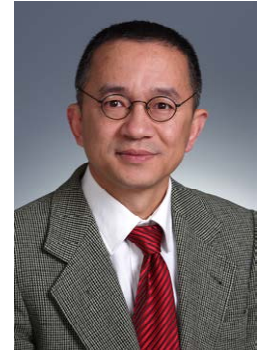


· 综 述 ·

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降解石油基塑料的微生物及微生物菌群

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摘要: 伴随着环境污染的日益严重, 处理“白色污染”成为人们面临的一个棘手难题, 而各种合成塑料因为应用广泛且很难降解成为其“主要元凶”。利用自然界存在的或者是进化产生的微生物可降解合成塑料是一种环境友好型的策略。以国家自然科学基金国际(地区)合作和交流(中欧组织间合作研究 NSFC-EU)项目“合成塑料降解转化微生物菌群”为基础, 总结近年来筛选到的能够降解合成塑料, 如聚乙烯(Polyethylene, PE)、聚丙烯(Polypropylene, PP)、聚苯乙烯(Polystyrene, PS)、聚氯乙烯(Polyvinyl chloride, PVC)、聚氨酯(Polyurethane, PUR)、聚对苯二甲酸乙二醇酯(Polyethylene terephthalate, PET)的纯细菌、纯真菌及微生物菌群的研究状况, 分析了各种微生物在石油基塑料降解中的作用, 讨论了微生物及其降解酶对合成塑料降解研究的优缺点。

关键词: 石油基塑料, 细菌, 真菌, 菌群, 生物降解

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Degradation of petroleum-based plastics by microbes and microbial consortia

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Abstract: Along with the increasingly serious environmental pollution, dealing with the “white pollution” issue, which is caused by the worldwide use of not readily-degradable or non-degradable synthetic plastics, has become a great challenge. It is an environmentally friendly strategy to degrade synthetic plastics using microorganisms that exist in nature or evolved under selection pressure. Based on the NSFC-EU International Cooperation and Exchanges Project “Bio Innovation of a Circular Economy for Plastics”, this review summarized the screening of bacteria, fungi and microbial consortia capable of degrading synthetic plastics such as polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), polyurethane (PUR), and polyethylene terephthalate (PET). We also analyzed the role of various microorganisms played in the degradation of petroleum-based plastics. Moreover, we discussed the pros and cons of using microorganisms and enzymes for degradation of synthetic plastics.

Keywords: petroleum-based plastics, bacteria, fungi, consortia, biodegradation

由于具有耐久性且成本较低,石油基塑料已被广泛应用,市场需求不断增加^[1-2]。根据Plastics-Europe最新统计,2019年全球塑料产量达到3.7亿t^[3]。然而,塑料的广泛使用不可避免地造成了对环境的负面影响,因为大部分塑料制品在自然环境中的降解率很低,很容易在环境中堆积,造成塑料污染问题^[4]。与此同时,塑料的不正确处理也会影响环境安全,给全球生态系统带来负担^[5-6]。例如,传统的塑料处理方法主要以填埋与焚烧方式为主,不仅耗能巨大、费时费力,而且填埋过程中产生的危险化学物质会污染地下水以及焚烧产生的二噁英等有害气体也会对大气等生态环境造成严重污染,存在巨大的安全隐患与应用局限^[7-9]。据报道,越来越多的塑料废物也在进入海洋环境,在2010年全球沿海国家就有多达1 200万t塑料进入海洋,这一数字还在稳步增长^[10]。这些进入海洋环境的塑料经过生物和非生物因素变成直径很小的微塑料,被海洋生物捕食后进入食物链,最终影响人类安全^[11]。尽管机械回收已成为主要的回收方法,并已被用于

再利用热塑性废料,但是在经过多个处理循环后,大多数回收材料的性能受到很大损害,其商业价值受到限制。除此之外,化学回收可以从塑料废物中回收单体和其他化合物,但其成功与否取决于工艺的可承受性和催化剂的效率,这是难以控制的^[12-13]。

虽然随着塑料污染问题受到越来越多的关注,很多方法被应用以缓解环境压力,如选择并有效地收集和回收日常生活中使用的不同塑料,减少微塑料的倾倒和垃圾掩埋,禁止使用非必要的塑料产品,以及限制塑料微粒在商业产品中的使用等。然而,目前塑料废物污染已成为严峻的环境和社会问题,开发处理塑料废物的新方法变得越来越迫切。近年来,有近千篇关于微生物降解塑料的报道涌现,许多塑料降解微生物被发现。越来越多的研究表明,生物法处理塑料污染问题具有很大的可行性^[14]。微生物降解为废弃塑料的“原位处理”提供了条件,高效的塑料降解微生物的筛选为发展塑料废物生物处理技术提供了帮助。

