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MINI-REVIEW

Genome analysis and -omics approaches provide new insights into the biodegradation potential of *Rhodococcus*

Jessica Zampolli[1] · Zahraa Zeaiter[1] · Alessandra Di Canito[1] · Patrizia Di Gennaro[1]

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Abstract

The past few years observed a breakthrough of genome sequences of bacteria of *Rhodococcus* genus with significant biodegradation abilities. Invaluable knowledge from genome data and their functional analysis can be applied to develop and design strategies for attenuating damages caused by hydrocarbon contamination. With the advent of high-throughput -omic technologies, it is currently possible to utilize the functional properties of diverse catabolic genes, analyze an entire system at the level of molecule (DNA, RNA, protein, and metabolite), simultaneously predict and construct catabolic degradation pathways. In this review, the genes involved in the biodegradation of hydrocarbons and several emerging plasticizer compounds in *Rhodococcus* strains are described in detail (aliphatic, aromatics, PAH, phthalate, polyethylene, and polyisoprene). The metabolic biodegradation networks predicted from omics-derived data along with the catabolic enzymes exploited in diverse biotechnological and bioremediation applications are characterized.

Keywords *Rhodococcus* · -omics · Biodegradation · Recalcitrant compounds · Gene cluster

Introduction

Rhodococcus genus is a heterogeneous group of microorganisms taxonomically associated with the *Actinobacteria* phylum, one of the largest within the bacteria domain (Ludwig et al. 2012). This phylum represents Gram-positive bacteria with high G-C content, often widespread in aquatic and terrestrial ecosystems. Bacteria of this group exhibit remarkable catabolic versatility and an array of unique enzymatic capabilities that reveal their environmental and biotechnological importance (Barka et al. 2016). For instance, they can degrade a large number of organic and xenobiotic compounds often toxic and recalcitrant categorized in different groups such as aliphatic, aromatic, and polyaromatic hydrocarbons and heterocyclic, halogenated, and nitro-substituted compounds (Martínková et al. 2009). Additional attributes of rhodococci are the modification of steroid, the enantio-selective synthesis, the production of amides from nitriles, the conversion of many plant secondary metabolites found in soil and rhizosphere, such as alkaloids, terpenes, and sterols, and the elimination of sulfur from coal and petroleum products (Larkin et al. 2006; Van Der Geize and Dijkhuizen 2004).

The genus of *Rhodococcus* represents an important reservoir of physiological and functional diversity. Their metabolites have a biotechnological and industrial significance. Indeed, they can produce carotenoids, wax esters, oils, biosurfactants, bioflocculation agents, and acrylamide (Jones and Goodfellow 2010). For instance, *Rhodococcus opacus* PD630 was shown to accumulate lipids in its cytosol due to its natural ability to tolerate and to utilize phenolic compounds, which emerges this strain as a promising microbial host for lignocellulose conversion into value-added products (Yoneda et al. 2016).

Moreover, rhodococci were characterized for their ability to thrive harsh environmental conditions and for their biodegradative potential with respect to very toxic and recalcitrant compounds. In this regard, *Rhodococcus jostii* RHA1 was assessed for its resistance to desiccation and nutrient starvation (LeBlanc et al. 2008; Patrauchan et al. 2012) by evaluating genes' expression through a proteomic approach while *Rhodococcus erythropolis* DCL14 was studied for its ability to tolerate various concentrations of water miscible (ethanol, butanol, and dimethylformamide, up to 50% *v*/*v*) and water immiscible solvents (dodecane, bis(2-ethylhexyl) phthalate, and toluene, up to 5% *v*/*v*) (De Carvalho et al. 2004).

✉ Patrizia Di Gennaro
patrizia.digennaro@unimib.it

[1] Department of Biotechnology and Biosciences, University of Milano-Bicocca, Piazza della Scienza 2, 20126 Milan, Italy

The recent awareness that *Rhodococcus* genus represents a promising potential in bioremediation, biotransformations, and biocatalysis applications is prompting research and interest from the scientific community in many countries of the world. Indeed, the number of publications and patents on rhodococci has increased during the last several years; in particular, research on the biotechnological exploitation of rhodococci has intensified significantly within the last 37 years, as illustrated by citation analysis (Fig. 1). Genome sequencing is the milestone in unveiling the bioremediation and biotechnological potential in bacteria. In fact, since the advent of genome sequencing two decades ago (Fleischmann et al. 1995), the technical and biological knowledge have increased. Nowadays, sequencing of bacterial genomes is a standard procedure, and the information from tens of thousands of bacterial genomes has had a major impact on our views of the bacterial world (Land et al. 2015) (Table 1). In regard to *Rhodococcus* genus, several public and private genome projects involving *Rhodococcus* members are now in progress due to their genome complexity, demonstrated by a multiplicity of catabolic genes and a high genetic redundancy of biosynthetic pathways often connected to a sophisticated regulatory network. Thus, combining functional genome studies with biochemical and physiological knowledge could enhance the exploitation of rhodococcal biotechnological use (Alvarez 2010).

This review intends to report new insights into the biodegradation potential of *Rhodococcus* genus based on genome analyses and "-omics" approaches. It summarizes the main characteristics of members of *Rhodococcus* genus and presents a collection of catabolic functions of different *Rhodococcus* species involved in the degradation and/or use of several toxic compounds, from hydrocarbons to several emerging contaminants as plasticizer compounds.

Genomics of *Rhodococcus* and "-omics studies"

This review presents an update collection of *Rhodococcus* genes and metabolic pathways involved in the biodegradation of main group of toxic compounds basing on "-omic" approaches. The suffix "-omics" mainly indicates studies undertaken on a genome-wide scale, including the genome itself (genomic), RNA transcription (transcriptomic), protein (proteomic), and metabolic products (metabolomic), relying on bioinformatic data.

