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自然环境中功能菌活的非可培养状态形成及复苏机制研究进展*

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摘 要 细菌在环境胁迫下可进入活的非可培养(viable but non-culturable,VBNC)状态,不再分裂但具有代谢活性,且能够在适宜条件下复苏.VBNC 状态限制了功能菌群在环境治理方面的作用,开展关于 VBNC 状态的形成及复苏机制研究可为激发 VBNC 状态功能菌在生物修复中发挥作用提供理论基础.本文对环境中 VBNC 状态细菌的表型、VBNC 状态的潜在污染降解菌、VBNC 状态细菌的形成及复苏机制进行综述,以期为进一步探究 VBNC 状态功能菌在环境中的作用提供科学依据,并为揭示 VBNC 状态功能菌对环境污染物降解及元素循环的贡献提供思路.

关键词 VBNC 状态,形成机制,复苏机制,潜在功能菌,环境治理.

Research progress on the formation and resuscitation mechanism of viable but non-culturable state of functional bacteria in natural environment

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Abstract: Bacteria are likely to enter into the viable but nonculturable (VBNC) state under environmental stress. They no longer divide but have metabolic activity, and resuscitate under suitable conditions. VBNC state limits the application of functional bacteria in environmental governance. Study on the formation and resuscitation mechanism of VBNC bacteria will provide a theoretical basis for stimulating the functional bacteria in bioremediation. In this paper, the phenotype of VBNC state bacteria, the potential pollution degradation bacteria of VBNC state, the formation and resuscitation mechanism of VBNC bacteria are reviewed, in order to provide a scientific basis for further exploring the role of functional bacteria in VBNC state in the environment and the insights into revealing the contribution of functional bacteria in VBNC state to pollution degradation and element cycles.

Keywords: VBNC, formation mechanism, resuscitation mechanism, potential function bacteria, environmental governance.

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活的非可培养(viable but non-culturable,VBNC)状态是微生物在恶劣环境下采取的一种特殊生存策略[1].在这种状态下,细胞不能生长和分裂,无法在培养基上形成菌落[2].1982 年,Xu 等首次在河口和海洋环境中发现此状态的霍乱弧菌(*Vibrio cholerae*)和大肠杆菌(*Escherichia coli*)[3],这引起了微生物学界的极大关注.随后越来越多的学者报道了关于 VBNC 状态的相关研究,但迄今为止这些研究主要集中于食品科学和基础医学领域,甚少关注环境中功能菌群的 VBNC 状态.

为了揭示环境中处于 VBNC 状态潜在功能菌的作用机制,需要突破其不可培养的限制,并且对 VBNC 状态细菌进行复苏,以便真正认识这些细菌在环境中的作用.目前研究者已利用复苏促进因子(resuscitation promoting factor,Rpf)从污染场地获得了一些之前未被分离的 VBNC 状态的污染降解菌[4].然而,通过与宏基因组分析结果进行比较,这些污染环境容纳的功能性细菌远远多于之前所认识的[5].因此,选择合适的方法成功复苏 VBNC 细菌是探索环境中潜在功能菌的关键一步.

本文从 VBNC 状态细菌的表型、环境中潜在功能菌的复苏、细菌 VBNC 状态的形成和复苏机制等方面进行了综述,以期为进一步探究潜在功能菌群如何有效发挥作用提供理论参考,同时为如何复苏 VBNC 状态细菌提供思路和方法.

1 细菌 VBNC 状态(VBNC status of bacteria)

细菌在遇到环境压力时,往往选择进入一种“休眠”状态,以此作为在不利条件下的生存策略[6].在许多物种中,休眠不是一个开关,而是一个随时间动态变化的渐进过程.这个过程可能是随机的,也可能是环境信号的结果[7].目前,在很多非产孢细菌中发现了这种休眠现象,即 VBNC 状态[8].

