

# 环境内分泌干扰物——邻苯二甲酸酯的生物降解研究进展\*

骆祝华<sup>1,2</sup> 黄翔玲<sup>1,2</sup> 叶德赞<sup>1,2\*\*</sup>

(国家海洋局<sup>1</sup>第三海洋研究所, <sup>2</sup>海洋生物遗传资源重点实验室 厦门 361005)

**摘要** 邻苯二甲酸酯是一类重要的合成有机物, 主要用作增塑剂来提高塑料树脂的机械性能尤其是柔韧性. 近年来, 由于在全球范围内的广泛使用, 在环境中的普遍存在, 以及其内分泌干扰毒性, 邻苯二甲酸酯受到了人们越来越多的关注. 微生物降解被认为是邻苯二甲酸酯完全矿化的主要途径. 邻苯二甲酸酯的降解机制与途径已经在细菌中得到广泛的研究, 但是在真菌领域的报道还很少见. 本文主要概述了邻苯二甲酸酯的环境污染现状, 介绍了其对人和动物的健康危害, 并且综述了邻苯二甲酸酯微生物降解的研究进展, 包括降解菌的种类、降解的代谢途径及相应的分子机制, 以及真菌对邻苯二甲酸酯的降解研究等. 图3 表6 参62

**关键词** 邻苯二甲酸酯; 内分泌干扰物; 生物降解; 增塑剂; 环境污染

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## Advances in Research of Biodegradation of Environmental Endocrine Disruptors-Phthalate Esters\*

LUO Zhuhua<sup>1,2</sup>, HUANG Xiangling<sup>1,2</sup> & YE Dezan<sup>1,2\*\*</sup>

(<sup>1</sup>The Third Institute of Oceanography, <sup>2</sup>Key Laboratory of Marine Biogenic Resource, State Oceanic Administration, Xiamen 361005, Fujian, China)

**Abstract** Phthalate esters (PAEs) are synthetic compounds used as plasticizers to improve mechanical properties of the plastic resin, particularly its flexibility and softness. In recent years, they have attracted increasing attention owing to their extensive use, ubiquity in the environment and endocrine-disrupting activity. Microbial degradation is the principal way for completely mineralization of phthalates in natural environments. The degradation mechanisms and pathways of phthalates have been consolidated by using bacteria while those using fungi have rarely been reported. This article mainly summarizes the pollution status of phthalates, introduces the health hazard of phthalates to humans and animals, and reviews the current research progress in the microbial degradation of phthalates, including species of phthalate-degrading bacteria, degradation pathways and related molecular bases, and fungal degradation of phthalates. Fig 3, Tab 6, Ref 62

**Keywords** phthalate ester; endocrine disruptor; biodegradation; plasticizer; environmental pollution

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邻苯二甲酸酯(Phthalates esters, PAEs)是一类重要的合成有机物(表1), 主要作为增塑剂, 即塑料生产中的改性添加剂, 用于增大产品的可塑性和提高产品的强度<sup>[1]</sup>. 近年来, 由于全球范围的广泛使用、在环境中普遍存在以及对人和动物的内分泌干扰毒性, PAEs受到了人们越来越多的关注<sup>[2,3]</sup>.

## 1 邻苯二甲酸酯的环境污染

PAEs主要用作塑料工业生产中的增塑剂, 用来提高一些塑料制品(如家具、日用容器、食品包装、医疗设备和儿童玩具等)的弹性和柔韧性<sup>[1]</sup>. 其中邻苯二甲酸二(2-乙基己基)酯(DEHP)作为全球用量最大的PAE类增塑剂, 广泛应用在儿童玩具、牙刷和服装的生产上. 作为增塑剂, PAEs在塑料制

品中的含量占20%~30%左右, 有时可高达50%<sup>[5]</sup>. 自上世纪50年代聚氯乙烯(PVC)面世以来, PAEs就得到大规模的生产. 目前, 它占了全球增塑剂市场的80%以上<sup>[6]</sup>. 除了作为增塑剂外, PAEs还广泛应用于油漆、粘合剂、驱虫剂、化妆品、香料和润滑剂的生产原料<sup>[1]</sup>. 在德国, 每年的PAEs的消耗达到4×10<sup>5</sup>吨, 其中DEHP的消耗占60%<sup>[7]</sup>.

作为增塑剂, 为了提供塑料产品必要的可塑性, PAEs并非共价聚合到塑料产品的基质中, 而是与聚烯烃类塑料分子之间由氢键或范德华力连接, 彼此保留各自相对独立的化学性质. 因此, 随着时间的推移, PAEs可由塑料中迁移到外环境, 造成对环境的污染<sup>[1,8]</sup>. 塑料制品的全球性大量应用导致PAEs在环境中无处不在. 目前, PAEs在全球主要工业国的生态环境中已达到了普遍检出的程度. 在土壤、河流、湖泊、海洋底质、饮用水、垃圾场乃至食品中都有检测到PAEs的存在<sup>[9,10]</sup>, PAEs已成为全球最普遍的污染物之一(表2).