Particularly, bacterial genomic information could be exploited on at least two levels, to (i) elucidate gene function of unknown enzymes and to (ii) understand the metabolic network of strains endowed with a broad catabolic diversity (Vilchez-Vargas et al. 2010). Hence, genomic analyses constitute an immense source for discovering and exploiting novel biocatalysts.

To date, among the bacterial genome completely sequenced and listed at https://www.ncbi.nlm.nih.gov/genome/browse/?report=2, 236 genome sequences belong to *Rhodococcus* sp. strains, of which 82 are fully sequenced. Accordingly, almost 80 publications on genome sequencing projects of *Rhodococcus* strains were released in the last four years in eminent journals, *Genome Announcement*. Furthermore, transcriptomic, proteomic, and metabolomic projects are in continuous increase.

Omics era elucidated the biotechnological potential of *Rhodococcus* genus in bioremediation and ecological applications. Since the first completely sequenced genome of *R. jostii* RHA1, *Rhodococcus* spp. showed to possess complex genomes that are among the largest available sequenced genomes in prokaryotes. In particular, RHA1 9.7 Mb genome is arranged in four linear replicons: one chromosome and three linear megaplasmids pRHL1, pRHL2, and pRHL3 (McLeod et al. 2006).

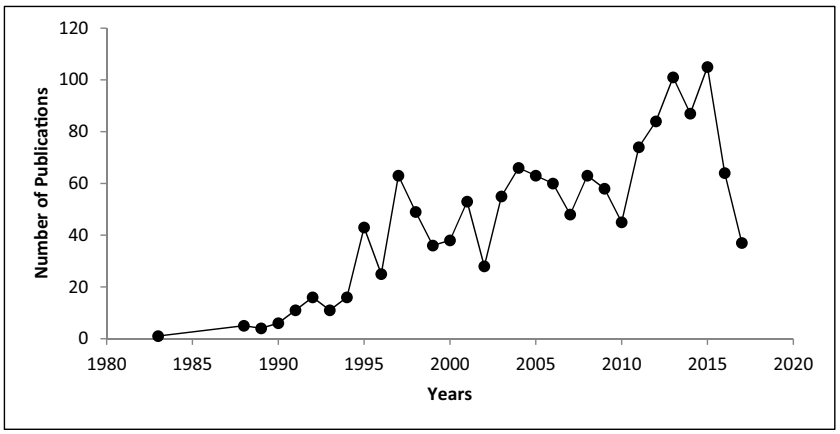

Fig. 1 Number of publications on *Rhodococcus* strains

Table 1 Omics approaches and biodegradative capabilities of different organic compounds reported in members of *Rhodococcus* genus

Compound	Strain	Type of -omic approach	Reference
Aliphatic hydrocarbons			
n-Hexadecane Commercial diesel oil Artificial mixture	*R. erythropolis* PR4	Genome comparative analysis and transcriptomic analysis	Sekine et al. 2006; Laczi et al. 2015
Medium- and long-chain alkanes	*R. aetherivorans* BCP1 and *R. opacus* R7	Genome sequencing and comparative analyses	Orro et al. 2015
Short-chain alkanes	*R. aetherivorans* BCP1	Transcriptional analysis and proteomic approach	Cappelletti et al. 2015
Sterol	*R. jostii* RHA1	Transcriptomic analysis	Rosłoniec et al. 2013; Kulig et al. 2015
Monoaromatic hydrocarbons			
BTEX	*R. aetherivorans* BCP1 and *R. opacus* R7	Genome sequencing and comparative analyses	Orro et al. 2015
	R. jostii DK17	Genome sequencing and comparative analysis	Yoo et al. 2012; Kim et al. 2002
o-Xylene	*R. opacus* R7	Genome analysis	Di Canito et al. 2018
Phenol	*R. opacus* PD630	Transcriptomic analysis (RNA-Seq)	Yoneda et al. 2016
2,4-DNT and 2,6-DNT	*R. pyridinivorans* NT2	Proteomic analysis	Kundu et al. 2016
Polycyclic aromatic hydrocarbons			
PCBs	*R. jostii* RHA1	Genomic and catabolic reconstruction; microarray hybridization	McLeod et al. 2006; Goncalves et al. 2006
Naphthalene	*R. opacus* M213	Genomic and metabolite analysis	Pathak et al. 2016
PAH	*R. aetherivorans* BCP1 and *R. opacus* R7	Genome sequencing and comparative analyses	Orro et al. 2015
Plasticizer compounds			
Phthalates	*R. jostii* RHA1	Transcriptomic	Hara et al. 2007
	R. jostii DK17		Choi et al. 2005
Isoprene	*R.* sp. AD45	Genomic	Crombie et al. 2015
Polyethylene	*R. ruber* C209	Transcriptomic	Gravouil et al. 2017
DTDB	*R. erythropolis* MI2	Genomic and proteomic	Khairy et al. 2016

Other *Rhodococcus* strains including *Rhodococcus opacus* R7 (Di Gennaro et al. 2014), *Rhodococcus opacus* M213 (Pathak et al. 2016), and *R. opacus* PD630 (Holder et al. 2011) have been also described for their large genomes (10.1, 9.19, and 9.17 Mbp, respectively) and their numerous plasmids (5 in R7, 2 in M213, and even 9 in PD630).

Moreover, it has been reported that a significant component (6%) of the biotransformations with potential biotechnological application (primarily xenobiotic reactions) recorded in the Minnesota biocatalysis/biodegradation database (http://http://umbbd.msi.umn.edu/) are assigned to *Rhodococcus* spp. This biotransformation activity in *Rhodococcus* genus is second only to *Pseudomonas* and it is achieved through numerous catabolic pathways.

In fact, a large number of enzymatic classes have been predicted to be involved in the degradation of aromatic compounds in RHA1; indeed genomic analyses revealed 203 oxygenases, of which 86 dioxygenases and 88 putative flavoprotein monooxygenases. Additionally, 50 hydroxylases, of which 28 putative cytochrome P450 hydroxylases putatively involved in aromatic and steroids compounds, have been also reported (McLeod et al. 2006).