1 降解塑料的细菌

许多细菌具有降解污染物的能力。细菌菌体微小,相对表面积很大,物质交换频繁、迅速,代谢十分活跃。各种细菌因其营养需求、能量来源、酶系统和代谢产物的不同,形成多种多样的代谢类型,可以适应复杂的外界环境。这些特性为细菌降解塑料提供了基础。近年来,科学家们从不同环境如土壤、海洋甚至是昆虫肠道中分离出一些细菌,它们可以对不同的塑料制品有不同程度的降解^[15-16](表1)。

聚对苯二甲酸乙二醇酯 (Polyethylene terephthalate, PET) 是一种由对苯二甲酸和乙二醇通过重复酯键构成的热塑性聚酯,广泛应用于食品包装材料、饮料瓶、涂料和纤维。最著名的能够降解 PET 的细菌是 2016 年由 Yoshida 等从 PET 垃圾场分离出的中温细菌 (*Ideonella sakaiensis* 201-F6),它能够在 30 °C 以无定形 PET 为主要碳源生长,并分泌两种酶将其降解为单体^[17]。2017 年, Fauziah 等从马来西亚红树林生态系统中分离出的芽孢杆菌 *Bacillus cereus* 和 *B. gottheilii* 都可降解 PET 微塑料^[18]。2020 年,研究人员从海水中分离到 1 株降解 PET 薄膜的弧菌 *Vibrio* sp.^[19]。这表明随着塑料污染日益严重,塑料降解菌的来源在不断扩展,不再局限于从土壤环境中分离获得。更多关于降解 PET 的细菌的研究聚焦于水解酶的鉴定、表征、表达和优化。PET 的重复酯键赋予它可以被一些脂酶和角质酶降解的特性。但由于高比例的芳香对苯二甲酸乙二酯单元降低了链的流动性, PET 的酯键水解性极低。另一方面, PET 是一种半结晶聚合物,而酶通常只能攻击非结晶区。例如, *I. sakaiensis* 来源的 IsPETase 通过不同宿主表达分泌,可以实现对 PET 薄膜的降解^[20]。但由于 IsPETase 结构稳定性和溶解性

较低, IsPETase 的应用很难达到工业要求。PET 的玻璃化转变温度 (T_g) 超过 65 °C^[21],在接近这个温度时,聚合物的非结晶部分变得灵活,更容易受到酶的攻击^[22]。因此降解 PET 的细菌和酶就要求在较高的温度下保持活性^[23]。研究发现,从土壤中分离出的嗜热裂孢菌 *Thermobifida* sp. 通过分泌一些嗜热酶,如 TfH、BTA2、Tfu_0882、TfCut1 和 TfCut2,在高温下 (50–70 °C) 能将 PET 降解^[24]。另外一种有效的嗜热酶是从叶枝堆肥中分离出的角质酶 (Leaf-branch compost cutinase, LCC)。通过在大肠杆菌 *Escherichia coli* 中过表达、纯化并表征后,证明了 LCC 的 PET 降解活性^[25]。随后有研究通过突变提高 LCC 热稳定性,开发了在 72 °C 降解 PET 的工艺,能够在 10 h 时内降解至少 90% 的 PET^[26]。2020 年, Yan 等研究发现可以通过嗜热厌氧菌——热梭菌 *Clostridium thermocellum* 高效表达分泌 LCC,显著降解 PET 薄膜^[27]。以上研究表明,工程化改造酶是提高 PET 降解效率的有效方法,同时嗜高温菌株对于 PET 的降解更具优势。

聚乙烯 (Polyethylene, PE) 根据聚合方法、分子量高低、链结构的不同,可以分为高密度聚乙烯 (High density polyethylene, HDPE)、低密度聚乙烯 (Low density polyethylene, LDPE) 及线性低密度聚乙烯 (Linear low density polyethylene, LLDPE)^[28]。已报道的能够降解 PE 的细菌包括不动杆菌 *Acinetobacter* sp.、食烷菌 *Alcanivorax* sp.、芽孢杆菌 *Bacillus* sp.、肠杆菌 *Enterobacter* sp.、苍白杆菌 *Ochrobactrum* sp. 和类芽孢杆菌 *Paenibacillus* sp. 等。研究者从垃圾回收站分离出的克雷伯氏肺炎杆菌 (*Klebsiella pneumoniae* CH001)、肠杆菌 (*Enterobacter* sp. bengaluru-btdsce01, *Enterobacter* sp. bengaluru-btdsce02) 和泛生菌 (*Pantoea* sp.

bengaluru-btdsce03), 分别能够降解 HDPE 和 LDPE^[29-30]。阴沟肠杆菌 (*E. cloacae* AKS7) 能够有效降解 LDPE, 且 AKS7 的细胞表面疏水性有利于生物膜形成, 提高 AKS7 对 LDPE 的降解能力^[31]。有研究还发现霍氏肠杆菌 (*E. hormaechei* LB-1) 能够降解 PE, 并产生酰胺类、有机酸等可溶性物质^[32]。这些研究表明, 肠杆菌属可能是降解 PE 的优势菌种。