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\*\*通讯作者 Corresponding author (E-mail: yedezan@public.xm.fj.cn)

表1 具有环境激素作用的PAEs化合物  
Table 1 PAEs with endocrine-disrupting activities

| 中文名称<br>Chinese name | 英文名称<br>English name             | 用途 <sup>[4]</sup><br>Uses <sup>[4]</sup>                                                                                                   |
|----------------------|----------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------|
| 邻苯二甲酸二甲酯 (I、II)      | Dimethyl phthalate (DMP)         | 塑料增塑剂; 驱蚊剂<br>Plastic plasticizer; anti-mosquito complex                                                                                   |
| 邻苯二甲酸二乙酯 (I、II)      | Diethyl phthalate (DEP)          | 塑料和合成橡胶等的增塑剂; 清漆的溶剂<br>Plasticizer for plastic and synthetic rubber; varnish solvent                                                       |
| 邻苯二甲酸二丙酯 (I)         | Di-n-propyl phthalate (DPP)      | 塑料增塑剂<br>Plastic plasticizer                                                                                                               |
| 邻苯二甲酸二丁酯 (I)         | Di-n-butyl phthalate (DBP)       | 塑料、合成橡胶、人造革等的常用增塑剂; 香料的溶剂; 卫生害虫驱避剂<br>Plasticizer for plastic, synthetic rubber, and artificial leather; perfume solvent; insect repellent |
| 邻苯二甲酸二戊酯             | Di-n-pentyl phthalate (DDP)      | 塑料增塑剂<br>Plastic plasticizer                                                                                                               |
| 邻苯二甲酸二己酯             | Dihexyl phthalate (DHP)          | 塑料增塑剂<br>Plastic plasticizer                                                                                                               |
| 邻苯二甲酸二辛酯 (I、II)      | Di-n-octyl phthalate (DOP)       | 塑料增塑剂<br>Plastic plasticizer                                                                                                               |
| 邻苯二甲酸丁苄酯             | Butylbenzyl phthalate (BBP)      | 塑料增塑剂; 涂料; 人造革材料<br>Plastic plasticizer; dope; artificial leather                                                                          |
| 邻苯二甲酸二(2-乙基己基)酯 (I)  | Di-2-ethylhexyl phthalate (DEHP) | 聚氯乙烯和氯乙烯共聚物的优良增塑剂; 硝酸纤维的软化剂<br>Plasticizer for PVC, chloroethylene polymer; softener of cellulose nitrate                                  |
| 邻苯二甲酸二环己烷酯           | Dicyclohexyl phthalate (DCHP)    | 丙烯酸稀释剂; 热粘合剂的可塑剂<br>Diluent for acrylic acid; plasticizer for hot-setting adhesive                                                         |

I. 美国环保局的优先污染物; II. 中国国家环境监测中心的优先污染物  
I. Priority pollutants listed by the United States Environmental Protection Agency; II. Priority pollutants listed by China National Environmental Monitoring Center

表2 一些PAEs在环境中的检出与浓度  
Table 2 Existence and concentration of several PAEs in the environment

| 邻苯二甲酸酯PAEs | 检出环境<br>Existence in environment | 浓度<br>Concentration             | 文献<br>References |
|------------|----------------------------------|---------------------------------|------------------|
| DMP        | 河流 River                         | 10 mg L <sup>-1</sup>           | 11               |
| DBP        | 井水 Well water                    | 0.19~9.26 µg L <sup>-1</sup>    | 12               |
|            | 食物 Foodstuffs                    | 0.09~0.19 mg kg <sup>-1</sup>   | 9                |
| BBP        | 食物 Foodstuffs                    | 0.017~0.019 mg kg <sup>-1</sup> | 9                |
| DEP        | 海洋底质 Sea sediment                | 0.049 µg L <sup>-1</sup>        | 13               |
|            | 生活垃圾 Household waste             | 18.2 mg kg <sup>-1</sup>        | 8                |
| DEHP       | 河流 River                         | 1.3~2.6 mg L <sup>-1</sup>      | 14               |
|            | 海洋底质 Sea sediment                | 0.18 µg L <sup>-1</sup>         | 13               |
|            | 生活垃圾 Household waste             | 16820.6 mg kg <sup>-1</sup>     | 8                |
|            | 垃圾渗滤液 Landfill leachate          | 346 µg L <sup>-1</sup>          | 15               |
|            | 食物 Foodstuffs                    | 0.11~0.18 mg kg <sup>-1</sup>   | 9                |

2 邻苯二甲酸酯的健康危害

PAEs共约30多种,大多是无色透明的油状液体,难溶于水,易溶于有机溶剂,属中等极性物质,可通过呼吸、饮食和皮肤接触进入人和动物体内。PAEs对人体健康的影响是一个慢性的过程,需要较长的时间才会出现,而且可能通过胎盘和授乳产生跨代影响,目前对其毒性的研究主要通过动物实验进行<sup>[16]</sup>。