The immense catabolic diversity shown by the members of this genus lies hidden in the multiplicity of catabolic genes, the high genetic redundancy of biosynthetic pathways, and the sophisticated regulatory networks of their genomes (Alvarez 2010). In this context, *Rhodococcus erythropolis* PR4 and *Rhodococcus* sp. DK17 showed a broad degradative abilities towards aliphatic and monoaromatic hydrocarbons, respectively (Laczi et al. 2015; Kim et al. 2002), while *R. opacus* R7 is capable of degrading the latter two as well as polycyclic aromatic hydrocarbons (Di Gennaro et al. 2010; Zampolli et al. 2014; Orro et al. 2015; Di Canito et al. 2018). Recently, -omic studies ascribed other metabolic traits to *Rhodococcus* members, such as the degradation of PCBs and steroids (chlorate and cholesterol) in *R. jostii* RHA1,

R. erythropolis PR4, *Rhodococcus opacus* B4, and *Rhodococcus aetherivorans* I24 (Goncalves et al. 2006; Swain et al. 2012; Alvarez 2010; Puglisi et al. 2010), the metabolism of isoprene (Crombie et al. 2015), short-chain alkanes, and chloroform (Ciavarelli et al. 2012; Cappelletti et al. 2015), and herbicides (Fang et al. 2016) in *Rhodococcus* sp. AD45, *Rhodococcus aetherivorans* BCP1, and *Rhodococcus* sp. MET, respectively.

Besides, intriguing degradative capabilities towards natural and synthetic polymers, organic sulfur compound as 4,4-dithiodibutyric acid (DTDB), and biodesulfurization of petroleum oil have been reported in *Rhodococcus rhodochrous* RPK1, *Rhodococcus erythropolis* MI2, and *Rhodococcus erythropolis* XP (Watcharakul et al. 2016; Gravouil et al. 2017; Khairy et al. 2016; Tao et al. 2011).

The huge repertoire of catabolic abilities of this genus could be also explained by horizontal gene transfer and gene duplication phenomena. In particular, the high frequency of recombination (homologous and illegitimate recombination) may trigger the flexibility of *Rhodococcus* genomes to easily acquire new functions (Larkin et al. 2006; Larkin et al. 2010). Indeed, catabolic genes, often identified on linear plasmids, have been found to contribute to degradation pathways together with genes located on the chromosome (Goncalves et al. 2006).

It is worth to mention that many *Rhodococcus* functional traits were unveiled combining omics data with genome comparative approaches providing insights in terms of phenotypes, metabolic capacities, and cellular response to different stress conditions. This has also contributed to elucidate phylogeny and evolutionary concepts and their relation.

Biodegradation of hydrocarbon compounds

Hydrocarbon compounds are widespread in the environment and originate from natural sources and anthropogenic activities. Large amounts of aromatic compounds were derived from decaying plant material (e.g., from lignin), soil weathering processes, and volcano emissions. Moreover, anthropogenic sources, in particular spillage of petroleum products, discharge of industrial effluents, and transport accidents, release significant amount of hydrocarbons into the environment (Martínková et al. 2009).

Mechanisms underpinning biodegradation of hydrocarbons have been described in several bacterial strains (Pérez-Pantoja et al. 2009; Juarkar et al. 2010). In particular, the aerobic biodegradation of hydrocarbon compounds has been well elucidated in several *Rhodococcus* strains (Field and Sierra-Alvarez 2004; Alvarez 2010). It was well established that aromatic compounds are catabolized through many upper and/or lower pathways leading to few central intermediates (catechol, protocatechuate, gentisate) (Fig. 2). The generated metabolites are then degraded to compounds involved in the TCA cycle through central pathways. The biodegradation of each class of hydrocarbons requires a specific enzyme due to the high diversity of the molecular structures of these compounds (Abbasian et al. 2016).

Aliphatic hydrocarbons

Rhodococcus strains are able to oxidize *n*-alkanes. Basically, the first step of oxidation is mainly performed by an alkane1-monooxygenase encoded by the *alkB* gene. This gene has been identified in well-known members of the *Rhodococcus* genus (e.g., *R. erythropolis*, *R. ruber*, *R. opacus*, *R. equi*, and *R. jostii*) and can be employed as a marker of this metabolism (Táncsics et al. 2014).

A multiplicity of *alkB* is reported in several *Rhodococcus* strains; indeed, it was identified at least four homologous alkane monooxygenase genes (*alkB1*, *alkB2*, *alkB3*, and *alkB4*) in two different rhodococcal strains (*Rhodococcus* strains Q15 and NRRLB-16531) (Whyte et al. 2002) and two different alkane hydroxylase systems (*alkBa* and *alkBb*) in *Rhodococcus ruber* SP2B (Amouric et al. 2010). Although the multiplicity of *alkB* in the genome of *Rhodococcus* spp. is frequently observed, this phenomenon is not universal for all members of the genus (Táncsics et al. 2014). Indeed, only one copy of *alkB* gene was identified in the genomes of *R. jostii* RHA1, (McLeod et al. 2006), *R. opacus* B4 (Sameshima et al. 2008), *R. aetherinovorans* BCP1 (Cappelletti et al. 2011; Cappelletti et al. 2013), and *R. opacus* R7 (Zampolli et al. 2014).