聚苯乙烯 (Polystyrene, PS) 是用苯乙烯合成的人造芳香族聚合物, 应用非常广泛。降解 PS 的细菌有产碱杆菌 *Alcaligenes* sp.、芽孢杆菌 *Bacillus* spp.、微杆菌 *Exiguobacterium* sp.、假单胞菌 *Pseudomonas* sp. 和沙门氏菌 *Salmonella* sp. 等。Mohan 和 Umamaheswari 等将从土壤中分离所得的芽孢杆菌 *Bacillus* spp. 和假单胞菌 *Pseudomonas* sp. 作用于 PS, 发现它们均能降解 PS^[33-34]。研究发现一些动物幼虫能够以 PS 为食, 且幼虫肠道内的微生物被认为是其能够以塑料为食的主要原因^[35-37]。我国研究人员曾经从黄粉虫和赤锥蝽的幼虫肠道分别分离出微小杆菌 (*Exiguobacterium* sp. YT2) 和不动杆菌 *Acinetobacter* sp., 它们都能够降解 PS, 且 YT2 作用于 PS 60 d 后可以检测到水溶性产物的存在^[38-39]。这一发现再次扩展了塑料降解菌的来源范围, 开发菌种筛选的新思路。

聚氨酯 (Polyurethane, PUR) 是由氨基甲酸酯键连接的二异氰酸酯和多元醇组成的聚合物。根据多元醇的不同可以制成不同特性的聚醚和聚酯 PUR。细菌降解 PUR 的报道出现时间相对较晚。1991 年, 从掩埋在土壤中的线性聚酯 PUR 表面分离出的细菌, 以交联聚酯 PUR 泡沫为唯一碳源, 发现只有棒状杆菌 (*Corynebacterium* sp. B12) 和

铜绿假单胞菌 (*P. aeruginosa* B16) 能降解交联聚酯 PUR^[40]。之后, 又陆续从土壤环境中发现了能够降解 PUR 的细菌。2013 年, 从土壤中分离出枯草芽孢杆菌 (*B. subtilis* MZA-75) 和铜绿假单胞菌 (*P. aeruginosa* MZA-85), 它们可以用线性聚酯 PUR 作为唯一碳源进行生长^[41-43]。随着聚酯 PUR 的降解研究进展, 科学家们发现一些细菌会分泌胞外酶对聚酯 PUR 起降解作用, 包括酰胺酶和酯酶^[44-45]。由于聚酯和聚醚聚氨酯结构的不同, 导致它们对微生物的耐受程度也有差异。2017 年研究者对分离自土壤的不同微生物降解聚醚 PUR 的能力进行了研究, 通过对降解能力的验证和对微生物培养前后的 PUR 特性进行比较分析, 表明聚醚 PUR 结构更稳定, 更难被降解^[46]。究其原因可能是聚酯 PUR 分子主链中的酯键要比聚醚 PUR 分子主链中的醚键更易受到微生物的攻击。

到目前为止, 关于生物降解聚丙烯 (Polypropylene, PP) 和聚氯乙烯 (Polyvinyl chloride, PVC) 的报道还很少。1993 年, 对微生物降解 PP 进行了首次尝试, 发现微生物能够降解 PP^[47]。近年来研究者从红树林不同深度的沉积物中分离得到芽孢杆菌 (*Bacillus* sp. 27) 和红球菌 (*Rhodococcus* sp. 36), 它们可定植于 PP 表面并利用 PP 作为碳源^[48]。2019 年, 研究者发现香茅醇假单胞菌 (*P. citronellolis* DSM50332) 和弯曲芽孢杆菌 (*B. flexus* DSM1320) 可以降解含有约 35% 添加剂的 PVC 薄膜^[49]。同年还在海洋中分离到 1 株对 PVC 有降解作用的芽孢杆菌 (*Bacillus* sp. AIIW2)^[50]。最近研究者又在黄粉虫肠道中分离出属于哈夫尼菌属 *Hafnia* 的菌株, 它能显著降低 PVC 的分子量并降解得到小分子物质^[51]。

