Central Metabolism of Species of the Genus *Rhodococcus*

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Abstract Metabolism of *Rhodococcus* has evolved for adapting to a wide range of nutritional conditions. This adaptation often involves the flexibility of the central metabolism, which usually provides energy and precursors for biosynthesis processes, either during growth or during non-replicative metabolically active periods. The pathways of central metabolism are almost identical across widely divergent organisms, which share essentially the same metabolic network. However, this network possesses species-specific components, which depends on the biology of rhodococci. The central metabolism of members of *Rhodococcus* genus in the context of their physiology is the main topic of this chapter. An overview of main pathways of the central metabolism and their link with other metabolic processes is given. Glycolytic pathways, gluconeogenesis, phosphoenolpyruvate-pyruvate-oxaloacetate node, tricarboxylic acid cycle (TCA), glyoxylate pathway, and some lithoautotrophic pathways are included.

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1 Introduction

Members of the genus *Rhodococcus* are aerobic non-sporulating bacteria widely distributed in diverse natural environments. They have been detected in tropical, artic, and arid soils, as well as marine and very deep-sea sediments (Whyte et al. 1999; Heald et al. 2001; Peressutti et al. 2003; Alvarez et al. 2004; Luz et al. 2004; Peng et al. 2008). One striking feature of rhodococci is the metabolic versatility with capabilities for biodegradation and transformation of a wide range of pollutant compounds, such as hydrocarbons, pesticides, and xenobiotics (Warhurst and Fewson 1994; Larkin et al. 2005; Martínková et al. 2009). This feature, in addition to their extraordinary environmental persistence and tolerance to stress conditions, makes such microorganisms promising candidates for in situ bioremediation of contaminated soil environments. Moreover, members of *Rhodococcus* are able to synthesize diverse compounds, such as surfactants, wax esters, and oils, which are of interest for biotechnological purposes. For these reasons, the studies on rhodococci have shown a significant increase within the last years. In this context, most knowledge on metabolism acquired during the last years has been focused principally on catabolism of diverse compounds and on the biosynthesis of lipids. For detailed overviews on these topics, the readers should refer to recent reviews (Larkin et al. 2005; Alvarez and Steinbüchel 2002; Alvarez 2006; Martínková et al. 2009; Yam et al. 2011). Despite the importance of the central metabolism for understanding the biology of rhodococci, this area has received little attention in comparison with the other metabolic processes mentioned above. The pathways of central metabolism are highly conserved among organisms; however, each species adopts a metabolic configuration specific to its biology. In this context, the pathways of central metabolism in rhodococci must provide energy and metabolic intermediates not only during growth periods but also in those environmental conditions in which cells do not grow but are still metabolically active. Under stress conditions, the physiology of rhodococci seems to depend on the metabolism of diverse storage compounds. Members of *Rhodococcus* genus possess an extensive capacity to synthesize and metabolize diverse storage compounds, such as triacylglycerols, wax esters, polyhydroxyalkanoates, glycogen, and polyphosphate (Anderson et al. 1995; Alvarez et al. 1997; Alvarez 2003; Hernández et al. 2008, 2017).

During the last years, numerous complete genome sequences of several *Rhodococcus* species such as *R. opacus* (strains B4 and PD630), *R. rhodochrous*, *R. pyridinivorans*, *R. ruber*, *R. hoagie* (*equi*), *R. erythropolis*, *R. qingshengii*, and *R. fascians*, among others, have been uploaded in public database. This fact reflects the importance of this genus as resources for biotechnological purposes or in the case of *R. hoagie* and *R. fascians* for their association with some pathologies of animals, humans, and plants. Among them, the genome sequence of *Rhodococcus jostii* strain RHA1 was the first sequence publicly available for screening and identification of genes and metabolic pathways (http://www.ncbi.nlm.nih.gov/genomes/lproks.cgi). For this reason, *R. jostii* RHA1 became a good model for understanding the genetics, physiology, and metabolism of the *Rhodococcus* genus. Strain RHA1 possesses one

of the largest bacterial genomes sequenced to date, containing 9.7 Mbp arranged in a linear chromosome (7,802,028 bp) and three linear plasmids: pRHL1 (1,123,075 bp), pRHL2 (442,536 bp), and pRHL3 (332,361 bp) (McLeod et al. 2006). RHA1 is a soil bacterium with the ability to degrade and transform polychlorinated biphenyls and other aromatic compounds (Masai et al. 1995; Van der Geize et al. 2007; Patrauchan et al. 2008). The large RHA1 genome contains a multiplicity of catabolic genes, a high genetic redundancy of biosynthetic pathways, and a sophisticated regulatory network. Based on peer-reviewed literature and a thorough genome examination of *R. jostii* RHA1 as model, this chapter summarizes some aspects of the central metabolism of species of the *Rhodococcus* genus, including glycolytic pathways and NADPH-generating systems, gluconeogenesis, and phosphoenolpyruvate-pyruvate-oxaloacetate node, the tricarboxylic acid cycle (TCA), and the glyoxylate shunt. In addition, some new information about the energy pathways is given.