In addition, other alkane hydroxylase systems have been described in *Rhodococcus*, including (i) P450 cytochrome alkane monooxygenase systems (CYP153) that hydroxylate C_5–C_{16} *n*-alkanes (van Beilen et al. 2006) and (ii) short-alkane monooxygenases that are involved in the oxidation of C_1–C_6 *n*-alkanes (Cappelletti et al. 2011). Cytochromes P450 (CYPs) are hemoproteins that oxidize a large number of chemical compounds, such as xenobiotics, antibiotics, steroids, terpenes, alkanes, fatty acids, and alkaloids in aerobiosis (Bernhardt and Urlacher 2014; Larkin et al. 2006). In fact, *Actinobacteria* display several P450 cytochromes distributed over 220 families. For instance, 25, 26, and 45 P450 cytochromes were reported in *R. jostii* RHA1 (McLeod et al. 2006), *R. opacus* R7 (Di Gennaro et al. 2014), and *R. aetherinovorans* BCP1, respectively (Cappelletti et al. 2013). Moreover, a *cyp125* gene was identified in *R. jostii* RHA1 genome. This gene is highly upregulated during growth on cholesterol and encodes a CYP125 that catalyzes the hydroxylation of the C26 atom of sterols through side-chain oxidation leading to the C26-carboxylic acid formation (Rosłoniec et al. 2013). Transcriptomic analysis of this strain suggested that the CYP257A1 (novel cytochrome P450 enzyme family) (Rosłoniec et al. 2009) is involved in sterol

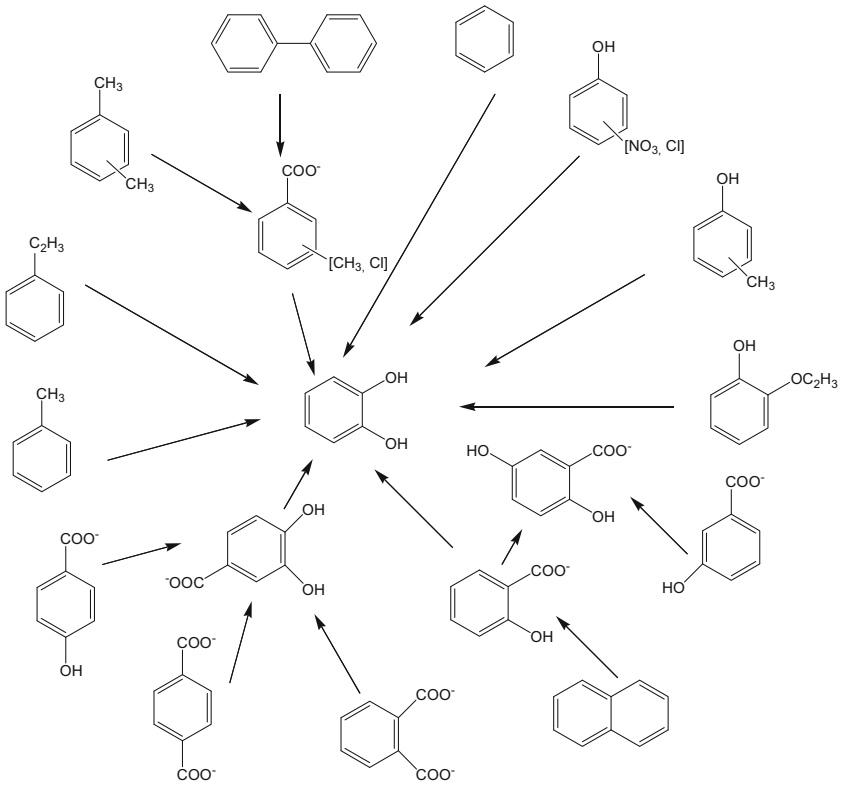

Fig. 2 Scheme of biodegradation pathways for aromatic hydrocarbon compounds in *Rhodococcus*

metabolism, while protein analysis showed that it *N*-demethylates the alkaloid substrate, i.e., dextromethorphan (Kulig et al. 2015). Another cytochrome P450 able to hydroxylate octane was described in *Corynebacterium* sp. 7E1C (in recent literature known as *Rhodococcus rhodochrous*) (Cardini and Jurtshuk 1970).

Besides, aerobic bacterial metabolism of gaseous and short-chain *n*-alkanes (ranged from ethane to *n*-butane) was reported in *Rhodococcus* genus. For instance, *R. aetherivorans* BCP1 was described for its ability to grow on gaseous *n*-alkanes as inducing condition for the co-metabolism with low chlorinated compounds. This strain presents two *prm* gene clusters: the first, *prm* gene cluster (*prmA,C,B,D* genes), is located on the chromosome and implicated in the degradation of propane and butane, and the second, *smo* gene cluster (soluble di-iron monooxygenase) (*smoA,B,D,C* genes), is positioned on the pBMC2 plasmid and involved in the degradation of short-chain *n*-alkanes (Cappelletti et al. 2015).

Aromatic hydrocarbons

The degradation of aromatic hydrocarbons employs the conversion of such substrates into a number of central intermediates, which are consequently subjected to ring cleavage. The reactions of the pathways consist in activation of the aromatic ring through oxygenases generating di- or hydroxylated intermediates. The subsequent ring cleavage of these intermediates can be catalyzed by either intradiol or extradiol dioxygenases (Martínková et al. 2009).

The BTEX system, comprising benzene, toluene, ethylbenzene, and xylene isomers, is listed as priority pollutants. Among BTEX-degrading bacteria, *Rhodococcus* sp. strain DK17, capable of degrading *o*-xylene and toluene, has been studied (Kim et al. 2002, 2010). The sequenced genome of this strain revealed that *akb* gene cluster is involved in the metabolism of these hydrocarbons. This cluster is composed of two genes encoding for the large subunit of an oxygenase (designated *akbA1a* and *akbA1b*) followed respectively by other two genes encoding for the small subunit (designated *akbA2a* and *akbA2b*). Within this cluster, three other genes *akbA3*, *akbA4*, and *akbB* encoding respectively for a reductase (AkbA4), a ferredoxin component (AkbA3), and a dehydrogenase (AkbB) were identified. Besides, in the same genomic region, *akbCDEF* genes encoding proteins involved in the ring cleavage (lower pathway) were reported. The latter genes are capable to hydroxylate aromatic compounds (i.e., ethylbenzene) to 2,3- and 3,4-*cis*-dihydrodiols and able to perform benzylic and aryl hydroxylations on *m*-xylene (Kim et al. 2002).