表 1 降解各种石油基塑料的细菌

Table 1 The bacteria capable of degrading petroleum-based plastics

Strain	Substrate	Biodegradation conditions	Biodegradability	References
<i>Ideonella sakaiensis</i> 201-F6	PET films	Reaction at 30 °C for 18 h ^a	PET was degraded to MHET and further degraded to terephthalic acid and ethylene glycol	[17]
<i>Bacillus cereus</i> , <i>Bacillus gottheilii</i> <i>Thermobifida fusca</i>	PET particles PET films	Incubate at 29 °C, 150 r/min for 40 d Reaction at 65 °C for 48 h ^a	The weight loss was 6.6% and 3.0%, respectively The weight loss was 12.9%±1.2% and 12.6%±0.2%, respectively	[18] [24]
<i>Escherichia coli</i>	PET	Reaction at pH 8.0, 50 °C ^{ab}	The degradation activity was 12.0 mg/(h·mg _{enzyme})	[25]
<i>Clostridium thermocellum</i>	PET films	Incubate at 60 °C for 14 d	More than 60.0% of PET film was converted into soluble monomer	[27]
<i>Klebsiella pneumoniae</i> CH001	HDPE films	Incubate at 120 r/min 30 °C for 60 d	The weight loss was 18.4%	[29]
<i>Enterobacter</i> sp. Bengaluru-btdsce01, <i>Enterobacter</i> sp. Bengaluru-btdsce02, <i>Pantoea</i> sp. bengaluru-btdsce03	LDPE	Incubate at 37 °C for 120 d	The weight loss of LDPE strips was 70.0%±4.0%, 68.0%±4.0%, 64.0%±4.0%; the weight loss of LDPE particles was 21.0%±2.0%, 28.0%±2.0%, 24.0%±2.0%, respectively	[30]
<i>Enterobacter cloacae</i> AKS7	LDPE films	Incubate at 30 °C for 45 d	The weight loss was 9.0%	[31]
<i>Enterobacter hormaechei</i> LB-1	PE films	Incubate at 30 °C for 60 d	The weight loss was 12.2%	[32]
<i>Bacillus</i> spp., <i>Pseudomonas</i> spp.	HIPS	Initial incubation at 50 °C for 20 min, then incubation at 30 °C for 4 d	The weight loss was 23.7% and <10.0%, respectively	[33]
<i>Pseudomonas</i> sp.	PS films	Incubate at 37 °C, 120 r/min for 30 d	Detected the degradation products	[34]
<i>Exiguobacterium</i> sp. YT2	PS films	Incubate for 60 d	The weight loss was 7.4%±0.4%; the molecular weight was reduced by 11.0%	[38]
<i>Acinetobacter</i> sp. AnTc-1	PS powder	Incubate at 27 °C for 60 d	The weight loss was 12.1%±1.4%; the molecular weight reduction by 13.0%–25.0%	[39]
<i>Corynebacterium</i> sp. B12, <i>Pseudomonas aeruginosa</i> B16	Polyester PUR foam	Incubate for 12 weeks	The weight loss was 15.8% and 9.3%, respectively	[40]
<i>Bacillus subtilis</i> MZA-75, <i>Pseudomonas aeruginosa</i> MZA-85	Polyester PUR films	Incubate at 150 r/min, 37 °C for 30 d	The weight loss was 20.0%	[41-43]
<i>Pseudomonas</i> sp.	PUR films	Incubate at 37 °C for 10 d ^a	The weight loss was 30.0% and 70.0% after incubating 5 and 10 d	[44]
<i>Escherichia coli</i>	PUR films	Incubate at 37 °C for 51 d ^{ab}	The weight loss was 33.0%	[45]
<i>Bacillus</i> sp. 27, <i>Rhodococcus</i> sp. 36	PP particle	Incubate at 29 °C for 40 d	The weight loss was 4.0% and 6.4%, respectively	[48]
<i>Pseudomonas citronellolis</i> DSM50332	PVC films	Incubate at 30 °C for 30 d	The weight loss was between (13.07%±0.36%)–(18.58%±0.01%)	[49]
<i>Bacillus</i> sp. AIIW2	PVC films	Incubate at 30 °C, 180 r/min for 3 months	The weight loss was 0.26%	[50]

Note: ^a The reaction condition of the enzyme secreted by the bacteria; ^b The *Escherichia coli* involved in the table is a host for heterologous expression of plastic-degrading genes.

目前,虽然在海洋、堆肥、其他水环境和昆虫肠道中都发现了塑料降解菌的存在,但由于土壤中微生物多样性较高,因此大多降解塑料的细菌都从土壤中分离获得,其中,肠杆菌属、芽孢杆菌属和假单胞菌属是降解各种塑料较常见的细菌。

2 降解塑料的真菌

真菌也具有粘附和利用塑料的潜力,它通过丝状结构延伸来寻找营养,在其他微生物难以到达的地方生长,适应不断变化的环境并能耐受多种类型的塑料污染物。它还可以促进塑料中不同化学键的形成,例如羰基、羧基和酯基,从而降低其疏水性^[52]。真菌是异养生物,通过吸收细胞外的营养物生长,并通过菌丝的胞吐作用释放消化酶,从而将大分子和有机分子分解成较小的有机化合物以吸收^[53]。真菌具有酶促系统、吸附能力,还能够产生天然生物表面活性剂,利用聚合物作为碳和电子的来源,分别为其提供细胞材料和能源^[54](表2)。

2015年,研究者将从垃圾场土壤中分离所得的微生物与PE粉末孵育后,发现黑曲霉 *Aspergillus niger* 和黄曲霉 (*A. flavus*) 对PE有降解作用^[55]。后来研究者分别从土壤中分离出棒曲霉 (*A. clavatus* JASK1)、米曲霉 (*A. oryzae* A5, 1)、黄曲霉 (*A. flavus*) 和土曲霉 (*A. terreus*),它们都能降解PE,且研究者通过对从不同采样点分离得到的细菌和真菌对PE薄膜的降解水平进行评估,确定真菌通常比细菌更易降解PE^[56-58],同样表明相对于其他真菌,曲霉对PE的降解更具优势。2017年,研究人员还从海洋中分离得到一株海洋真菌 *Zalerion maritimum*,与PE颗粒接触一段时间后,PE颗粒的大小和重量都有所下降,表明该真菌同样具有PE降解性^[59]。