2 Glycolytic Pathways and NADPH-Generating Systems

Glycolysis (**E**mbden-**M**eyerhof-**P**arnas pathway, EMP) is a common pathway for glucose oxidation used by eukaryotic cells and some aerobic and facultative anaerobic bacteria, but not by Archaea. In this pathway, glucose is split into two molecules of pyruvic acid with the formation of two ATP molecules. Many aerobic bacteria and archaea use an alternative glycolytic pathway called **E**ntner-**D**oudoroff pathway (ED pathway), which produces glyceraldehyde 3-P, pyruvate, and only one ATP molecule from each molecule of glucose. This pathway is found in prokaryotes such as *Pseudomonas*, *Azotobacter*, *Rhizobium*, and other Gram negatives. However, the occurrence of the ED pathway has been also described in actinobacteria (Gunnarsson et al. 2004; Borodina et al. 2005; Juarez et al. 2017).

The analysis of the *R. jostii* RHA1 genome revealed the occurrence of key genes involved in glycolytic pathways. RHA1 strain is able to use both EMP and ED glycolytic pathways, to catabolize carbohydrates. Figure 1 and Table 1 show an overview on the pathways and enzymes/genes implicated in the glucose oxidation by this strain.

The RHA1 genome also contains all necessary genes/enzymes for the **P**entose **P**hosphate **P**athway (PPP) (Fig. 1 and Table 1). This pathway produces the precursor ribose 5-phosphate used in nucleic acid biosynthesis as well as erythrose 4-phosphate for the synthesis of aromatic amino acids. In addition, the PPP has been linked with the production of NADPH used in anabolic processes (Spaans et al. 2015).

Whereas the glucose metabolism by EMP generates two reducing equivalents in the form of NADH, the ED and PPP pathways generate one and two reducing equivalents in the form of NADPH, respectively. The initial step of ED and PPP pathways involves the conversion of glucose 6-phosphate in 6-phosphogluconolactone catalyzed by the enzyme glucose-6-phosphate dehydrogenase (Zwf), in which NADPH is generated.