Another studied BTEX-degrading bacteria is *R. jostii* RHA1 (Seto et al. 1995). This strain efficiently assimilates ethylbenzene (ETB), isopropylbenzene (IPB), and biphenyl (BPH) via a common pathway. Transcriptomic analysis revealed that three gene clusters *bph*, *etb1*, and *etb2*, identified on large linear plasmids, are induced by BPH, ETB, and IPB,

which indicates that all these hydrocarbons are catabolized by the same enzymes and the genes are controlled by a common regulatory system.

Basically, these compounds are converted to benzoate and 2-hydroxypenta-2,4-dienoate (HPD) in four successive steps catalyzed by (i) four-component biphenyl dioxygenase (ferredoxin reductase, ferredoxin, and two-subunit terminal oxygenase coded by *bphA1A2A3A4*, *etbAa1Ab1*, and *etbAa2Ab2Ac*; (ii) dihydrodiol dehydrogenase (*bphB1* and *bphB2*); (iii) 2,3-dihydroxybiphenyl dioxygenase (*bphC1* and *etbC*); and finally 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoatehydrolase (*bphD1*). ETB degradation results in propionate and HPD. HPD is further metabolized to pyruvate and acetyl coenzyme A (CoA) through the lower biphenyl pathway divided into three steps (encoded by *bphGFE* in *R. jostii* RHA1) (Martínková et al. 2009; Goncalves et al. 2006) while benzoate is metabolized to succinate and acetyl-CoA through the benzoate pathway.

It was found that *bphA1A2A3A4* and *etbAa1Ab1* genes are under the control of a two-component system encoded by *bphS* and *bphT* genes, which encode the sensor histidine kinase and response regulator, respectively. Indeed, the transcriptional induction of the *bphA1* promoter by biphenyl, benzene, alkylbenzenes, and chlorinated benzenes requires *bphS1T1* genes. In a further study, Takeda et al. showed that the inducing-substrate spectrum of BphS1 includes substrates of BphS2. However, the only difference between *bphS1T1* and *bphS2T2* systems is that BPH is an inducing substrate only for the *bphS1T1* (Takeda et al. 2004). Therefore, they suggested that in the presence of a single-ring aromatic compound, such as ethylbenzene, both *bphS1* and *bphS2* are responsible for the transcriptional activation of degradation genes in RHA1.

Recently, a particular BTEX degradation pathway was found in *R. opacus* R7. This strain was found to metabolize *o*-xylene with the same dioxygenase system previously identified in DK17 (*akb*) and in RHA1 (*etb*) strains (allocated on two plasmids, pPDG5 and pPDG2). Furthermore, R7 was shown to possess numerous monooxygenases/phenol hydroxylases included in the *o*-xylene degradation pathway, highlighting the redundancy of oxygenases genes in this strain (Di Canito et al. 2018).

Phenols

Phenolic compounds (e.g., cresols), released and accumulated in the environment, pose serious health hazards to living organisms (Michałowicz and Duda 2007).

A wide range of phenols are degraded by *Rhodococcus* strains. The degradation begins by the conversion of phenol compound to catechol by phenol hydroxylase, which is further metabolized through *ortho*- or *meta*-cleavage. Basically, catechol 1,2-dioxygenase, an *ortho*-cleaving enzyme, and catechol 2,3-dioxygenase, a *meta*-cleaving enzyme, are proteins involved in the β-ketoadipate pathway in which the bacterial degradation of catechol to central metabolic intermediates occurs (Guzik et al. 2011; Szőköl et al. 2014; Zídková et al. 2013). In *Rhodococcus erythropolis* CCM2595, the phenol hydroxylase enzyme was described; it consists of two subunits encoded by the *pheA1* and *pheA2* genes clustered with the *pheR* gene, coding for an AraC-type transcriptional regulator (activator) (Zídková et al. 2013). These genes were found to be induced by phenol and other aromatic compounds such as *p*-chlorophenol, *p*-nitrophenol, resorcinol, and *p*-cresol (Fialova et al. 2003). Moreover, this gene cluster was found in *R. jostii* RHA1 in two copies, on the chromosome and on the plasmid pRHL1 (McLeod et al. 2006).

Other *Rhodococcus* strains with phenol degradation ability were reported. For instance, *Rhodococcus erythropolis* UPV-1 is efficiently capable of degrading PAHs, phenol, and a mixture of *o*-, *m*-, and *p*-cresols (Irvine et al. 2000). In addition, *Rhodococcus opacus* 1CP is able of degrading *p*-cresols through 4-methylcatechol and 3-methyl-*cis*,*cis*-muconate via *ortho*-pathway. This strain possesses also another catechol-1,2dioxygenase activity, in addition to dioxygenases with specificities for 4-chlorophenol and *p*-toluate (Kolomytseva et al. 2007).

Interestingly, an important biotechnological trait attributed to *Rhodococcus* genus is the capability of accumulating lipids to high extent in their cytosol, up to 80% in *R. opacus* PD630 (Holder et al. 2011). Recent study employing omics techniques has shown a positive correlation between phenolic compound degradation and lipid production in *Rhodococcus rhodochrous*. This could explain the capacity of *Rhodococcus* strains to utilize recalcitrant compounds including complex polyaromatic structure (Shields-Menard et al. 2017).