在较早的年代,科学家们就已经对有关真菌对于PUR的降解作出了探究。1968年,科研工

作者第一次进行了真菌降解PUR的研究,将PUR薄膜贴在平板表面,平板上分别接种了7种真菌,观察结果得出,这些真菌均能在聚酯PUR表面大量生长,但不能在聚醚PUR表面生长^[60]。因此可以推测,对于真菌而言,聚醚PUR更难降解。Álvarez-Barragán等在2016年以PUR为唯一碳源筛选出8株降解菌,其中6株属于芽枝状枝孢霉 *Cladosporium cladosporioides*,另外2株被鉴定为烟曲霉 (*A. fumigatus*) 和产黄青霉 *Penicillium chrysogenum*,其中 *C. cladosporioides* sp. T1.PL降解率最高^[61]。研究者们后来分别从垃圾场中分离出一些曲霉菌,它们可以降解线性聚酯PUR^[62-64]。2018年,又在漂浮塑料碎片上确定了3个腐生真菌能够降解PUR,其中芽枝状枝孢霉 (*C. cladosporioides*) 最高效^[65]。同年,Oprea等在染病的落叶中发现一株能够降解聚醚PUR的真菌——细极交链孢霉 *Alternaria tenuissima*^[66]。2019年Magnin等从含PUR的肥料中筛选真菌,发现有3种真菌能够以PUR为唯一碳源,其中青霉 (*Penicillium* sp.) 降解率最高^[67]。从以上研究可以看出,PUR的复杂结构引起了许多研究人员的兴趣。通过实验探究发现,曲霉菌是PUR降解的优势菌株,其次是青霉菌和孢霉菌。

目前关于真菌对PVC、PS和PET的降解的研究报道还很少。2018年,我国科研人员从黄粉虫肠道中分离出能够降解PS的黑曲霉 (*Aspergillus niger* KHJ-1)^[68]。最近,头孢霉菌 *Cephalosporium* sp. 对PS的降解作用也被研究证实^[69]。2019年,球毛壳霉 *Chaetomium globosum* 被用来研究对PVC的降解情况,孵育后可以观察到该菌在PVC上粘附并生长^[70]。同年,研究者通过在毕赤酵母 *Pichia pastoris* 细胞中表达PETase开发了一种全细胞生物催化系统,提高了它的降解效率^[71],这表明研究者可以通过在真菌系统中表达PET降解酶,使一些真菌获得降解PET的能

表 2 降解石油基塑料的真菌

Table 2 The fungi capable of degrading petroleum-based plastics

Strain	Substrate	Biodegradation conditions	Biodegradability	References
<i>Streptomyces</i> sp., <i>Aspergillus niger</i> , <i>Aspergillus flavus</i>	LDPE particle	Incubate at 30 °C, 120 r/min for 6 months	The weight loss was 46.7%, 26.0% and 16.0%, respectively	[55]
<i>Aspergillus oryzae</i> A5, 1	LDPE	Incubate at 28 °C for 16 weeks	The weight loss was 36.40%±5.53%	[57]
<i>Aspergillus flavus</i> , <i>Aspergillus terreus</i>	LDPE particle	Incubate for 9 months in soil, 4 months in synthetic medium	In soil, the weight loss was 30.6% and 13.1%, respectively; in synthetic medium, the weight loss was 14.3% and 11.4%, respectively	[58]
<i>Zalerion maritimum</i>	PE pellets	Incubate at 25 °C, 120 r/min for 28 d	The size of the pellets decreased	[59]
<i>Aspergillus niger</i> QM386, <i>Aspergillus flavus</i> QM380, <i>Aspergillus versicolor</i> QM432, <i>Penicillium</i> <i>funiculosum</i> QM391, <i>Pullularia pullulans</i> QM279c, <i>Trichoderma</i> sp. QM365, <i>Chaetomium</i> <i>globosum</i> QM459	PUR films	Incubate at 30 °C for 3 weeks	All could grow on the surface of the polyester PUR film, and could not grow on the surface of the polyether PUR film	[60]
<i>C. cladosporioides</i> sp. T1.PL	Polyester PUR	Incubate at 30 °C for 14 d	The weight loss was 87.0%	[61]
<i>Aspergillus tubingensis</i>	Polyester PUR films	MSN agar plates were incubated at 37 °C for 4 d; liquid MSN medium was incubated at 37 °C for 20 d, then kept at room temperature for 2 months; PUR in soil was incubated at room temperature for 4 months	Degrade linear polyester PUR film on MSN agar plate and form holes on its surface; in liquid MSN medium containing 2% glucose, the bacteria can degrade linear polyester PUR film into fragments; in soil clearly observe small holes, corrosion, cracks, loss of tensile strength and surface roughness	[62]
<i>Aspergillus</i> sp. S45	Polyester PUR films	Incubate at 30 °C for 28 d	The weight loss was 20.0%	[63]
<i>Aspergillus niger</i>	PUR sheets	Incubate at room temperature for 3 months	The weight loss was 2.18%	[64]
<i>C. cladosporioides</i>	PUR	Incubate for at least 3 weeks in the dark at room temperature	Transparent circle expansion rate was approximately 4 mm/d	[65]
<i>Penicillium</i> sp.	PUR	Incubate at 30 °C for 2 months	The weight loss was 8.9%	[67]
<i>Aspergillus niger</i> KHJ-1	PS particle	Incubate at 30 °C for 60 d	The weight loss was 4.29%	[68]
<i>Cephalosporium</i> sp.	PS strips	Incubate at 28 °C, 120 r/min for 8 weeks	The weight loss was 2.17%±0.16%	[69]
<i>Chaetomium globosum</i>	PVC films	Incubate at 28 °C for 28 d	The fungus adhered and grew on PVC	[70]
<i>Pichia pastoris</i>	PET films	Reaction at 30 °C for 18 h ^{ab}	The conversion rate was increased by about 36 times	[71]