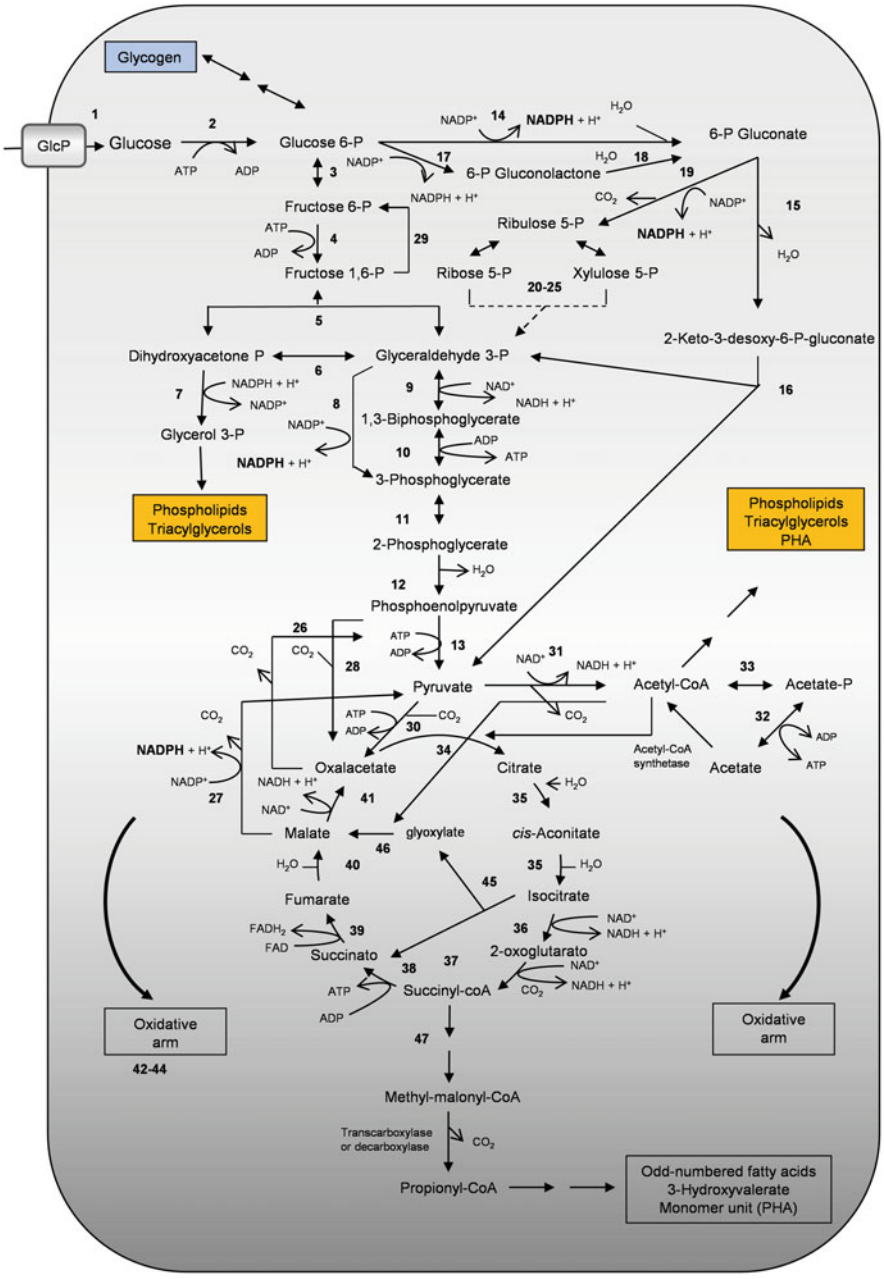

Fig. 1 Main pathways involved in central metabolism of *Rhodococcus jostii* RHA1 deduced from the genome database

Table 1 Main genes involved in the central metabolism of *R. jostii* RHA1

Reaction number[a]	Gene ID	Gene name	Predicted function based on gene annotation	Metabolic pathway
1	*RHA1_RS11555*	*glcP*	Glucose transporter major facilitator superfamily (MFS)	Glucose uptake
2	*RHA1_RS05385*		Glucokinase	Glycolysis (EMP-ED), glycogen degradation
2	*RHA1_RS06585*		Glucokinase	Glycolysis (EMP-ED), glycogen degradation
2	*RHA1_RS20805*		Glucokinase	Glycolysis (EMP-ED), glycogen degradation
3	*RHA1_RS43575*	*pgi*	Glucose-6-phosphate isomerase	Glycolysis (EMP), gluconeogenesis
3	*RHA1_RS43120*	*pgi*	Glucose-6-phosphate isomerase	Glycolysis (EMP), gluconeogenesis
3	*RHA1_RS44310*	*pgi*	Glucose-6-phosphate isomerase	Glycolysis (EMP), gluconeogenesis
3	*RHA1_RS27160*	*pgi*	Glucose-6-phosphate isomerase	Glycolysis (EMP), gluconeogenesis
4	*RHA1_RS00295*		Phosphofructokinase	Glycolysis (EMP)
4	*RHA1_RS31690*	*pfkA*	6-Phosphofructokinase	Glycolysis (EMP)
4	*RHA1_RS33150*		1-Phosphofructokinase	Glycolysis (EMP)
5	*RHA1_RS27000*	*fba*	Class II fructose-bisphosphate aldolase	Glycolysis (EMP), gluconeogenesis
5	*RHA1_RS26975*	*fba*	Fructose-bisphosphate aldolase	Glycolysis (EMP), gluconeogenesis
6	*RHA1_RS35040*	*tpiA*	Triose-phosphate isomerase	Glycolysis (EMP)
7	*RHA1_RS31815*	*gpsA*	Glycerol-3-phosphate dehydrogenase [NAD(P)+] 2	Glycerol 3-phosphate synthesis
7	*RHA1_RS11710*	*gpsA*	Glycerol-3-phosphate dehydrogenase [NAD(P)+] 1	Glycerol 3-phosphate synthesis
8	*RHA1_RS16630*	*tadD*	NADP+-dependent Glyceraldehyde-3-phosphate dehydrogenase	Glycolysis (EMP/ED)
9	*RHA1_RS35030*	*gap2*	Glyceraldehyde 3-phosphate dehydrogenase	Glycolysis (EMP), gluconeogenesis
10	*RHA1_RS35035*	*pgk*	Phosphoglycerate kinase	Glycolysis (EMP), gluconeogenesis
11	*RHA1_RS04205*		Phosphoglycerate mutase	Glycolysis (EMP), gluconeogenesis
11	*RHA1_RS06270*	*gpmA*	Phosphoglycerate mutase	Glycolysis (EMP), gluconeogenesis

(continued)

Table 1 (continued)