Polyaromatic hydrocarbons

A wide range of PAH compounds are catabolized by bacteria of *Rhodococcus* genus, i.e., fluoranthene, pyrene, and chrysene (Xu-Xiang et al. 2006). Basically, PAH degradation initiates in the cytosol by the action of intracellular dioxygenases. Subsequently, PAHs are oxidized to *cis*-dihydrodiols, which in turn are re-oxidized to aromatic dihydroxy compounds (catechols) and eventually channeled through the *ortho* or *meta*-cleavage pathways (Cerniglia 1984; Smith 1990). PAH metabolic pathway has been investigated in many *Rhodococcus* strains by growing the bacterium on naphthalene (the simplest and most soluble PAH). The latter is considered a model compound to investigate the ability of bacteria to degrade PAHs. For instance, *R. opacus* R7 degrades naphthalene through the dioxygenation of the aromatic ring, via 1,2-dihydro-1,2-dihydroxynaphthalene, that is further oxidized to salicylate and gentisate (Di Gennaro et al. 2010). In this pathway, two genes, *narAa* and *narAb*, encoding

respectively for the large (NarAa) and the small (NarAb) components of the naphthalene dioxygenase are involved. These genes are clustered with *rub1*, *rub2*, *rub1bis*, *narB*, and *orf* (*orf7*) genes, encoding for three rubredoxins, a naphthalene dihydrodiol dehydrogenase, and a protein of unknown function, respectively. In addition, two regulatory genes (*narR1*, *narR2*) were described in R7 encoding for putative regulatory proteins NarR1 and NarR2, belonging to GntR and NtrC families, accordingly. Nonetheless, no LysR-type regulatory gene was reported. The final aromatic ring cleavage in this strain proceeds through the gentisate pathway, encoded by *gen* and *sal* genes (Di Gennaro et al. 2010). Moreover, the lower pathway of naphthalene degradation was found in two copies, the first is on pPDG4 plasmid and the second on pPDG1 plasmid, which lacks *genL* gene (Orro et al. 2015). Besides, salicylate metabolism in R7 involves *genC*, *genB*, and *genA* genes located upstream the genes associated with gentisate degradation. In particular, *genA*, *genB*, *genC*, *genH*, *genI*, and *genL* encode for salicylate CoA ligase, salicylate CoA synthetase, salicylate hydroxylase, gentisate 1,2-dioxygenase, 3-maleylpyruvate isomerase, and a protein of unknown function, respectively.

Naphthalene degradation pathway has been also described in *Rhodococcus* sp. NCIMB12038. In particular, *nar* region is involved in this degradation; it is composed of *narA* and *narB* genes which are transcribed in several units. Contrariwise, these genes are transcribed as single units in P200 and P400 strains (Kulakov et al. 2005).

Other genetic determinants that likely participate in naphthalene (and *o*-xylene) degradation were reported by Martínková et al. (2009). For instance, in *Rhodococcus opacus* TKN14 (Kulakov et al. 2000), *nid* gene cluster was identified; it is composed of *nidAB*, *nidE*, and *nidF* genes encoding for the subunits of oxygenase, rubredoxin, and auxiliary protein, respectively (Maruyama et al. 2005). *nid* genes were found to be induced by *o*-xylene and are supposed to be involved in the degradation pathways of a wide range of aromatic hydrocarbons in *Rhodococcus* species.

Thanks to -omics approach a peculiar naphthalene metabolism has been described in *R. opacus* M213, which occurs via *o*-phthalate and salicylate pathways. Conversely to naphthalene pathways previously described in other rhodococci, which occur via gentisate pathway in NCIMB 12038, B4 and R7 and through catechol pathway in P200 and P400 strains. The metabolism of naphthalene in M213 occurs via the cyclization of 2-hydroxycinnamic acid or 2-carboxycinnamic acid and eventually oxidizing into phthalic anhydride or phthalic acid. It is particularly interesting that several genes associated with this peculiar biodegradation pathway were found on genome islands (GEIs), such as small and large subunits of naphthalene dioxygenase (NDO), *cis*-naphthalene dihydrodiol dehydrogenase gene, and a putative naphthalene degradation regulatory protein (Pathak et al. 2016).

Degradation of plasticizer compounds

Plasticizers and their derivatives are among the prime examples of emerging compounds. A large amount of these compounds derives from anthropogenic sources, in particular discharge of industrial activities (Gravouil et al. 2017; Koutny et al. 2006). In this paragraph, we focused on -omic studies related to the degradation of phthalate, phthalate esters (PEs), polyisoprene, and 4,4-dithiodibutyric acid (DTDB) in *Rhodococcus* strains (Table 2).

Phthalate

After decades of global industrial use as plasticizers, phthalate esters are now recognized as ubiquitous toxicologically significant environmental pollutants (Hara et al. 2007).

Among strains of *Rhodococcus* genus, *R. jostii* RHA1 is able to grow on a variety of monoalkyl PEs, including methyl, butyl, hexyl, 2-ethylhexyl phthalates, and terephthalate (Hara et al. 2010). This strain is able to degrade phthalate (PTH), terephthalate, and 4-hydroxybenzoate via protocatechuate *ortho*-cleavage pathway (Patrauchan et al. 2005). Nevertheless, it can also degrade terephthalate via the catechol branch of the 3-oxoadipate pathway (Hara et al. 2007).

Transcriptomic analysis revealed the metabolic pathway of PTH in RHA1. Two identical functional copies of *pad* genes

Table 2 Enzymes and/or pathways involved in biodegradative metabolism of representative plasticizer compounds identified in *Rhodococcus* members

Degradation substrate	Enzyme and/or pathway	Strain name	Reference
Phthalates	*pad*, *pat* genes	*R. jostii* RHA1	Hara et al. 2007
		R. sp. DK17	Choi et al. 2005
Polyethylene	Alkane pathway (*alk*) Laccase	*R. ruber* C208	Gravouil et al. 2017
Isoprene	Isoprene pathway (*iso*)	*R.* sp. AD45	Crombie et al. 2015
Polyisoprene	*lcp* genes	*R. rhodochrous* RPK1	Watcharakul et al. 2016
DTDB	*nox* gene	*R. erythropolis* MI2	Khairy et al. 2016