PAEs对生物体的急性毒性不大,小鼠口服LD<sub>50</sub>值为800~1 600 mg kg<sup>-1</sup>, Ames试验呈阴性<sup>[17]</sup>。但在大剂量下,PAEs对动物有致畸、致癌和致突变作用<sup>[18]</sup>。例如,Agarwal等研究了DEHP对成年小白鼠的致畸与致突变影响,发现经DEHP处理后的小白鼠表现有显性致死和致突变作用<sup>[19]</sup>。1982年,美国国家毒理规划署的实验报告(National Toxicology Program)确证了大白鼠和小白鼠长期吸收DEHP可引发肝癌<sup>[20]</sup>。此外,动物实验还表明某些邻苯二甲酸酯还会引起动物的肝、肾损伤

<sup>[21]</sup>。

近来的研究指出,PAEs主要的危害在于环境激素作用,可在极低的浓度下干扰人和动物的内分泌系统<sup>[10]</sup>。其对内分泌系统的扰乱是通过雌激素受体(Estrogen receptor)介导的反应,通过与雌激素受体结合,作用于DNA中雌激素反应元件(Estrogen responsive element)激活基因的转录,产生雌激素效应<sup>[22]</sup>。动物实验表明,作为内分泌干扰物,PAEs的生化效应表现为过氧化酶体增生、足细胞毒性、肝脏促进作用、抗雄激素、体外雌激素活性等<sup>[23]</sup>。对动物生殖方面的扰乱主要是使精囊萎缩、精子数量减少以至于精子形成中止、生殖能力下降、后代数量减少、体重下降、子宫粘膜组织增生等<sup>[24]</sup>。

由于PAEs对人体健康的潜在危害,一些环境监控组织,包括美国环保署和中国国家环境监测中心都把它列为优先污染物加以控制<sup>[25]</sup>。目前被认为有环境激素作用的邻苯二甲酸酯有10种,其名称与用途见表1。

3 邻苯二甲酸酯的生物降解

PAEs在环境中的水解、光解的速率非常缓慢,属于难降解物质.因此,微生物降解被认为是自然环境中PAEs完全矿化的主要过程<sup>[1]</sup>.

3.1 邻苯二甲酸酯的细菌降解

近年来,PAEs的细菌降解已经得到广泛的研究,大量高效降解PAEs的菌株已经从各类环境中分离得到,包括红树林、土壤、海洋、河流与废水处理厂的活性污泥等(表3).但是,并非所有的菌株都能完全矿化PAEs,部分菌株只能将PAEs转化成单酯,或者进一步转化成邻苯二甲酸.利用各种各样的PAEs降解菌构建成的细菌菌群显示出更好的PAEs的降解能力,可以通过不同菌株的协同作用完全矿化PAEs(表4).Vega 和 Bastide等<sup>[26]</sup>研究了一个由*Arthrobacter* sp.和*Sphingomonas paucimobilis*两种细菌构建的菌群对邻苯二甲酸二甲酯(DMP)的降解能力,发现*Arthrobacter* sp.能够将DMP转化为邻苯二甲酸单甲酯(MMP),但不能将MMP进一步转化为邻苯二甲酸(PA),但是这个菌株却有PA的降解能力.而另一个菌株*Sphingomonas paucimobilis*能够将MMP转

化为PA,并且能完全降解PA,但是却不能完成DMP到MMP的转化.因此,利用由这个两个菌株构建成的菌群能够很好的完全矿化DMP.

同时,细菌对PAEs的代谢途径也得到深入的研究(图1, 2).对大多数细菌菌株来说,在PAEs的好氧降解过程中,酯键的水解是一个共同的起始步骤.在微生物酯酶作用下,PAEs水解形成邻苯二甲酸单酯,再生成邻苯二甲酸.而邻苯二甲酸的降解是完成整个降解过程的关键步骤.细菌对邻苯二甲酸的降解通常有两条途径,革兰氏阴性菌通过邻苯二甲酸4,5双加氧酶的作用氧化邻苯二甲酸生成顺式-4,5-二羟基-4,5-二氢邻苯二甲酸,然后脱氢生成4,5-二羟基邻苯二甲酸,后者再通过脱羧生成原儿茶酚<sup>[48]</sup>.而革兰氏阳性菌则是在邻苯二甲酸的碳3-4位氧化和脱氢生成3,4-二羟基邻苯二甲酸,最后再通过脱羧形成原儿茶酚<sup>[49, 50]</sup>.原儿茶酚这一许多芳香族化合物代谢途径中的重要中间代谢物可通过正位或偏位开环形成相应的有机酸,进而转化成丙酮酸、琥珀酸、草酰乙酸等进入三羧酸循环,最终转化为CO<sub>2</sub>和H<sub>2</sub>O.如*Pseudomonads fluorescens*和*P. putida*通过原儿茶酚3,4双加氧酶的作用正位开环氧化原儿茶酚生成3-羧基-顺,顺-粘康