Reaction number[a]	Gene ID	Gene name	Predicted function based on gene annotation	Metabolic pathway
11	*RHA1_RS31045*		Phosphoglycerate mutase	Glycolysis (EMP), gluconeogenesis
12	*RHA1_RS28200*	*eno*	Enolase	Glycolysis (EMP), gluconeogenesis
13	*RHA1_RS04835*	*pyk*	Pyruvate kinase	Glycolysis (EMP-ED)
13	*RHA1_RS12545*	*pyk*	Pyruvate kinase	Glycolysis (EMP-ED)
13	*RHA1_RS15635*	*pyk*	Pyruvate kinase	Glycolysis (EMP-ED)
13	*RHA1_RS23435*	*pyk*	Pyruvate kinase	Glycolysis (EMP-ED)
14	*RHA1_RS12070*		PQQ-dependent sugar dehydrogenase	Glucose degradation (PPP-ED pathway)
14	*RHA1_RS31700*		PQQ-dependent sugar dehydrogenase	Glucose degradation (PPP-ED pathway)
15	*RHA1_RS11570*	*edd*	Phosphogluconate dehydratase	Glucose degradation (ED)
16	*RHA1_RS11565*	*eda*	KHG/KDPG aldolase	Glucose degradation (ED)
17	*RHA1_RS02710*	*zwf1*	Glucose-6-phosphate 1-dehydrogenase	PPP/ED pathway
17	*RHA1_RS11575*	*zwf2*	Glucose-6-phosphate 1-dehydrogenase	PPP/ED pathway
17	*RHA1_RS27530*	*zwf3*	Glucose-6-phosphate 1-dehydrogenase	PPP/ED pathway
17	*RHA1_RS49040*	*zwf4*	Glucose-6-phosphate 1-dehydrogenase (NADP+)	PPP/ED pathway
17	*RHA1_RS44335*	*zwf5*	Glucose-6-phosphate 1-dehydrogenase	PPP/ED pathway
17	*RHA1_RS43115*	*fgd*	Glucose-6-phosphate dehydrogenase coenzyme-F420	PPP/ED pathway
17	*RHA1_RS43570*	*fgd*	Glucose-6-phosphate dehydrogenase coenzyme-F420	PPP/ED pathway
18	*RHA1_RS35055*	*pgl*	6-Phosphogluconolactonase	PP pathway
19	*RHA1_RS02715*	*gnd*	Phosphogluconate dehydrogenase (decarboxylating)	PP pathway
19	*RHA1_RS17785*	*gnd*	Phosphogluconate dehydrogenase (decarboxylating)	PP pathway
19	*RHA1_RS35365*	*gnd*	Phosphogluconate dehydrogenase ((NADP(+)-dependent, decarboxylating))	PP pathway

(continued)

Table 1 (continued)

Reaction number[a]	Gene ID	Gene name	Predicted function based on gene annotation	Metabolic pathway
19	*RHA1_RS43110*	*gnd*	Phosphogluconate dehydrogenase (decarboxylating)	PP pathway
20	*RHA1_RS34980*	*rpe*	Ribulose-phosphate 3-epimerase	PP pathway
21	*RHA1_RS14120*		Ribose-5-phosphate isomerase	PP pathway
21	*RHA1_RS06675*		Ribose 5-phosphate isomerase	PP pathway
22	*RHA1_RS49045*	*tal*	Transaldolase	PP pathway
22	*RHA1_RS36885*	*tal*	Transaldolase	PP pathway
23	*RHA1_RS03880*		Transketolase, C-terminal subunit	PP pathway
23	*RHA1_RS03885*		Transketolase, N-terminal subunit	PP pathway
23	*RHA1_RS18640*		Transketolase, N-terminal subunit	PP pathway
23	*RHA1_RS18645*		Transketolase, C-terminal subunit	PP pathway
23	*RHA1_RS35075*		Transketolase	PP pathway
24	*RHA1_RS16375*		Sugar phosphate isomerase/ epimerase	PP pathway
24	*RHA1_RS25680*		Sugar phosphate isomerase/ epimerase	PP pathway
24	*RHA1_RS22195*		Sugar phosphate isomerase/ epimerase	PP pathway
24	*RHA1_RS14555*		Sugar phosphate isomerase/ epimerase	PP pathway
24	*RHA1_RS10065*		Sugar phosphate isomerase/ epimerase	PP pathway
25	*RHA1_RS22585*		Class II aldolase	PP pathway
25	*RHA1_RS34445*		Class II aldolase	PP pathway
26	*RHA1_RS25350*		Phosphoenolpyruvate carboxykinase	Gluconeogenesis/ anaplerotic pathway
27	*RHA1_RS02430*		NAD-dependent malic enzyme	Gluconeogenesis/ anaplerotic pathway
27	*RHA1_RS12570*		NAD-dependent malic enzyme	Gluconeogenesis/ anaplerotic pathway
27	*RHA1_RS29320*		NADP-dependent malic enzyme	Gluconeogenesis/ anaplerotic pathway
27	*RHA1_RS44255*		NADP-dependent malic enzyme	Gluconeogenesis/ anaplerotic pathway
27	*RHA1_RS39800*		NADP-dependent malic enzyme	Gluconeogenesis/ anaplerotic pathway
28	*RHA1_RS35050*	*ppc*	Phosphoenolpyruvate carboxylase	Anaplerotic pathway