Note: ^a The reaction condition of the enzyme secreted by the fungi; ^b The *Pichia pastoris* involved in the table is a host for heterologous expression of plastic-degrading genes.

力,而且降解效率可能会得到有效提升。最近又从海洋环境中分离出能降解 PET 的曲霉菌 *Aspergillus* sp.^[19],再一次证实了全球塑料污染范围不断变广,也给世界敲响了警钟,解决塑料污染刻不容缓!

3 降解塑料的菌群

使用纯菌株的优势在于,它可以研究代谢途径或评估不同环境条件对塑料降解的影响。此外,还可以密切监控功能性菌株降解塑料的过程以及塑料中发生的变化^[52]。然而,与纯分离物降解塑料相比,微生物菌群因其高效且生态友好的降解特性而具有相当大的优势。当前的研究表明微生物菌群可以通过共代谢提高降解效率。目前利用自然菌群有效降解塑料的研究已经有了一定进展,而将具有不同属性的菌种配制成菌群的策略也成为了研究热点^[72](表 3)。

研究表明,自然界天然组成的菌群对一些塑料具有降解性。近年来,研究者分离出了一些自然菌群,经鉴定分别由不同的菌种组成,它们都可以降解 PE^[73-76]。Anwar 等从土壤中分离得到了菌群,包括假单胞菌属 (*P. otitidis*)、蜡状芽孢杆菌 (*B. cereus*) 和足棘皮杆菌 *Acanthopleurobacter pedis*,并进一步通过一系列方法分析了不同孵育时间下菌群对 PVC 的降解情况^[77]。最近, Giacomucci 等从海洋样品中富集了 16 个厌氧菌群,并对不同菌群降解 PVC 的能力进行了评估^[78]。一系列研究表明,自然菌群对不同的塑料都有降解性,且通过菌群中菌株的相互作用,可能会达到比单一菌株更有效的降解结果。这是相对于纯菌株而言菌群降解塑料逐渐成为科学家们研究热点的原因所在。

2017 年, Syranidou 等对人工菌群降解塑料的能力进行了探索。将自然风化后的 PS 薄膜与海洋中上层自然菌群 (Indigenous, INDG) 或经生物增强 (Bioaugmented, BIOG) 的菌群进行孵育,

发现红球菌 *Rhodococcus* sp.、希瓦氏菌 *Shewanella* sp. 和假单胞菌 *Pseudomonas* sp. 组成的菌群可以在 PS 上生长,并以 PS 作为碳源^[79]。之后,他们又在过滤盐水中,分两阶段进行微观实验,结果表明 INDG 和 BIOG 处理的聚合物链发生了断裂^[80]。这表明不仅自然菌群能够降解塑料,人工菌群对塑料也有降解能力。