carried on two large linear plasmids, pRHL1 and pRHL2, are employed. These genes encode a ring-hydroxylating 3,4-dioxygenase system (PadAaAbAcAd), a dihydrodiol dehydrogenase (PadB), a decarboxylase (PadC), and a regulatory protein (PadR). Moreover, close to *pad* genes, *pat* genes were identified, which encode an ABC transport system (PatABCD) and an ester hydrolase (PatE) (Patrauchan et al. 2012) and are highly upregulated in presence of phthalate rather than terephthalate. Interestingly, in a further study involving *patB* gene knockout, ATP-binding cassette (ABC) transporter was identified necessary for the uptake of phthalate and monoalkyl phthalate esters in RHA1. Moreover, in the same study, it was demonstrated that PatE which is a member of monoalkyl PE hydrolase family is able to specifically hydrolyze monoalkyl PEs to phthalate, rather than terephthalate or other PEs (Hara et al. 2010). In addition, other genes named *tpa* gene were identified to be putatively involved in terephthalate (TPA) uptake and degradation. The *tpa* genes encode a transport protein (TpaK), the large and small subunits plus the reductase of a ring-hydroxylating 1,2-dioxygenase system (TpaAaAbB), a dihydrodiol dehydrogenase (TpaC), and a regulatory protein (TpaR). Interestingly, the nearly identical regions of pRHL1 and pRHL2 were also identified to nearly identical regions that are duplicated in pDK2 and pDK3 in *Rhodococcus* sp. strain DK17 (Choi et al. 2005), which have also been shown to encode PTH and TPA degradation proteins.

Polyethylene

Polyethylene (PE) is a notable synthetic polymer used worldwide, particularly in the packaging industry. PE is stable and tends to accumulate in natural environment, representing by itself the majority of all plastic wastes (Gravouil et al. 2017). The degradation of PE in nature is a complex procedure in which hydrocarbon chains are oxidized into short aliphatic fragments (e.g., complex mixture of alkanes, alkenes, ketones, aldehydes, alcohols, carboxylic acids); subsequently, these fragments are mineralized by specific microorganisms from the environment (Koutny et al. 2006).

Species of *Rhodococcus* genus have been associated with PE degradation in several studies (Gravouil et al. 2017; Santo et al. 2013). For instance, Sivan and colleagues reported their ability to grow as biofilm on PE-oxidized films. Nevertheless, the molecular mechanism of PE consumption has not been revealed (Sivan et al. 2006).

Recent study based on transcriptomics and lipidomics approaches pointed out the consumption pathways of polyethylene degradation in *Rhodococcus ruber* C208. In this context, non-oxidized short-chain PE and oxidized PE samples resulting from abiotic treatment induce the pathways related to alkane degradation and β-oxidation of fatty acids in *R. ruber* C208. This observation revealed these pathways as being the bulk for the consumption of PE. The induction of these pathways could be explained by the presence of short aliphatic fragments in the samples, which could be recognized as natural inducers (Gravouil et al. 2017).

Abiotic treatment of PE (thermo and photo-oxidization) releases mixture of fragments displaying a wide range of lengths and oxidation levels. Fragments of low molecular weight are more likely to be consumed than PE films by *Rhodococcus* species.

Santo and colleagues have reported the role of laccase enzyme in catalyzing the biotic oxidation of PE and consequently in increasing the amount of metabolized short fragments. Indeed, the authors identified a copper-binding laccase in *R. ruber* C208, which is able, when overproduced, to induce a reduction of the PE Mw and Mn of 20% and 15%, respectively (Santo et al. 2013). In addition, Gravouil and colleagues identified three close homologs to laccase in the RNA library of *R. ruber* C208, but interestingly, none of them were shown to be significantly upregulated in the presence of the PE samples, regardless their oxidation levels (Gravouil et al. 2017).

Polyisoprene

Natural rubber is synthetized in large amount by many plants and is used in the production of tires, sealings, and latex gloves. The main component of rubber is the poly(*cis*1,4-isoprene) (Sharkey 1996). Nonetheless, the economic importance of rubber and the enormous amount of rubber waste materials released into the environment, only a limited number of studies have investigated its biodegradation in bacteria.

Van Hylckama Vlieg and colleagues revealed the genes involved in isoprene utilization in *R.* sp. AD45. In this strain, the consumption of isoprene includes its oxidation to epoxide, conjugation with glutathione, and dehydrogenation steps. The authors identified the presence of 10 genes, where two of them encode enzymes involved in isoprene degradation: a glutathione S-transferase with activity towards 1,2-epoxy-2-methyl-3-butene (*isoI*) and a 1-hydroxy-2-glutathionyl-2-methyl-3-butene dehydrogenase (*isoH*) (Van Hylckama Vlieg et al. 2000). Another glutathione S-transferase gene (*isoJ*) was reported. The latter acts with 1-chloro-2,4-dinitrobenzene and 3,4-dichloro-1-nitrobenzene, but not with 1,2-epoxy-2-methyl-3-butene. In addition, the authors identified downstream of *isoJ*, six genes (*isoABCDEF*) that encode for a putative alkene monooxygenase, and they figured out an amino acid sequence encoded by an additional gene (*isoG*) which shows high homology with respect to α-methylacyl-coenzyme A racemase (Van Hylckama Vlieg et al. 2000).

Subsequently, Crombie and colleagues have identified the complete set of inducible genes responsible for isoprene degradation. The authors reported the genome of *R.* sp. AD45. They found that genes involved in isoprene metabolism are concentrated in a small region on a megaplasmid, containing a relatively large number of transposases (Crombie et al. 2015). Their

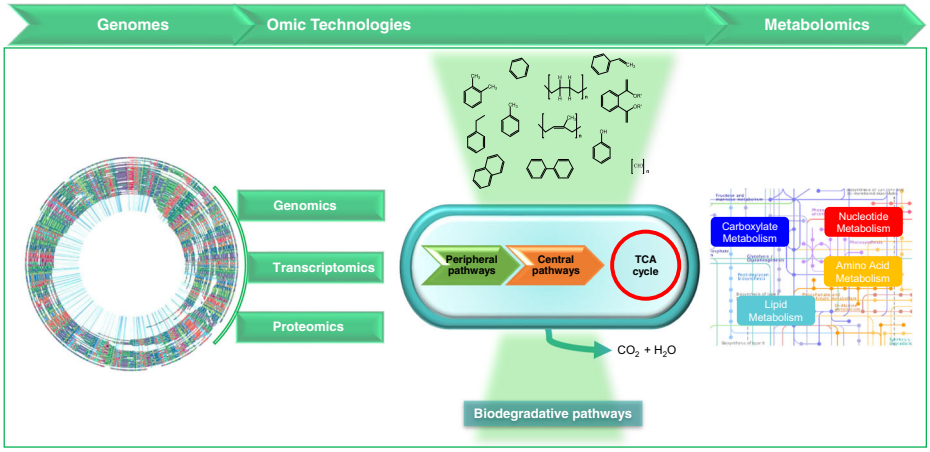

Fig. 3 Schematic representation of relationship between omics technologies and *Rhodococcus* biodegradative pathways