表3 降解PAEs的细菌菌株  
Table 3 Bacterial species capable of degrading PAEs

| 分离环境<br>Isolation sources                                 | 细菌菌株<br>Bacterial species        | 降解底物<br>Degradation substrates   | 文献<br>References |
|-----------------------------------------------------------|----------------------------------|----------------------------------|------------------|
| 红树林<br>Mangrove                                           | <i>Pseudomonas fluorescens</i>   | DBP, BBP                         | 27, 28           |
|                                                           | <i>Rhodococcus ruber</i>         | DMP, DMIP, DMTP                  | 29               |
|                                                           | <i>Pasturella multocida</i>      | DMTP                             | 21, 30           |
|                                                           | <i>Sphingomonas paucimobilis</i> | DMTP                             | 21               |
|                                                           | <i>Klebsiella oxytoca</i>        | DMTP                             | 31               |
| 土壤<br>Soil                                                | <i>Bacillus</i> sp.              | DMP                              | 32               |
|                                                           | <i>Rhodococcus rhodochrous</i>   | DEHP                             | 33               |
|                                                           | <i>Gordonia</i> sp.              | BBP                              | 34               |
|                                                           | <i>Arthrobacter</i> sp.          | DMP                              | 26               |
| 海洋<br>Marine                                              | <i>Viarovorax paradoxus</i>      | DMIP, DMTP                       | 35, 36           |
|                                                           | <i>Sphingomonas yanoikuyae</i>   | DMTP                             | 36               |
| 河流<br>River                                               | <i>Corynebacterium</i> sp.       | DEP, DPrP, DBP, BBP, DPP         | 37               |
|                                                           | <i>Acinetobacter</i> sp.         | DEHP, DBP                        | 38               |
| 废水处理厂的活性污泥<br>Activated sludge from waste treatment plant | <i>Sphingomonas</i> sp.          | DEP, DPrP, DBP, BBP, DPP         | 37, 39           |
|                                                           | <i>Comamonas acidovorans</i>     | DMTP                             | 40               |
|                                                           | <i>Pseudomonas fluorescens</i>   | DMP, DEP, DnBP, DiBP, DnOP, DEHP | 41               |
|                                                           | <i>Rhodococcus erythropolis</i>  | DMIP                             | 42               |
|                                                           | <i>Rhodococcus ruber</i>         | DMIP                             | 42               |
|                                                           | <i>Pseudomonas fluorescens</i>   | DMP, D <sub>n</sub> BP           | 43, 44           |
|                                                           | <i>Pseudomonas aeruginosa</i>    | DMP                              | 43               |
|                                                           | <i>Brevibacterium</i> sp.        | DMP                              | 43               |

表4 降解PAEs的细菌菌群  
Table 4 Bacterial consortia capable of degrading PAEs

| 分离环境<br>Isolation sources                                 | 细菌菌群<br>Bacterial consortia                                                                                 | 降解底物<br>Degradation substrates | 文献<br>References |
|-----------------------------------------------------------|-------------------------------------------------------------------------------------------------------------|--------------------------------|------------------|
| 土壤 Soil                                                   | <i>Arthrobacter</i> sp. and<br><i>Sphingomonas paucimobilis</i>                                             | DMP                            | 26               |
|                                                           | <i>Pseudomonas</i> sp.,<br><i>Aeromonas</i> sp.,<br><i>Arthrobacter</i> sp. and<br><i>Bacillus</i> sp.      | DMTP                           | 45               |
| 废水处理厂的活性污泥<br>Activated sludge from waste treatment plant | <i>Pseudomonas fluorescens</i> ,<br><i>Pseudomonas aureofaciens</i> and<br><i>Sphingomonas pacuimobilis</i> | DMP                            | 46, 47           |
|                                                           | <i>Xanthomonas maltophilia</i> and<br><i>Sphingomonas pacuimobilis</i>                                      | DMP                            | 46, 47           |