VBNC 状态是微生物对温度波动、营养缺乏、紫外线辐射、有机污染物和重金属等不利环境条件作出的适应性反应[9-10],该状态限制了细胞的生长和分裂.在这种状态下,细菌仍然存活并保持一定的代谢活性,但无法在常规细菌培养基上形成菌落[2, 11].进入 VBNC 状态的细胞主要表现出形态和代谢改变,包括体积缩小、细胞膜组成和细胞壁结构的改变[12],以及大分子合成、营养物质运输和呼吸速率水平降低等[9].同时,它们具备了更强的抵抗力,且一旦条件适宜,又可复苏并随后再生[13].Oliver 等研究数据显示,一些细菌在 VBNC 状态下具有更强的抗性,可以抵御热、氧化作用、渗透压、pH、乙醇、抗生素和重金属等各种胁迫,并保持恢复到可培养状态的能力[14].

在研究 VBNC 状态的过程中,研究者还发现了一种过渡状态——持留(persister)状态[15].这种状态主要是针对抗生素这种新型环境污染物的一种抵抗状态,因为多数的报道集中于通过使用大剂量抗生素处理培养物来进行研究[16-17].但有研究表明,持留化(persistence)也是由多种因素引起的,包括饥饿[18]、碳源转换、氧化应激、DNA 损伤和 pH 等[19-20].考虑到各种环境压力会导致持留化,持留化很可能已经演变成一种普通的环境压力反应,而不仅仅是抗生素耐受.有研究报道,饥饿同样可以诱导持留细胞的形成,并且随着时间的推移,持留细胞逐渐转化成 VBNC 状态(图 1)[15].Ayrapetyan 等在 2015 年提出[21],环境中有很多功能菌在环境胁迫下,首先会进入持留状态,在消除压力后能迅速复苏.然而,如果细菌继续暴露在长期的压力下,其休眠程度会增加,进而转变成 VBNC 状态,该状态则需要更多的时间去复苏.这些发现都说明持留状态可能是具有环境选择压力下的某些细菌进入 VBNC 状态的一种短暂过渡状态.

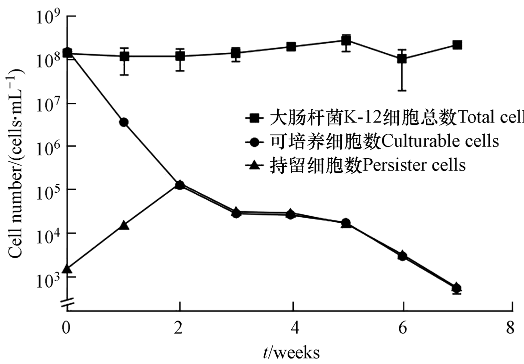

图 1 VBNC 诱导条件下,可培养细胞和持留细胞的时间变化[15]

Fig.1 Temporal change in VBNC culturable and persister cells[15]

2 环境中 VBNC 状态的潜在功能菌(Potential functional bacteria in VBNC state in the environment)

一直以来,由于各种人为因素,例如家用清洁剂、抗生素、有机物和重金属等污染的不合理排放,使

许多功能性微生物进入"休眠"状态而无法发挥其生物降解功能,这对生物资源的开发和利用是一个巨大的损失.尤其是近年来多环芳烃(PAHs)和多氯联苯(PCB)等持久性有机污染物不断释放到环境中,严重威胁着自然生态系统和人类健康[22-23].目前不少学者已利用 Rpf 从这些污染环境中复苏培养了一些 VBNC 细菌,且从中获得多种具有除磷、除臭、絮凝、降解有机污染物等环境功能的高效菌种(表 1 中序号 1—10),但仍发现污染区还存在大量 VBNC 状态的潜在功能菌(表 1 中序号 11—18).