Skariyachan 等从垃圾加工厂分离菌群,将筛选得到的 3 株最佳菌株配制为人工菌群,发现该人工菌群可以降解 LDPE^[30]。他们又从塑料污染点的牛粪中分离出死谷芽孢杆菌 (*B. vallismortis* bt-dsce01)、假单胞菌 (*P. protegens* bt-dsce02)、寡养单胞菌 (*Stenotrophomonas* sp. bt-dsce03) 和类芽孢杆菌 (*Paenibacillus* sp. bt-dsce04)。它们组成的人工嗜热菌群与 LDPE 和 HDPE 共同孵育后,可以观察到明显的重量损失^[72]。研究发现将芽孢杆菌 *Bacillus* sp. 和假单胞菌 *Pseudomonas* sp. 共培养,与经预处理的 PP 薄膜孵育后,PP 表面形成生物膜^[81]。2018 年,研究者从污水处理厂筛选菌群,发现由一些杆菌组成的菌群能够显著降解 LDPE、HDPE、PP^[82]。2019 年,有报道称香茅醇假单胞菌 (*P. citronellolis*) 和弯曲芽孢杆菌 (*B. flexus*) 能在 PVC 薄膜表面形成生物膜^[49]。人工菌群成功降解塑料的发现使研究者们可以将不同的菌株按比例自由搭配,从而得到最优菌群,达到理想的降解效果。近年, Yin 等从黄粉虫 *Tenebrio molitor* 肠道中分离得到不动杆菌 (*Acinetobacter* sp. NyZ450) 和芽孢杆菌 (*Bacillus* sp. NyZ451),这两株菌按比例共培养,与 PE 薄膜孵育 30 d, PE 薄膜重量损失 18%。经过平板菌落计数法和电子显微镜观察表明 NyZ450 在共培养中占主导地位^[83]。2021 年,孙超岷等从青岛近海采集了上千份塑料垃圾,经过大量筛选发现 1 个在 PET 和 PE 表面具有很明显的定植和降解能力的菌群^[84]。作者从该菌群中分离出 3 株具有明显降解塑料能力的细菌,将它们按照一定比

表 3 降解各种石油基塑料的菌群

Table 3 The microbial consortia capable of degrading petroleum-based plastics

Strain	Substrate	Biodegradation conditions	Biodegradability	References
<i>Comamonas</i> , <i>Delftia</i> , <i>Stenotrophomonas</i>	LDPE	Incubate at 28 °C for 90 d	Metabolic activity and cell viability remained until the end of the experiment and the viscosity area of PE decreased by 6.7%	[73]
<i>Bacillus</i> spp., <i>Pseudomonas</i> spp.	HDPE	Incubate at 30 °C for 4 weeks	The weight loss was 23.14%±0.24%	[74]
<i>Bacillus</i> sp., <i>Paenibacillus</i> sp.	PE	Incubate at 30 °C for 60 d	The weight loss was 14.7%, and the average particle size was reduced by 22.8%	[75]
<i>Agios Onoufriou</i> and <i>Kalathas</i>	PE	Incubate at 25 °C, 120 r/min for 6 months	The weight loss was 19.0%	[76]
3 marine consortia incubated in different media	PVC	Static incubation at 20 °C for 7 months	The weight loss was 11.67%±0.58%, 10.71%±0.54%, 6.26%±0.31%, respectively	[78]
<i>Rhodococcus</i> sp., <i>Shewanella</i> sp., <i>Pseudomonas</i> sp.	PS	Incubate for 6 months	The weight loss after BIOG and INDG treatment was 4.7% and 2.3%, respectively	[79]
<i>Enterobacter</i> sp. bengaluru-btdsce01, <i>Enterobacter</i> sp. bengaluru-btdsce02, <i>Pantoea</i> sp. bengaluru-btdsce03	LDPE	Incubate at 45 °C for 120 d	The weight loss of LDPE strips and LDPE particles was 81.0%±4.0% and 38.0%±3.0%, respectively	[30]
<i>Bacillus vallismortis</i> bt-dsce01, <i>Pseudomonas</i> <i>protegens</i> bt-dsce02, <i>Stenotrophomonas</i> sp. bt-dsce03, <i>Paenibacillus</i> sp. bt-dsce04	LDPE, HDPE	Incubate at 55 °C for 120 d	The weight loss of LDPE films, pellets, HDPE films and pellets was 75.0%±2.0%, 55.0%±2.0%, 60.0%±3.0% and 43.0%±3.0%, respectively	[72]
<i>Bacillus</i> , <i>Pseudomonas</i>	PP	Incubate at 28 °C for 1 year	The weight loss was 1.95%±0.18%	[81]
<i>Aneurinibacillus</i> <i>aneuriniolyticus</i> btDSCE01, <i>Brevibacillus agri</i> btDSCE02, <i>Brevibacillus</i> sp. btDSCE03, <i>Brevibacillus brevis</i> btDSCE04	LDPE, HDPE, PP	Incubate at 50 °C for 140 d	The weight loss of LDPE, HDPE and PP strips was 58.2%±2.0%, 46.6%±3.0% and 56.3%±2.0%, respectively, and the weight loss of LDPE, HDPE and PP particles was 45.7%±3.0%, 37.2%±3.0% and 44.2%±3.0%, respectively	[82]
<i>Pseudomonas citronellolis</i> , <i>Bacillus flexus</i>	PVC	Incubate for 45 d	The average molecular weight was reduced by 10.0%	[49]
<i>Acinetobacter</i> sp. NyZ450, <i>Bacillus</i> sp. NyZ451	PE films	Incubate at 23 °C, 180 r/min for 30 d	The weight loss was 18.0%	[83]
<i>Exiguobacterium</i> sp., <i>Halomonas</i> sp., <i>Ochrobactrum</i> sp.	PET and PE films	Incubate for 2 weeks	Both PET and PE films were fully degraded into small pieces	[84]