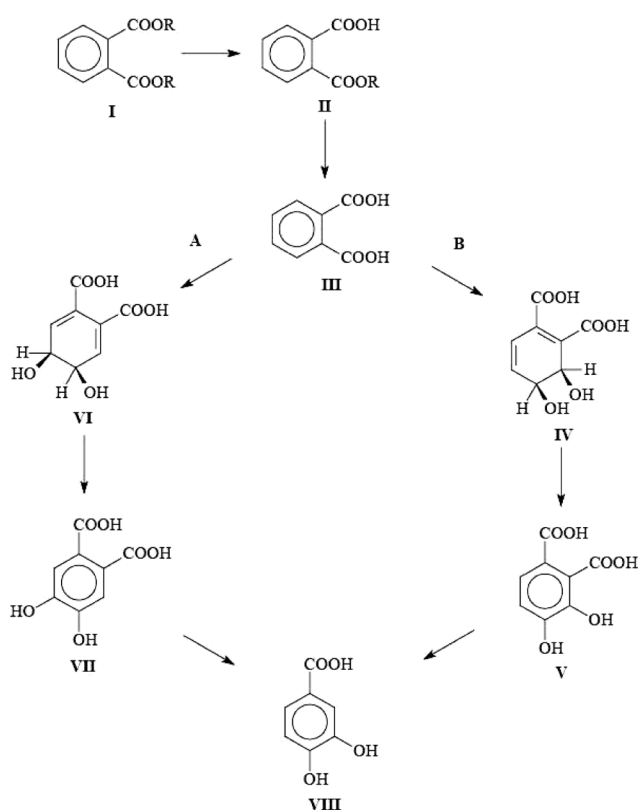


图1 PAEs的细菌好氧降解途径——从邻苯二甲酸酯到原儿茶酚<sup>[54]</sup>

(A. 革兰氏阴性菌转化途径<sup>[48]</sup>; B. 革兰氏阳性菌转化途径<sup>[49]</sup>)

Fig. 1 Transformation pathway of PAEs to protocatechuate by aerobic bacteria<sup>[54]</sup>

(A. Transformation pathway by Gram-negative bacteria<sup>[48]</sup>; B. Transformation pathway by Gram-positive bacteria<sup>[49]</sup>)

I. *ortho*-phthalate ester; II. Monophthalate ester; III. Phthalic acid; IV. *cis*-3,4-dihydroxy-3,4-dihydrophthalate; V. 3,4-dihydroxyphthalate; VI. *cis*-4,5-dihydroxy-4,5-dihydrophthalate; VII. 4,5-dihydroxyphthalate; VIII. Protocatechuate

酸,后者再经由 $\beta$ -酮己二酸途径转化为琥珀酸与乙酰辅酶A进入三羧酸循环代谢。而对于*P. testosterone*和*Arthrobacter keyseri*,它们通过原儿茶酚4,5-双加氧酶的催化氧化原儿茶酚生成2-羟基-4-羧基粘康半醛,后者再通过一系列的酶促反应最后转化为草酰乙酸与丙酮酸从而进入三羧酸循环<sup>[51, 52]</sup>。

而PAEs在厌氧条件下的生物降解途径研究较少。Kleerebezem等<sup>[53]</sup>研究了产烷细菌菌群对PAEs的厌氧降解过程,发现至少有3类细菌参加了PAEs的降解过程。首先是通过发酵细菌(Fermentative organisms)将PAEs转化为乙酸盐与氢,然后由嗜乙酸甲烷产生菌(Acetoclastic methanogens)转化乙酸盐为甲烷和碳酸氢盐,最后通过氢利用产甲烷菌(Hydrogenotrophic methanogens)的作用下还原碳酸氢盐产生甲烷。

此外,邻苯二甲酸酯细菌降解的遗传机制也得到了深入的研究,这些研究主要集中在对参与从邻苯二甲酸到原儿茶酚降解的酶基因进行克隆和鉴定。其中*Arthrobacter keyseri* 12B与*Burkholderia cepacia* DBO1分别是革兰氏阳性和阴性菌株中遗传机制研究得最为透彻的两株邻苯二甲酸降解菌。

在*A. keyseri* 12B中,确认了一个130 kb的质粒编码邻苯二甲酸的完全代谢<sup>[49]</sup>。在这个质粒中确定了5个遗传单位:*pht*

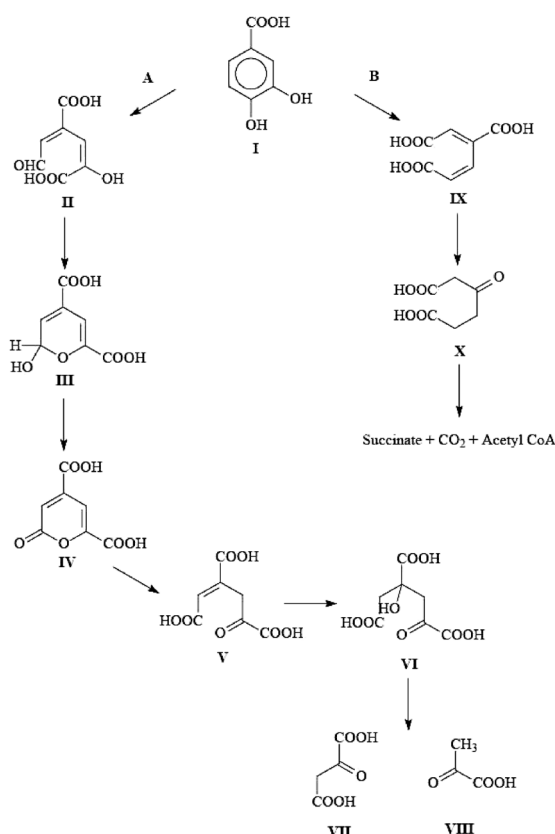


图2 PAEs的细菌好氧降解途径——原儿茶酚的降解<sup>[54]</sup>

(A. 偏位开环途径<sup>[49, 51]</sup>; B. 正位开环途径<sup>[51]</sup>)