(continued)

Table 1 (continued)

Reaction number[a]	Gene ID	Gene name	Predicted function based on gene annotation	Metabolic pathway
29	*RHA1_RS28640*	*glpX*	Fructosa 1,6-bisphosphatase class II	Gluconeogenesis
30	*RHA1_RS31870*	*pycA*	Pyruvate carboxylase	Anaplerotic pathway
31	*RHA1_RS02650*		Pyruvate dehydrogenase E1 component	Acetyl-CoA synthesis
31	*RHA1_RS02655*		Dihydrolipoyllysine-residue succinyltransferase (PDH)	Acetyl-CoA synthesis
31	*RHA1_RS05135*		Pyruvate oxidase	Acetyl-CoA synthesis
31	*RHA1_RS05795*	*aceE1*	Pyruvate dehydrogenase (acetyl-transferring), homodimeric type	Acetyl-CoA synthesis
31	*RHA1_RS07665*	*pdhA*	Pyruvate dehydrogenase E1 component alpha subunit	Acetyl-CoA synthesis
31	*RHA1_RS07670*		Alpha-ketoacid dehydrogenase subunit beta	Acetyl-CoA synthesis
31	*RHA1_RS07675*		2-Oxo acid dehydrogenase subunit E2	Acetyl-CoA synthesis
31	*RHA1_RS10445*	*lpdA*	Dihydrolipoyl dehydrogenase	Acetyl-CoA synthesis
31	*RHA1_RS12550*	*aceE*	Pseudo	Acetyl-CoA synthesis
31	*RHA1_RS14255*	*lpdA*	Dihydrolipoyl dehydrogenase	Acetyl-CoA synthesis
31	*RHA1_RS16000*		MFS transporter	Acetyl-CoA synthesis
31	*RHA1_RS16020*		Pyruvate dehydrogenase	Acetyl-CoA synthesis
31	*RHA1_RS16085*	*lpdA*	Dihydrolipoyl dehydrogenase	Acetyl-CoA synthesis
31	*RHA1_RS16090*		Alpha-ketoacid dehydrogenase subunit beta	Acetyl-CoA synthesis
31	*RHA1_RS16095*	*pdhB1*	Pyruvate dehydrogenase E1 component beta subunit	Acetyl-CoA synthesis
31	*RHA1_RS16100*	*pdhA*	Pyruvate dehydrogenase (acetyl-transferring) E1 component subunit alpha	Acetyl-CoA synthesis
31	*RHA1_RS16380*	*lpdA*	Dihydrolipoyl dehydrogenase	Acetyl-CoA synthesis
31	*RHA1_RS16385*		2-Oxo acid dehydrogenase subunit E2	Acetyl-CoA synthesis
31	*RHA1_RS16390*		Alpha-ketoacid dehydrogenase subunit beta	Acetyl-CoA synthesis

(continued)

Table 1 (continued)

Reaction number[a]	Gene ID	Gene name	Predicted function based on gene annotation	Metabolic pathway
31	*RHA1_RS16395*	*pdhA*	Pyruvate dehydrogenase (acetyl-transferring) E1 component subunit alpha	Acetyl-CoA synthesis
31	*RHA1_RS27135*		NAD(P)/FAD-dependent oxidoreductase	Acetyl-CoA synthesis
31	*RHA1_RS30615*		NAD(P)H-quinone dehydrogenase	Acetyl-CoA synthesis
31	*RHA1_RS37395*		Hypothetical protein	Acetyl-CoA synthesis
31	*RHA1_RS42945*	*lpdA*	Dihydrolipoyl dehydrogenase	Acetyl-CoA synthesis
31	*RHA1_RS42970*		2-oxo acid dehydrogenase subunit E2	Acetyl-CoA synthesis
31	*RHA1_RS42975*		Alpha-ketoacid dehydrogenase subunit beta	Acetyl-CoA synthesis
31	*RHA1_RS42980*		Thiamine pyrophosphate-dependent dehydrogenase E1 component subunit alpha	Acetyl-CoA synthesis
32	*RHA1_RS10720*		Acetate kinase	AK-PTA pathway
33	*RHA1_RS10725*		Phosphate acetyltransferase	AK-PTA pathway
34	*RHA1_RS01765*	*citA1*	Citrate (Si)-synthase	TCA cycle (oxidative)
34	*RHA1_RS07835*		Possible citrate synthase, C-terminal	TCA cycle (oxidative)
34	*RHA1_RS24420*	*citA2*	Citrate (Si)-synthase	TCA cycle (oxidative)
34	*RHA1_RS24440*		Citrate (Si)-synthase	TCA cycle (oxidative)
34	*RHA1_RS34010*		Citrate synthase	TCA cycle (oxidative)
35	*RHA1_RS11725*	*acnA1*	Aconitate hydratase	TCA cycle (oxidative/reductive)
35	*RHA1_RS35180*	*acnA2*	Aconitate hydratase	TCA cycle (oxidative/reductive)
35	*RHA1_RS36835*	*acnA3*	Aconitate hydratase	TCA cycle (oxidative/reductive)
36	*RHA1_RS02995*		Isocitrate dehydrogenase (NADP+)	TCA cycle (oxidative/reductive)
36	*RHA1_RS30490*		Isocitrate dehydrogenase (NADP+)	TCA cycle (oxidative/reductive)
37	*RHA1_RS29380*	*odhA*	2-Oxoglutarate dehydrogenase, E1 and E2 components	TCA cycle (oxidative)
38	*RHA1_RS27190*	*sucC*	Succinate CoA ligase β-subunit	TCA cycle (oxidative)