表 1　环境中 VBNC 状态的功能菌

Table 1　Functional bacteria in VBNC state in the environment

序号 Serial number	环境中的功能菌 Functional bacteria in the environment			环境中的功能菌的状态 Status of functional bacteria in the environment	
	菌种 Species	功能 Function	参考文献 References	进入或退出 VBNC 状态 Enter or exit VBNC status	参考文献 References
1	*Arthrobacter* sp.节杆菌属	降解联苯、絮凝	[24,27]	添加 Rpf 复苏分离	[24,27]
2	*Streptomyces* sp.链霉菌属	降解多环芳烃	[25]	添加 Rpf 复苏分离	[26]
3	*Chryseobacterium* sp.金黄杆菌属	絮凝	[27]	添加 Rpf 复苏分离	[27]
4	*Arthrobacter liuii* 刘志恒氏节杆菌	除臭、絮凝、降解多氯联苯	[28]	添加 Rpf 复苏分离	[28]
5	*Rhodococcus biphenylivorans* 嗜联苯红球菌	降解联苯	[29]	添加 Rpf 复苏分离	[30]
6	*Pseudomonas aeruginosa* 铜绿假单胞菌	降解多环芳烃	[31]	富集培养复苏分离	[31]
7	*Bacillus* sp. 芽孢杆菌属	降解联苯	[32]	添加藤黄微球菌的胞外有机物复苏分离	[32]
8	*Alcaligenes* sp. 产碱菌属	降解联苯	[32]	添加藤黄微球菌的胞外有机物复苏分离	[32]
9	*Microbacterium* sp. 微杆菌属	降解多环芳烃	[32]	添加藤黄微球菌的胞外有机物复苏分离	[32]
10	*Achromobacter* sp. 无色杆菌属	降解联苯	[32]	添加藤黄微球菌的胞外有机物复苏分离	[32]
11	*Serratia* sp.沙雷氏菌属	降解多环芳烃	[25]	进入 VBNC 状态	[33]
12	*Halomonas* sp.盐单胞菌属	降解苯酚	[34]	进入 VBNC 状态	[35]
13	*Burkholderia* sp.伯克氏菌属	降解多环芳烃	[36]	进入 VBNC 状态	[37]
14	*Mycobacterium* sp.分枝杆菌属	降解有机氯化物、多环芳烃	[38]	进入 VBNC 状态	[39]
15	*Novosphingobium* sp. 新鞘氨醇杆菌属	降解多环芳烃	[40]	进入 VBNC 状态	[40]
16	*Bacillus cereus* 蜡样芽孢杆菌	降解苯酚	[41]	进入 VBNC 状态	[42]
17	*Pseudomonas putida* 恶臭假单胞菌	降解多环芳烃	[43]	进入 VBNC 状态	[44]
18	*Acinetobacter calcoaceticus* 乙酸钙不动杆菌	降解多环芳烃	[45]	进入 VBNC 状态	[44]

注:潜在功能菌是指具有环境功能却无法发挥作用的细菌,目前潜在功能菌的研究主要集中在污染区,当以污染物为唯一碳源对其进行富集培养时,可以发现污染物的降解率很高,但利用平板进行分离时却没有菌落长出,并且宏基因组数据显示这些细菌多为 uncultured bacterium.本文中潜在功能菌指具有功能但处于 VBNC 状态而无法发挥作用的细菌.

表 1 中序号 1—10 是目前文献报道已经成功复苏并分离出来的 VBNC 功能菌种,其中 1—5 是通过添加 Rpf 复苏并分离,6 是通过富集培养复苏并分离,7—10 是通过添加藤黄微球菌的胞外有机物复苏并分离;表 1 中序号 11—18 是文献中通过实验和宏基因组数据所发现的在污染区处于 VBNC 状态的潜在环境菌属或菌种,它们由于环境压力进入 VBNC 状态而无法发挥作用.

Potential functional bacteria refers to the bacteria that have environmental functions but cannot function. At present, the research on potential functional bacteria is mainly concentrated in contaminated areas. When the pollutants are enriched and cultured as the only carbon source, it can be found that the degradation rate of the pollutants is very high, but no bacterial colonies grow out when separated by a plate. Moreover, the metagenomics sequencing data shows that these bacteria are mostly uncultured. In this paper, the potential functional bacteria refers to the bacteria that have environmental functions but are in the state of VBNC and cannot function.

Numbers 1—10 in Table 1 are VBNC functional strains that have been successfully recovered and isolated by current literature reports. Among them, 1—5 are recovered and isolated by adding recovery promotion factor Rpf, 6 is recovered and isolated through enrichment culture, 7—10 are the recovery and separation of the extracellular organic matter of *Micrococcus luteus*; the numbers 11—18 in Table 1 are the potential functional bacteria or species found in the contaminated area in the VBNC state through experiments and metagenomic data in the literature. They cannot function due to the environmental pressure entering the VBNC state.