Fig. 2 Degradation pathway of protocatechuate by aerobic bacteria<sup>[54]</sup>

(A. *meta*-cleavage pathway<sup>[49, 51]</sup>; B. *ortho*-cleavage pathway<sup>[51]</sup>)

I. Protocatechuate; II. 2-hydroxy-4-carboxymuconic semialdehyde-hemiacetal; III. 2-pyrone-4,6-dicarboxylate; IV. 4-oxalomesaconate; V. 4-oxalocitramate; VI. 4-oxalocitramate; VII. Pyruvate; VIII. Pyruvate; IX. 3-carboxy-cis, cis-muconate; X.  $\beta$ -ketoadipate.

操纵子编码邻苯二甲酸到原儿茶酚的转化;*pcm*操纵子编码从原儿茶酚到丙酮酸和草酰乙酸的进一步代谢;一个推测的*ptr*操纵子编码一个可能是邻苯二甲酸,或原儿茶酚,或邻苯二甲酸酯的转运蛋白;*pehA*基因,一个可能的邻苯二甲酸酯水解酶基因;*tnpR*基因,转座子解离酶基因。其中*pht*操纵子(*phtBAaAbAcAdCR*)是革兰氏阳性菌中报道的第一个邻苯二甲酸催化基因操纵子。在这个操纵子中,*phtAaAbAcAd*基因簇(氧化酶大亚基,氧化酶小亚基,铁氧还蛋白,和铁氧化蛋白还原酶)编码一个三组分的双加氧酶,邻苯二甲酸3,4-双加氧酶。编码顺式-3,4-二氢-3,4-二羟基邻苯二甲酸脱氢酶(*phtB*)和3,4-二羟基邻苯二甲酸脱羧酶的基因(*phtC*)分别位于*phtAaAbAcAd*基因簇的上下游。调控基因(*phtR*)位于操纵子的后面。此外,*pht*操纵子在*Terrabacter* sp. DBF63中也得到克隆与描述<sup>[55]</sup>。该操纵子(*phtA1A2BA3A4CR*)与*A. keyseri* 12B中的*pht*操纵子高度相似,除了*phtB*基因所在的位置有所不同。此外,Stingley等在*Mycobacterium vanbaalenii* PYR-1也克隆了一个*pht*操纵子(*phtRAaAbBacAd*)<sup>[50]</sup>。然而,该操纵子无论在基因排布与核酸序列上都与前面两株菌有很大的不同。其编码调控蛋白的基因(*phtR*)的位置与转录方向都与另两株菌完全不同。此外,该操纵子没有脱羧酶基因,所以*M. vanbaalenii*

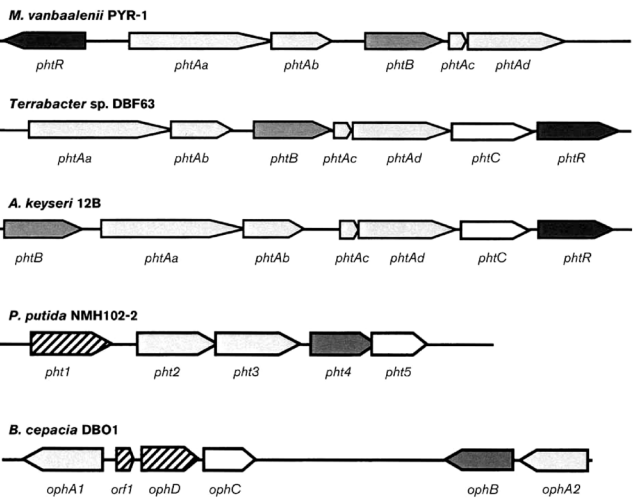


图3 若干细菌菌株的邻苯二甲酸降解操纵子的比较<sup>[50]</sup>  
Fig. 3 Comparison of operons containing phthalate-degrading genes from several bacterial strains<sup>[50]</sup>

