具有新的生物学功能,对微量新脂肽的鉴定比较困难,需要较高的分离纯化技术、结构分析技术。

## 5 结论

脂肽是微生物分泌的重要化合物,具有抗菌、抗病毒、抗炎症、抗肿瘤等多种功能,其结构、功能及合成调控研究已取得重要进展,其应用潜力巨大,可用于种植业、养殖业、食品、医药、石油工业等领域。未来脂肽的发展需要在新型脂肽的发现、高产活性脂肽的生产、脂肽生产的低成本技术研发及合成脂肽的安全性等方面加强。

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