3 VBNC 状态形成的分子机制(Molecular mechanisms of VBNC state formation)

目前,针对 VBNC 状态形成机制的研究主要集中在一些模式菌中,学者在漫长的研究过程中发现它的形成机制很复杂,涉及众多基因和代谢途径,被普遍认同的机制包括严紧反应(stringent response)、蛋白酶对抗毒素的降解和毒素-抗毒素系统(toxin−antitoxin system,TA system),这些机制并不是独立作用,它们之间通常相互联系并协同作用.需要注意的是,严紧反应和蛋白酶对抗毒素的降解已被证明直接参与了持留细胞的形成,对于更深的休眠状态——VBNC 状态的形成没有给予明确的说明.随后越来越多的证据表明,它们同样在 VBNC 状态的控制上发挥着重要的作用[46-47].而 TA 系统则通过调控毒素和抗毒素的比例控制细胞进入不同程度的“休眠”状态.以上机制的关键是鸟苷五磷酸或鸟苷四磷酸[(p)ppGpp]信号分子,它对整个机制的调控至关重要.另外,一些调控因子在功能菌 VBNC 状态的形成过程中也发挥着重要作用,如 *Labrenzia aggregata* 细菌,它可以将硝酸盐还原为氮气参与到氮循环过程中,并且还可以去除有机废水和高含量硝酸盐废水中的氮,以此减少河流氮污染和富营养化问题.Xu 等将 *Labrenzia aggregata* LZB033 的 *rpoN* 突变体与野生型处于相同的胁迫条件下培养,2 d 后发现缺失 *rpoN* 基因的菌株会更快地丧失可培养性,这表明 RpoN 是该菌株在压力条件下存活的重要调节因子[48].最近,有研究表明,再生延迟体、核糖体调节因子(RMF)和冬眠促进因子(HPF)也有助于解释 VBNC 状态的形成及复苏,这些因子与(p)ppGpp 的响应和抗毒素的降解等过程密切相关[49-50].

3.1 严紧反应和 TA 系统

严紧反应是细菌在氨基酸饥饿条件下的主要调节方式,通过基因 *relA* 合成(p)ppGpp 作为信号分子,调节细胞分裂、转录和翻译等细胞过程[51].该过程同蛋白酶对抗毒素的降解和 TA 系统协同作用,诱导细菌进入 VBNC 状态(图 2)[7].细菌在受到环境压力时,(p)ppGpp 合成酶 RelA 或 SpoT 被激活,调控细胞内(p)ppGpp 水平升高,而(p)ppGpp 能够抑制外切聚磷酸酶(PPX)降解多聚磷酸盐(polyphosphates,PolyP),使 PolyP 得到积累,积累的 PolyP 激活 Lon/Clp 蛋白酶,开始降解抗毒素,进而通过改变抗毒素与毒素的比例来影响 VBNC 状态的形成(图 2).

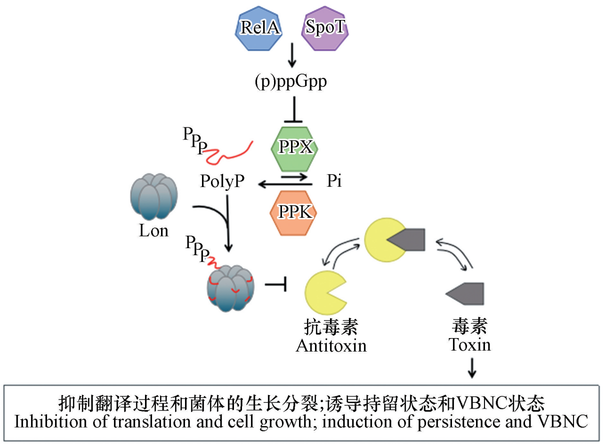

图 2 持留状态和 VBNC 状态形成机制[7]

(注:RelA 或 SpoT 的激活使细胞内(p)ppGpp 水平升高,(p)ppGpp 能够抑制外切聚磷酸酶(PPX)降解多聚磷酸盐(polyphosphates),导致多聚磷酸盐的积累,Lon/Clp 蛋白酶被多聚磷酸盐激活,开始降解抗毒素,从而影响 TA 系统相关基因的表达.游离的毒素抑制翻译过程和菌体的生长分裂,进而调控持留状态和 VBNC 状态的形成.)