表5 若干细菌菌株中的邻苯二甲酸酯降解相关基因及其功能

| Table 5 Phthalate-degrading genes and their corresponding functions in several bacterial strains |                                                                  |
|--------------------------------------------------------------------------------------------------|------------------------------------------------------------------|
| 基因 Genes                                                                                         | 基因功能 Gene functions                                              |
| 革兰氏阳性菌 Gram-positive bacteria                                                                    |                                                                  |
| <i>Arthrobacter keyseri</i> 12B                                                                  | <i>Mycobacterium vanbaalenii</i> PYR-1                           |
| <i>phtAa</i>                                                                                     | <i>phtAa</i> Phthalate dioxygenase large subunit                 |
| <i>phtAb</i>                                                                                     | <i>phtAb</i> Phthalate dioxygenase small subunit                 |
| <i>phtAc</i>                                                                                     | <i>phtAc</i> Ferredoxin subunit                                  |
| <i>phtAd</i>                                                                                     | <i>phtAd</i> Ferredoxin reductase                                |
| <i>phtB</i>                                                                                      | <i>phtB</i> cis-3,4-dihydro-3,4-dihydroxyphthalate dehydrogenase |
| <i>phtC</i>                                                                                      | <i>phtC</i> 3,4-dihydroxyphthalate decarboxylase                 |
| <i>phtR</i>                                                                                      | <i>phtR</i> Transcriptional regulator                            |
| 革兰氏阴性菌 Gram-negative bacteria                                                                    |                                                                  |
| <i>Burkholderia cepacia</i> DBO1                                                                 | <i>Pseudomonas putida</i> NMH102-2                               |
| <i>ophA1</i>                                                                                     | <i>pht2</i> Phthalate dioxygenase reductase                      |
| <i>ophA2</i>                                                                                     | <i>pht3</i> Phthalate dioxygenase                                |
| <i>ophB</i>                                                                                      | <i>pht4</i> 4,5-dihydro-4,5-dihydroxyphthalate dehydrogenase     |
| <i>ophC</i>                                                                                      | <i>pht5</i> 4,5-dihydroxyphthalate decarboxylase                 |
| <i>orf1, ophD</i>                                                                                | <i>pht3</i> Putative phthalate transporter                       |

PYR-1只能将邻苯二甲酸转化为3,4-二羟基邻苯二甲酸。

由于革兰氏阴性菌中参与最初的邻苯二甲酸双加氧过程的酶不同于革兰氏阳性菌，因此，编码这些酶的基因也完全不同。*Burkholderia cepacia* DBO1是研究最为透彻的革兰氏阴性邻苯二甲酸降解菌，参与催化过程的酶和基因已经得到深入的研究。Chang和Zylstra克隆并测序了DBO1中参与从邻苯二甲酸到原儿茶酚转化的基因<sup>[48]</sup>。有趣的是，其它细菌中的邻苯二甲酸代谢基因都是组成一个功能单位的操纵子，而该菌的5个与邻苯二甲酸降解相关的基因是分散组织并基于转录方向排列成3个转录单位：分别是*ophA1*（邻苯二甲酸加氧酶还原酶）、*ophDC*（转录蛋白和4,5-二羟基邻苯二甲酸脱羧酶）和*ophA2B*（邻苯二甲酸加氧酶和顺式-二氢邻苯二甲酸脱氢酶）。编码邻苯二甲酸双加氧酶的氧化酶和还原酶两个组分的基因*ophA1*和*ophA2*分别位于序列对应的两端，中间间

隔了7 kb。与之相比，*Pseudomonas putida* NMH102-2的邻苯二甲酸降解基因(*pht12345*)则是串成一个操纵结构并以同一方向转录的<sup>[52]</sup>。各菌株的邻苯二甲酸降解基因的排布和功能见图3和表5。这些基因的克隆和功能鉴定将有助于我们更好地了解 and 补充邻苯二甲酸酯代谢的生化机制，并且为进一步研究代谢过程中的关键酶打下基础。

3.2 邻苯二甲酸酯的真菌降解

与细菌相比，邻苯二甲酸酯降解在真菌方面的研究还很少见。现在对于真菌降解的研究主要有两方面，一方面是利用真菌产生的纯酶试剂进行降解研究，另一方面是用纯培养菌株进行降解研究。

韩国的一个研究小组做了大量的邻苯二甲酸酯的纯酶降解研究(表6)。他们选择了*Fusarium oxysporum* f. sp. *pisi*产生的几丁质酶(Cutinase)和*Candida cylindracea*产生的酯酶(Esterase)来对各类的邻苯二甲酸酯进行纯酶降解的比较研究。发现与酯酶相比，几丁质酶对邻苯二甲酸酯的降解有2个优点：1) 几丁质酶对PAEs的降解开始的更早，速率更快，而

且酯水解的活性更加稳定；2) 几丁质酶产生的降解产物是无毒的，而酯酶产生的降解产物对细菌细胞却有氧化毒性，并能破坏细菌细胞的蛋白质合成。这两种酶的PAEs降解产物毒性的不同与其降解产物的组成有关。

纯培养真菌菌株对邻苯二甲酸酯降解的研究极少。最早的报道始于1991年，*Sclerotium rolfsii*可将邻苯二甲酸二甲酯(DMTP)经由邻苯二甲酸单甲酯(MMTP)转化为邻苯二甲酸(TPA)，但是TPA是其代谢终产物，进一步的降解没有发生<sup>[60]</sup>。与之相比，Ganji等报道*Aspergillus niger*可以完全降解DMTP，它可将DMTP转化成MMTP、TPA和原儿茶酚，最后原儿茶酚偏位开环完全降解为CO<sub>2</sub>和H<sub>2</sub>O<sup>[61]</sup>。邻苯二甲酸酯酶的活性在两个菌株的菌丝体中都有检测到，而原儿茶酚双加氧酶的活性还在*A. niger*的菌丝体中检测到。