(continued)

Table 1 (continued)

Reaction number[a]	Gene ID	Gene name	Predicted function based on gene annotation	Metabolic pathway
38	*RHA1_RS27195*	*sucD*	Succinate CoA ligase α-subunit	TCA cycle (oxidative)
38	*RHA1_RS17525*		CoA binding protein (previously annotated as probable succinate CoA ligase)	TCA cycle (oxidative)
39	*RHA1_RS05045*	*sdhB1*	Succinate dehydrogenase iron-sulfur protein	TCA cycle (oxidative)
39	*RHA1_RS05050*	*sdhA1*	Succinate dehydrogenase flavo-protein subunit	TCA cycle (oxidative)
39	*RHA1_RS05055*		Possible succinate dehydrogenase	TCA cycle (oxidative)
39	*RHA1_RS23305*		Hypothetical protein	TCA cycle (oxidative)
39	*RHA1_RS27810*		FAD-binding protein	TCA cycle (oxidative)
39	*RHA1_RS30525*	*sdhB2*	FAD-binding protein	TCA cycle (oxidative)
39	*RHA1_RS30530*	*sdhA2*	Succinate dehydrogenase	TCA cycle (oxidative)
39	*RHA1_RS30535*		Probable succinate dehydrogenase hydrophobic membrane anchor protein	TCA cycle (oxidative)
39	*RHA1_RS30540*		Probable succinate dehydrogenase (cytochrome)	TCA cycle (oxidative)
39	*RHA1_RS36845*		Ferredoxin	TCA cycle (oxidative)
39	*RHA1_RS39385*		Probable succinate dehydrogenase hydrophobic membrane anchor protein	TCA cycle (oxidative)
39	*RHA1_RS47620*	*sdhC*	Succinate dehydrogenase cyto-chrome β-subunit	TCA cycle (oxidative)
39	*RHA1_RS39395*		Ferredoxin	TCA cycle (oxidative)
39	*RHA1_RS39400*	*sdhB3*	Succinate dehydrogenase Fe-S protein subunit	TCA cycle (oxidative)
40	*RHA1_RS28635*	*fumC*	Fumarate hydratase class II	TCA cycle (oxi-dative/reductive)
40	*RHA1_RS28815*		Fumarate hydratase class I	TCA cycle (oxi-dative/reductive)
40	*RHA1_RS39380*		Fumarate hydratase, class I	TCA cycle (oxi-dative/reductive)
41	*RHA1_RS30520*	*mdh*	Malate dehydrogenase	TCA cycle (oxi-dative/reductive)
41	*RHA1_RS32320*		Malate dehydrogenase (acceptor)	TCA cycle (oxi-dative/reductive)

(continued)

Table 1 (continued)

Reaction number[a]	Gene ID	Gene name	Predicted function based on gene annotation	Metabolic pathway
42	*RHA1_RS00955*		Citrate lyase	TCA cycle (reductive)
42	*RHA1_RS01540*		CoA ester lyase	TCA cycle (reductive)
42	*RHA1_RS03190*		Aldolase	TCA cycle (reductive)
42	*RHA1_RS03695*		CoA ester lyase	TCA cycle (reductive)
42	*RHA1_RS03710*		CoA ester lyase	TCA cycle (reductive)
42	*RHA1_RS11960*		Possible citrate lyase beta subunit	TCA cycle (reductive)
42	*RHA1_RS13995*		CoA ester lyase (previously annotated as citrate (pro-3S)-lyase)	TCA cycle (reductive)
42	*RHA1_RS14330*		Citrate lyase beta subunit	TCA cycle (reductive)
42	*RHA1_RS29250*		CoA ester lyase	TCA cycle (reductive)
42	*RHA1_RS29815*		CoA ester lyase	TCA cycle (reductive)
42	*RHA1_RS32175*		CoA ester lyase	TCA cycle (reductive)
42	*RHA1_RS38600*		Possible citrate lyase beta subunit (C-terminal)	TCA cycle (reductive)
42	*RHA1_RS39235*		Aldolase	TCA cycle (reductive)
43	*RHA1_RS11675*		2-Oxo acid ferredoxin oxidoreductase subunit beta	TCA cycle (reductive)
44	*RHA1_RS11825*		FAD-binding dehydrogenase	TCA cycle (reductive)
44	*RHA1_RS40180*		FAD-binding dehydrogenase	TCA cycle (reductive)
44	*RHA1_RS40490*		FAD-binding dehydrogenase	TCA cycle (reductive)
45	*RHA1_RS10355*		Isocitrate lyase	Glyoxylate pathway
46	*RHA1_RS04300*	*glcB*	Malate synthase G	Glyoxylate pathway
46	*RHA1_RS12540*		Malate synthase G	Glyoxylate pathway
47	*RHA1_RS35295*	*mutA*	Methylmalonyl-CoA mutase small subunit	Methylmalonyl-CoA pathway
47	*RHA1_RS35300*	*mutB*	Methylmalonyl-CoA mutase	Methylmalonyl-CoA pathway