Fig.2 Formation mechanism of persister and VBNC bacteria[7]

(Note:Activation of RelA or SpoT causes an increase in intracellular (p)ppGpp levels, resulting in the inhibition of exopolyphosphatase (PPX) which responsible for degrading the polyphosphates (PolyP). This results in accumulation of PolyP as a result of the constitutive activity of polyphosphate kinase (PPK). Lon/Clp protease is activated by PolyP and begins to degrade antitoxins, which affects the expression of toxin-antitoxin related genes. Free toxins inhibit translation and division of the bacteria, thereby regulating the formation of VBNC state.)

TA 系统除了与严紧反应协同作用来调节细胞过程外,还在细菌适应其他外界压力和毒性等方面发挥重要作用,主要受一种称为条件协同作用机制的调控[19].条件协同性是指毒素同时具有其同源 TA 操

纵子的辅抑制因子和去抑制因子的能力[52].在快速生长的非应激细胞中,[抗毒素]>[毒素],TA 复合物与 TA 启动子紧密结合,强烈地抑制 TA 操纵子,使毒素活性被多余的抗毒素抑制(毒素的辅抑制因子作用).然而,在外界环境压力下,细胞内高水平的(p)ppGpp 破坏了抗毒素的稳定性,使[抗毒素]<[毒素],毒素使启动子结合的 TA 复合物失稳,从而解除了抑制作用,介导 TA 操纵子的转录(毒素去抑制因子作用),进而导致毒素和抗毒素的比例增加,抑制细胞翻译和生长[53-54].

3.2　核糖体二聚体机制

在暴露于某些环境胁迫条件下,细菌通过核糖体调节因子(RMF)和冬眠促进因子(HPF)的共同介导,将两个 70S 核糖体聚在一起形成一个翻译不活跃的、休眠的 100S 复合体,主动抑制翻译过程,从而适应环境的变化[50].

2020 年,Song 等提出了(p)ppGpp 核糖体二聚体模型[55],即在应激条件下,细菌通过(p)ppGpp 调节 RMF 和 HPF,使 70S 核糖体通过形成二聚体迁移为 100S 复合体,主动抑制翻译过程,诱导细胞持久性.大肠杆菌在营养缺乏等应激等条件下,促使基因 *relA* 和 *spoT* 合成(p)ppGpp,(p)ppGpp 通过激活 RMF 的表达改变活跃 70S 核糖体的形状,使两个 70S 聚在一起转化为不活跃的 90S 核糖体二聚体复合物.(p)ppGpp 再诱导 HPF 将 90S 二聚体复合物转化为不活跃的 100S 核糖体复合物,使其"头对头"连接,诱导细胞进入持留状态[56-57].这一过程与 Matzov 等研究结果一致,但 Matzov 等还强调了 100S 核糖体复合物具有物种特异性[58].在大肠杆菌中,进入持留状态需要 RMF 和 HPF 两个蛋白质因子,而在金黄色葡萄球菌中不具有 RMF,只需要 HPF 就可将 70S 核糖体聚在一起形成 100S 复合物,使其"肩并肩"连接,诱导持留性.

另外,细菌在通过核糖体二聚体机制进入持留状态过程中,一些调控因子也起到一定的作用.Kline 等观察到在盐、乙醇和热应激作用下,基因 *hpf* 通过 Sigma B(σB)调控,其表达增加了大约 10 倍[50].Chaturongakul 等研究结果也显示,σB 和 σH 调节单核细胞李斯特菌中基因 *hpf* 的表达,并加强 HPF 在革兰氏阳性菌耐应力中的作用[59].此外,Basu 等通过对表达谱分析得出结论[49],σB 和(p)ppGpp 对 *hpf* 的表达具有正调控和负调控作用,并且受温度的强烈影响.

综上,在营养限制[60]、抗生素胁迫[61]、热应激[50]等应激条件下,细胞通过(p)ppGpp、Sigma 因子、RMF 和 HPF 等因子的共同作用,使 70S 核糖体聚在一起形成翻译不活跃的 100S 核糖体复合物,抑制代谢和翻译过程,维持其存活.

3.3　再生延迟体

再生延迟体(regrowth-delay body)是一种可逆的亚细胞结构[62],这种结构是由细胞极颗粒(cell-pole granule)组成并在细菌生长稳定期后期形成,它能够选择性地隔离多种细胞生长所必需的蛋白质,从而使细胞处于持留状态.当细菌细胞退出再生延迟期并恢复生长时,再生延迟体就会溶解,同时释放被隔离的蛋白质以恢复其功能.因此,进一步了解能够有效促进再生延迟体溶解的条件后,也许能够使这些细菌细胞在特定条件下培养.