此外，Pardeepkumar等报道*A. niger*也能完全矿化

表6 *Fusarium oxysporum* f. sp. *pisi*的几丁质酶(cutinase)和*Candida cylindracea* 的酯酶(esterase)对PAEs的酶降解  
Table 6 Enzymatic degradation of several PAEs by two purified fungal enzymes, *Fusarium oxysporum* f. sp. *pisi* cutinase and *Candida cylindracea* esterase

| 底物<br>Substrates       | 酶<br>Enzymes     | 降解速率<br>Degradation rate | 降解产物<br>Degradation products                 | 降解产物毒性<br>Toxic effects of degradation products                                                                     | 文献<br>References |
|------------------------|------------------|--------------------------|----------------------------------------------|---------------------------------------------------------------------------------------------------------------------|------------------|
| 邻苯二甲酸二(2-乙基己基)酯 (DEHP) | 几丁质酶<br>Cutinase | 快<br>Fast                | 邻苯二甲酐<br>IBF                                 | 无毒<br>Non-toxic                                                                                                     | 56               |
|                        | 酯酶<br>Esterase   | 慢<br>Slow                | 邻苯二甲酐, 未确定的化合物<br>IBF, unidentified compound | 有毒, 对细菌细胞造成氧化压力并损害蛋白合成<br>Toxic hazard, causing oxidative stress and damage to protein synthesis in bacterial cells |                  |
| 邻苯二甲酸二丙酯 (DPrP)        | 几丁质酶<br>Cutinase | 快<br>Fast                | 邻苯二甲酐<br>IBF                                 | 无毒<br>Non-toxic                                                                                                     | 57               |
|                        | 酯酶<br>Esterase   | 慢<br>Slow                | 邻苯二甲酐, 邻苯二甲酸单丙酯<br>IBF, PrMP                 | 有毒, 对细菌细胞造成氧化压力并损害蛋白合成<br>Toxic hazard, causing oxidative stress and damage to protein synthesis in bacterial cells |                  |
| 邻苯二甲酸二戊酯 (DPeP)        | 几丁质酶<br>Cutinase | 快<br>Fast                | 邻苯二甲酐<br>IBF                                 | 无毒<br>Non-toxic                                                                                                     | 58               |
|                        | 酯酶<br>Esterase   | 慢<br>Slow                | 邻苯二甲酐, 邻苯二甲酸单戊酯<br>IBF, PeMP                 | 有毒, 对细菌细胞造成氧化压力并损害蛋白合成<br>Toxic hazard, causing oxidative stress and damage to protein synthesis in bacterial cells |                  |
| 邻苯二甲酸丁基苯甲基酯(BBP)       | 几丁质酶<br>Cutinase | 快<br>Fast                | 邻苯二甲酐, 未确定的化合物<br>IBF, unidentified compound | 无毒<br>Non-toxic                                                                                                     | 59               |
|                        | 酯酶<br>Esterase   | 慢<br>Slow                | 邻苯二甲酐, 邻苯二甲酸丁基苯基酯<br>IBF, BMP                | 有毒, 对细菌细胞造成氧化压力并损害蛋白合成<br>Toxic hazard, causing oxidative stress and damage to protein synthesis in bacterial cells |                  |

DMP<sup>[62]</sup>. 它可由MMP、PA和原儿茶酚将DMP完全降解. 特别要指出的是, 在这个报道中原儿茶酚通过正位开环完全降解为CO<sub>2</sub>和H<sub>2</sub>O. 这一结果不同于*A. niger*对DMTP降解的报道<sup>[61]</sup>, 在该报道中, 原儿茶酚通过偏位开环途径降解. 同时, 与Ganji等的报道一样<sup>[61]</sup>, 邻苯二甲酸酯酶和原儿茶酚双加氧酶的活性也都有在*A. niger*的菌丝中检测到.

4 结语

邻苯二甲酸酯是人们长期大量生产、使用塑料制品所造成的一种环境有机污染物. 虽然人们目前已经认识到它的危害, 但是在实际的生产和生活中仍离不开它. 因此, 当前的研究重点一方面应放在如何使其与塑料结合更加紧密, 使其不易从塑料中分离出来, 进入环境; 另一方面应研究如何降解进入环境中的邻苯二甲酸酯, 减少环境污染. 微生物降解被认为是消除环境中邻苯二甲酸酯污染的有效途径. 但是, 现阶段对邻苯二甲酸酯的降解的研究主要还是在实验室内, 如何将实验室所取得的研究成果应用于现实环境中, 解决目前环境中邻苯二甲酸酯的污染, 是需要研究的一个重要方向.

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