例进行复配,成功获得一个能稳定共存并具有显著降解 PET 和 PE 塑料垃圾的菌群。该菌群尤其喜好降解 PE 塑料,两周时间可以将 PE 降解为碎片。

在微生物菌群降解塑料的研究中,不管是从环境中获取的天然菌群或是人工配制的菌群大多都以芽孢杆菌属和假单胞菌属为主要研究对象,表明芽孢杆菌属和假单胞菌属不仅能够单独降解塑料,还可以在菌群中与其他微生物相互作用,促进塑料的有效降解。

总体而言,塑料降解菌株似乎分散在整个微生物发育树中,其中主要的一些细菌门和真菌门都包含已报道可以降解塑料的菌种,但目前还没有发现古生菌具有塑料降解能力。大多数具有塑料降解能力的菌种都是细菌,它们主要属于变形菌门、放线菌门、厚壁菌门、拟杆菌门和蓝细菌;而已报道的真菌也主要属于子囊菌门、担子菌门和毛霉菌门。据统计,变形菌门是塑料降解菌中最常观察到的细菌门,其中最常见的是假单胞菌属,属于 γ 变形菌。据报道,在所有已报道的 66 种塑料中, γ -变形菌可以降解其中 43 种塑料,包括大多数大规模生产的合成塑料,如 PE、PET、PP、PS、PVC 和 PUR。而假单胞菌属就可以降解 35 种不同的塑料类型。假单胞菌适应性强,用途广泛,在环境中无处不在,这些数据更突出了其对塑料降解的重要作用和潜力^[85]。

4 挑战与前景展望

塑料的生物降解研究虽然已经发展了一段时间,但仍存在一些急需攻克的难题,导致塑料的生物降解技术难以在实际应用中发挥作用。限制塑料降解的因素有很多。首先,塑料是由不同单体聚合而成的大分子聚合物,很难被微生物吸收同化,且作为一种固体废物,微生物或酶只能从塑料表面开始发挥作用,其致密交联结构导致生物利用率很低^[86]。其次,塑料的降解速率也受到其形态的显著影响,包括结晶度和物理形态^[52]。

研究表明,聚合物的非晶态部分更容易被酶降解,因为非结晶区在接近玻璃化转变温度的条件下,其分子链会更活跃,酶的可及性增加,从而提高降解速率^[4]。最后,塑料表面疏水性也是限制塑料降解的主要因素之一,微生物定植以及胞外酶的活性会随着疏水性的增加而受到抑制^[7]。表面亲水性的增加,具有更高的表面能,与水的接触角更低,从而促进微生物附着,并加快降解速度。

另外,目前对于塑料降解的研究往往需要借助一些间接方法如计算质量损失、鉴定表面修饰、检测代谢产物或观察微生物生长来确定^[86]。然而,这些常用的分析方法不仅各有利弊^[2],其所得的结果也可能是由生物降解以外的原因引起,因此对塑料降解的解释力有限^[86]。除此之外,在筛选菌株的过程中也会遇到挑战。例如,通过微生物降解研究发现,目前分离得到的菌株大多效率较低,菌株分泌的酶活性普遍偏低,且酶量也不高,这都限制了塑料在环境中的生物降解^[16]。

因此,从环境中分离筛选得到更多能够高效降解塑料的微生物以及从微生物中鉴定更多的降解酶,并通过定向改造提高酶促效率仍然是未来研究工作的重点。为了解决塑料生物降解研究所面临的诸多挑战,我们认为在未来研究中可以重点关注以下几个方向:一是在材料方面,可以通过一些预处理方法,如机械破碎增加比表面积或紫外照射增加塑料的亲水性,从而提高降解速率。二是在微生物和酶的设计方面,提高酶的热稳定性以及筛选耐高温降解菌。合理的蛋白质工程和定向进化对于提高解聚酶的活性和稳定性是必要的^[87]。此外,研究者们还必须克服目前用于评估塑料的生物降解性方法的缺点,寻找将量化塑料表面元素组成与结构信息相结合的分析方法,从而即使在反应速率很慢且不能立即反映到重量变化的情况下,也能够量化涉及塑料聚合结构生化反应的速率。

最后,虽然现在科学家们已经筛选到了许多

降解塑料的微生物,但其降解机制尚不清楚,未来可以对降解机制进行深入研究,在合成塑料降解微生物的挖掘及关键解聚酶进化的基础上,融入生物合成的思路^[88],一方面可以改善塑料污染,另一方面可以重新利用塑料解聚后的单体,通过构建塑料解聚及其再利用生产生物塑料的人工微生物菌群,实现废弃塑料的解聚与资源化的偶联^[89-92]。目前,笔者实验室已经筛选到能够利用 PET 单体对苯二甲酸的菌株,并将其改造用于合成可降解塑料 (Poly- β -hydroxybutyrate PHB)^[93]。这种偶联的实现不仅可以减轻环境压力,同时将塑料垃圾“变废为宝”,创造更高的经济效益,达到“解塑再用”的目的。

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