同样的道理,在环境治理方面,通过促进 VBNC 细菌再生延迟体的溶解,也许能找到预防和控制功能菌群进入 VBNC 状态并使污染降解菌在环境修复中表现出较高降解活性的方法.目前对于再生延迟体的研究还处于初级阶段,有许多问题值得探讨,包括触发再生延迟体形成和溶解的关键信号分子是什么?以及它们是如何选择性地隔离多种蛋白?

4　VBNC 状态复苏的分子机制(Molecular mechanisms of VBNC state resuscitation)

VBNC 细胞的复苏是一个非常复杂的过程,其方法因细菌各自的特性而大不相同[63],一些细菌仅靠消除诱导应激即可复苏,而另一些细菌则依赖于特定的群体感应信号[64]、Rpf 和特殊化合物或只能在与其共生的生物体中进行复苏[65].尽管 VBNC 细胞的复苏机制还不清楚,但根据这些方法使细菌从 VBNC 状态成功复苏的研究已经取得了很大的成果,随着分子生物学的发展,人们对 VBNC 细胞复苏机制的认识也逐渐加深.

4.1　复苏促进因子

Rpf 是一种高度保守的蛋白质[66],可以促进高 G+C 含量的革兰氏阳性菌[67-68]和一些革兰氏阴性

菌[69]的复苏.目前,已经有多篇文献报道 Rpf 及其同系物在功能菌的复苏中起关键作用[26, 70],特别是控制着放线菌门中大部分菌种的生长繁殖和复苏[71-72].Panutdaporn 等研究结果表明,Rpf 作为细胞因子,在通过生长细胞分泌到培养基中时,与 VBNC 状态菌体表面的受体蛋白结合,通过促进细胞生长而触发复苏[73].随后 Ruggiero 等研究发现,Rpf 启动细胞复苏是由于其溶壁活性直接降解 VBNC 细胞壁肽聚糖,而并非与表面受体蛋白结合[74].关于后者目前有两种观点:一种观点认为 Rpf 需要降解对菌体生长有抑制作用的肽聚糖,从而促进细胞分裂和生长恢复;另一种观点认为 Rpf 降解的肽聚糖产物可能与其他因素相互作用,并作为"第二信使"刺激 VBNC 细胞的复苏和生长[66].

最近,Ye 等研究发现添加 Rpf 的培养液可以复苏 VBNC 状态的 *Rhodococcus biphenylivorans* strain $TG9^T$,并促进此功能菌对 PCB 的降解[30].出现这一结果的主要原因是外源性 Rpf 刺激了 $TG9^T$ 中 *rpf* 同源基因(*rpfA*、*rpfD* 和 *rpfE*)的内源性表达,从而使 VBNC 状态的 $TG9^T$ 细胞复苏.$TG9^T$ 细胞的再生和增殖又导致细菌数量的积累和 PCB 分解代谢基因(*bphA* 和 *bphC*)的强烈表达,最终促进了 PCB 降解效率的提高.但其并没有解释 Rpf 如何作用来刺激菌体的复苏,因此 Rpf 是直接作用于肽聚糖来复苏 VBNC 状态功能菌还是作用于肽聚糖后释放其他信号因子引起细胞复苏还不清楚.这也是目前未能发现更多潜在功能菌的原因.在未来的研究中,可以结合分子手段来分析 Rpf 的作用机制,依据不同菌种对症下药,使其复苏并高效发挥作用.