analyses showed high level of transcription of 22 contiguous genes when induced by isoprene or epoxyisoprene, suggesting their involvement in isoprene metabolism, including the monooxygenase, glutathione transferase, dehydrogenase (IsoH), and glutathione biosynthesis genes. In addition, the authors showed that only two aldehyde dehydrogenases, a disulfide reductase, and hypothetical protein are induced by isoprene, suggesting that this cluster may contain all the genes specific to this metabolism. Finally, they demonstrated that the previous identified genes were only induced by isoprene in a strain able to oxidize isoprene to epoxyisoprene, demonstrating that isoprene itself was not the inducing molecule. Hence, they suggested that *isoGHIJABCDEF* are co-transcribed as an operon with a promoter upstream of *isoG* (Crombie et al. 2015).

Watcharakul and colleagues identified a gene coding for the latex-clearing protein (*lcp*) in *Rhodococcus rhodochrous* strain RPK1, which is putatively involved in the initial oxidative attack on the polyisoprene polymer. This gene was also found in many *Actinobacteria* and all known rubber-degrading *Actinobacteria* for which the genome sequences are available (Nanthini et al. 2015; Rose et al. 2005). In addition, a hypothetical *lcp* gene in the genomes of *Rhodococcus rhodochrous* strain MTCC11081, *Rhodococcus* sp. MK3027, and *Rhodococcus* sp. ARG-BN062 was identified. Particularly, the predicted Lcp amino acid sequence of this gene includes the DUF2236 domain which constitutes the central part of most Lcp proteins. Moreover, by isolating and characterizing the Lcp protein of *R. rhodochrous* RPK1, the authors pointed out the presence of different Lcp subgroups which vary among them by metal ion dispositions and spectroscopic properties (Watcharakul et al. 2016).

4,4-Dithiodibutyric acid

4,4-Dithiodibutyric acid (DTDB) is a synthetic organic sulfur compound employed in diverse biochemical fields (Imbernon et al. 2015; Jang and Keng 2006). However, the biotechnological significance of this compound is its application in studies for the improvement of polythioester (PTE) synthesis. Although DTDB is non-toxic compound, its biodegradation results in the formation of very harmful metabolite: the poly(4-mercaptobutyric acid) (4MB), which is a PTE precursor substrate (Khairy et al. 2016). Hence, investigating DTDB catabolism in bacteria is necessary for the generation of metabolically engineered PTE production strains. *R. erythropolis* strain MI2 has been investigated for its rare ability to use DTDB as sole carbon source and electron donor for aerobic growth (Khairy et al. 2016). Genome and proteome analysis of this strain has clarified the DTDB degradation by elucidating the 4MB catabolism pathway. Basically, DTDB is cleaved by the action of Nox (RERY_03780) to 4MB, which is then oxidized forming 4-oxo-4-sulfanylbutyric acid via the LLMMI2 F420-dependent enzyme (RERY_05640) encoded by one of the 126 monooxygenase-encoding genes found in MI2 genome. The ultimate step is supposed to be a desulfurization reaction. Thus, 4-oxo-4-sulfanylbutyric could either be degraded by SQRMI2 (RERY_02710) or the putative desulfhydrase (RERY_06500) which forms succinic acid and volatile hydrogen sulfide. H_2S could be oxidized by SQR and then reacts with a free thiol group forming a sulfurhydrate-containing compound. The latter may be oxidized forming thiosulfite by protein encoded by RERY_02720, which is then oxidized by the putative rhodanese-related sulfurtransferase (RERY_02740), giving sulfite and succinic acid as final products. Sulfite could be oxidized to sulfate prior to be transported outside the cell through the transmembrane exporter protein (RERY_02750), while succinic acid is most probably utilized for growth (Khairy et al. 2016). More studies are needed to verify the postulated enzymatic reactions.

Conclusions

In the last decades, -omics data have increased our understanding of the biological aspects, genetics, and metabolic pathways that could be exploited for biotechnological and bioremediation

applications (Khairy et al. 2016; Juwarkar et al. 2010). Traditional approaches of genetics and biochemistry have been applied to *Rhodococcus* bacteria. Nonetheless, understanding the mechanisms and pathways of hydrocarbons biodegradation have been limited. Nowadays, omics techniques with advanced high-throughput analytical technologies have a special impact in the wake of predicting and constructing metabolic pathways and since the possibility to exploit constructed pathways and gene functions in biodegradation and bacterial biotechnological potential (Fig. 3) (Pathak et al. 2016; Yoneda et al. 2016). With this aim, combination of molecular tools and markers could be useful in bioremediation processes.

Recent achievements of combining genomic, proteomic, and bioinformatics approaches provided insights not only on gene functions, degradation pathways, and molecular mechanisms, but also in the genome-wide gene expression of *Rhodococcus* bacteria in various environmental conditions (Kim et al. 2018). However, further researches (i.e., cell physiology and regulatory studies, environmental stress response) are required in the field of hydrocarbon degradation in *Rhodococcus*. The analysis of generated data from currently available sequenced genomes along with genetics and molecular biology approaches will allow to investigate in depth both biodegradation properties and biotechnological potential of these bacteria, which have been recognized as a untapped source of genetic and functional diversity.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in this study were in compliance with ethical standards. This article does not contain any studies with human participants or animals performed by any of the authors.

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