[a]Numbers refer to reactions shown in Fig. 1.

Nguyen et al. (2017) have characterized an alternative enzyme that catalyzes this step in *R. jostii* RHA1 called F420-dependent glucose-6-phosphate dehydrogenase (FGD). This enzyme uses low redox potential cofactor that could play an important role in the central metabolism and xenobiotics degradation.

The NADPH is an essential reducing equivalent in most biosynthetic pathways such as the fatty acid biosynthesis (Spaans et al. 2015). Recent proteomic and transcriptomic analyses (Dávila Costa et al. 2015; Juarez et al. 2017) of *R. jostii* RHA1 under lipid accumulation conditions (low nitrogen levels) showed a significant induction of genes/proteins that participate in ED and PPP pathways. Likewise, studies conducted in *R. opacus* PD630 using ^{13}C also show the activation of these pathways during lipid accumulation (Hollinshead et al. 2015). In addition, MacEachran and Sinskey (2013) identified and characterized an enzyme that contributes to the formation of NADPH, a non-phosphorylative glyceraldehyde dehydrogenase enzyme, which catalyzes the direct conversion of the glyceraldehyde 3-P to 3-phosphoglycerate (Fig. 1). This enzyme was also induced specifically during lipid accumulation. On the other hand, the occurrence of $NADP^+$-dependent malic enzymes (reaction 27 in Fig. 1 and Table 1) and their effect on lipid accumulation have been described recently in the RHA1 strain (Hernández and Alvarez 2018). Then, the NADPH necessary for lipid biosynthesis seems to come from several pathways in these bacteria.

Glycolytic pathway also contributes with key precursors for lipid biosynthesis such as triacylglycerols (TAG) in oleaginous *Rhodococcus* species. For example, the glycolytic intermediate dihydroxyacetone phosphate is converted to glycerol 3-phosphate by the NADP-dependent glycerol 3-phosphate dehydrogenase. This enzyme is upregulated in *R. jostii* RHA1 under TAG-accumulating condition (Dávila Costa et al. 2015).

All genes involved in the carbohydrate metabolism are widely distributed throughout the RHA1 genome. However, some genes such as the *tpiA*, *gap2*, and *pgk* involved in the glycolytic pathways are clustered in the genome. Similarly, the genes *RHA1_RS11570* coding for a phosphogluconate dehydratase (Edd) and *RHA1_RS11565* coding for a KHG/KDPG aldolase (Edda), both involved specifically in the ED pathway, are clustered in RHA1 genome with the gene coding for glucose-6-phosphate dehydrogenase (Zwf). In this same cluster, it is located in the gene *glcp* (*RHA1_RS11555*), involved in glucose uptake components in *R. jostii* RHA1 (Araki et al. 2011).

3 Glycogen Synthesis and the Link with the Central Metabolism

The ability to synthesize and accumulate glycogen has been studied based on bioinformatics and/or experimental approaches in different members of the *Rhodococcus* genus such as *R. jostii* (Hernández et al. 2008; Tajparast and Frigon

2015), *R. opacus* (Hernández and Alvarez 2010; Hernández et al. 2013), *R. erythropolis*, *R. ruber*, *R. equi*, and *R. fascians* (Hernández and Alvarez 2010; Yano et al. 2016). In general, the total content of glycogen in those microorganisms reached up to 2–6% of cellular dry weight. The studied strains accumulated glycogen during exponential growth phase, and the content tends to decrease during stationary growth phase. The occurrence of glycogen accumulation during exponential growth phase has been reported a long time ago in other actinomycetes such as *Mycobacterium smegmatis* (Belanger and Hatfull 1999) and *Corynebacterium glutamicum* (Seibold et al. 2007).