4.2 群体感应

群体感应(quorum sensing)是一种细胞间的通讯行为,它使细菌能够根据周围微生物群落的细胞密度和物种组成变化,集体地改变行为并做出适应性的调节.这一过程通过一种被称为自诱导因子的信号分子的产生和分泌来实现[75].由基因 *luxR* 编码的 LuxR 是一种转录调控蛋白,它能够调控毒性基因的表达和细菌生物膜的形成等多种细菌生理过程[76].RpoS 则是 RNA 聚合酶的一个亚基,由 *rpoS* 基因编码并表达,在细菌应对环境胁迫时起主要调节作用[77].当环境条件允许时,由群体感应介导的过程可以作用于 VBNC 细胞,触发 *luxR* 和 *rpoS* 等相关基因的表达,从而使其复苏[78-79].Ayrapetyan 等发现自诱导因子 AI-2 可以在体内和体外复苏创伤弧菌的 VBNC 细胞,但在 *luxS* 或 *rpoS* 基因突变体的 VBNC 培养体系中菌体却无法复苏,待向培养物中添加外源 AI-2 便可重新唤醒 VBNC 细胞[64].另外,群体感应过程中 LuxR 增强了 *rpoS* 基因的表达,且随着复苏的进程,*rpoS* 基因的表达显著增加,进而使 RpoS 活性增加并引起过氧化氢酶(KatG)过量表达,这使得细胞能够抵御培养基中的过氧化氢而进行生长和复苏[64, 80-81].这些结果都说明 LuxR 和 RpoS 是 AI-2 介导 VBNC 菌体复苏的重要参与者.除此之外,LuxS 和 AI-3 等因子在 VBNC 细胞的复苏过程中同样发挥着重要作用,如 Kendall 等总结的 3 种群体感应系统[82],包括 LuxR 过程、LuxS/AI-2 系统、以及 AI-3/epinephrine/norepinephrine 系统,每种系统起关键作用的因子不同.研究还发现 OxyR 作为一种重要的调节蛋白,在抗氧化中起着关键作用,能够调控菌体的氧化应激反应[83].Liao 等研究发现,群体感应系统可以触发过氧化氢酶的表达,使菌体从 VBNC 状态复苏,而不依赖于 OxyR 调控因子[84].这个结果同 Ayrapetyan 等[64]研究结果一致,充分证实了群体感应在 VBNC 细菌复苏过程中的重要性.当然,这些因子不是独立地发挥作用,不能排除群体感应外与 VBNC 相关的其他复苏机制的影响,如严紧反应、TA 系统和 Rpf 等.

另外一方面,调控群体感应过程可以诱导活性细菌进入 VBNC 状态.水体污浊和散发臭味是一种常见的环境问题.水体表面由于细菌生物膜的存在,使大量生物幼体附着在膜上而造成小型或大型的生物污损.黄等研究发现群体感应与细菌生物膜的形成息息相关[85].对于环境治理来说,可以通过调控群体感应过程诱导水体表面细菌进入 VBNC 状态,抑制生物膜的形成进行生物防污.关于群体感应中的哪些因子或途径在调控生物膜的形成过程中发挥关键性作用有待于进一步研究.因此更加有必要研究 VBNC 细菌的群体感应机制,为发现环境中更多的潜在功能菌及高效开展环境治理提供重要参考.

4.3 丙酮酸-可代谢碳源

丙酮酸是一种抗氧化剂[86],可以清除过氧化氢[87]和羟基自由基[88],防止脂质过氧化[89].因此,利用这一特点,一些研究已经将丙酮酸描述为复苏的主要促进剂[2, 90].但近年来的报道显示,丙酮酸不仅仅是因为其可以降解细胞内过氧化物,它还可以作为可代谢碳源,并激活某些物质的代谢途径而触发 VBNC 细胞的复苏[86].Mu 等利用丙酮酸,已成功从海洋沉积物中分离出多种潜在的功能菌,其中包括

Pseudomonas putida 和 *Halomonas shengliensis*,这两种细菌可以降解多环芳烃和芳香族化合物,在生物修复中发挥重要的作用[90].最近,Vilhena 等研究也证实了丙酮酸作为可代谢碳源可以使 VBNC 细胞复苏这一结论,并发现 BtsSR 和 YpdAB 系统对丙酮酸存在下 VBNC 细胞的复苏至关重要[91].其研究结果显示,细菌在复苏过程中,通过组氨酸激酶-反应调节系统 BtsS/BtsR 和 YpdA/YpdB 来感知刺激,进而介导丙酮酸/H^+转运体 BtsT 迅速吸收丙酮酸,然后触发细胞内 DNA 和蛋白质的合成,启动细胞的复苏.通过组学手段进一步发现,缺乏这种系统的 VBNC 细胞在丙酮酸的存在下无法复苏,因为其无法恢复 DNA 和蛋白质的合成.因此,可以考虑利用该机制微调细菌对丙酮酸的吸收,来抑制/促进细菌 VBNC 的发生/复苏.

5 结论与展望(Conclusion and perspective)

随着环境污染问题的频繁爆发,功能菌群在生物修复方面的作用越来越重要.然而,实际环境中存在着各种物理、化学等胁迫因素,这些都使得实验室中分离出的高效菌种进入 VBNC 状态而无法在实际场地环境修复和污染治理中表现出较高的活性[92].如 Fida 等于 2017 年发现一株 PAH 降解菌株 *Novosphingobium* sp. LH128 在接种到菲污染土壤中后迅速进入 VBNC 状态使其无法发挥降解功能[40].因此掌握细菌进入和退出 VBNC 状态的分子机制,对于如何预防功能菌群进入 VBNC 状态,以及如何激发 VBNC 细胞在生物修复中充分发挥作用具有重要的实际意义.

自 VBNC 状态发现以来,学者们陆续提出对 VBNC 状态的检测和识别方法,例如,光学法、PCR 法和生物传感器法等.这些方法各有利弊,可根据不同菌体的特性进行选择,同时要考虑到菌体的诱导和复苏因素,而这些又与 VBNC 的形成和复苏机制紧密联系.所以,在实际应用中,不仅要针对不同菌体选择最佳复苏方式,还要结合最优的检测方法来评估 VBNC 功能菌复苏后的环境修复作用.另外,无论是 VBNC 菌体的形成还是复苏都是可逆的,细菌可以通过逆向调控 VBNC 形成机制来促进细胞的复苏,也可以通过逆向调控复苏机制来诱导 VBNC 细胞的形成.关于 VBNC 状态机制的研究已经取得了一定进展,尤其是对潜在功能菌的发掘.在这个方面,研究者主要利用 Rpf 复苏和培养污染物降解菌,并对其进行分离和研究.但环境污染问题种类多样,包括水体污染、土壤污染、化学污染和生物污染等,不同污染区的污染物和土著的微生物群落也不尽相同.所以,想要挖掘更多的功能菌种,还需对 VBNC 细菌不同的复苏机制及其具体调控通路进行深入的探究.现在大多数文献采用单一转录组学或蛋白质组学方法,即便对两者进行整合分析也很难实现信息补足和功能验证[93-94].这些结果只能说明部分基因或蛋白质参与了细胞 VBNC 状态的形成,并不足以揭示其具体的作用机制.

因此,在未来的研究中,可以对以下三个方面进行深入探究,包括:(1)环境中的潜在功能菌普遍存在于不同的污染场所,许多未发掘的 VBNC 细菌具有很好的污染降解能力.因此,可以根据上述复苏机制,利用 Rpf、群体感应分子和丙酮酸等在污染场所对 VBNC 细菌进行复苏.由于不同污染区的细菌感知刺激并传递信号的途径不同,所以需要对本地细菌建立小的培养体系,研究不同因子的添加对其复苏及污染物降解的影响,根据 VBNC 细菌对不同因子的响应,选择合适的复苏方式提高本地细菌的降解能力,达到加速生物修复污染物的目的.(2)在控制污损现象方面,可以通过将水体表面的微生物诱导进入 VBNC 状态,使其无法形成生物膜进行治理.在细菌进入 VBNC 状态的过程中,涉及众多基因和蛋白质分子,如何选择合适的方法来调节关键性因子或形成再生延迟体隔离生长所需的蛋白质分子使其进入 VBNC 状态需要进一步探究.同时在诱导水体表面微生物进入 VBNC 状态时,如何避免其他有益微生物进入该状态也是一个值得研究的问题.(3)在实际场地治理中,接种目标功能菌种并不能使治理效果达到理想状态,这可能是降解菌由于污染物的强选择压力迅速进入 VBNC 状态所致.微生物群落复杂,利用群体感应提高其污染治理能力是一种有效途径.那么目标菌种如何在多种细菌产生信号分子的情况下进行有效的复苏有待于进一步的探究.对以上研究方向的深入研究,对于揭示 VBNC 状态功能菌在环境污染的治理和控制以及在物质循环方面的贡献具有重要的理论意义和实践价值.

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