Similarly to other microorganisms, genes of glycogen synthesis and degradation in *Rhodococcus* species are usually arranged in clusters and seem to be non-redundant (*glgC*, *glgA*, *glgB*, *glgX*) (Hernández et al. 2008; Hernández and Alvarez 2010). In addition, as occur in other actinobacteria such as *Mycobacterium* and *Streptomyces* (Kalscheuer et al. 2010; Elbein et al. 2010), *Rhodococcus* species also contain the alternative GlgE α-glucan pathway mediated by the *glgE* gene (Hernández et al. 2008). Glycogen may have a role as metabolic intermediate since it is accumulated mainly during the exponential growth phase by cells and is mobilized later in the stationary phase; thus, glycogen has been proposed as a carbon capacitor for glycolysis during exponential growth (Belanger and Hatfull 1999). Glycogen may be a part of a mechanism for controlling sugar excess in *Rhodococcus* or may act as part of a sensing/signaling mechanism. Persson et al. (2007) proposed that the expression of some genes in *E. coli*, such as the universal stress protein *uspA* involved in the carbon starvation conditions or stationary phase, is regulated by glycolytic intermediates such as fructose-6-phosphate. Alteration in the pool size of phosphorylated sugars of the upper glycolytic pathway may ensure expression of stress proteins preceding the complete depletion of the external carbon source and growth arrest (Persson et al. 2007). Thus, glycogen formation may act to attenuate phosphorylated sugar signals and to protect cells from sudden increases in fluxes of sugars.

Transcriptome and proteome analyses in RHA1 and PD630 strains have shown that gene expression and/or protein abundance associated with glycogen synthesis remains constant between culture conditions with high or low nitrogen levels, which suggest that glycogen synthesis is not essentially regulated at the transcriptional level in those conditions (Dávila Costa et al. 2015; Chen et al. 2014). On the contrary, proteins associated with glycogen synthesis seem to be regulated at a posttranslational level. In this context, Cereijo et al. (2016) reported an allosteric regulation of ADP-glucose pyrophosphorylase of RHA1 strain, a key regulatory step in bacterial glycogen metabolism, being the glucose-6P, mannose-6P, fructose-6P, ribose-5P, and phosphoenolpyruvate, the major activators, whereas the NADPH and 6P-gluconate behaved as main inhibitors of this enzyme. The authors also suggested a potential role of glycogen as a temporal reserve that provides a pool of carbon able to be re-routed to produce long-term storage of lipids under certain conditions in oleaginous species of *Rhodococcus*. In contrast, Dávila Costa et al. (2015) reported a higher abundance of enzymes involved in glycogen degradation, such as glycogen phosphorylase (GlgP, EC 2.4.1.1), glycogen debranching enzyme (GlgX,

EC3.2.1.33), and phosphoglucomutase (PGM,EC5.4.2.2) during cell cultivation under conditions leading to lipid accumulation (low-nitrogen conditions), which suggest that glycogen degradation can be regulated at a transcriptional level. In conclusion, glycogen metabolism seems to respond to a complex regulation at both transcriptional and posttranslational levels and probably in orchestration with the synthesis of other compounds such as exopolysaccharides and lipids. In this context, recent studies have described the potential role of a capsular α-glucan-like compound exported from internal glycogen in the pathogenic strain of *M. tuberculosis* in virulence by evading the host immune response (Koliwer-Brandl et al. 2016). Although a capsular α-glucan-like compound have not been specifically described in *Rhodococcus*, it is known that several species of this genus are able to produce exopolysaccharides of varied nature (Perry et al. 2007; Hernández et al. 2017).

4 Gluconeogenesis and the Phosphoenolpyruvate-Pyruvate-Oxaloacetate Node

The metabolic link between glycolysis/gluconeogenesis and the TCA cycle is represented by the PEP-pyruvate-oxaloacetate node, also called the anaplerotic node (Sauer and Eikmanns 2005) (Fig. 1). This node comprises a set of reactions that direct the carbon flux into appropriate directions in a flexible manner. The node represents a relevant switch point for carbon flux distribution within the central metabolism (Sauer and Eikmanns 2005).

During growth on acetate, fatty acids or ethanol, which enters to the central metabolism via the acetyl-CoA or as TCA cycle intermediates, the intermediates malate and oxaloacetate must be converted to pyruvate and phosphoenolpyruvate (PEP) for the synthesis of sugars. Once PEP is formed, the synthesis of sugar phosphates is accomplished by reversible reactions of glycolysis (gluconeogenesis). Under gluconeogenic conditions, the TCA cycle intermediates oxaloacetate and malate must be decarboxylated (C4-decarboxylation) to form pyruvate and PEP, which serve as precursors for sugar phosphate synthesis. The formation of PEP can be achieved either by the PEP carboxykinase enzyme (Pck) directly or oxaloacetate decarboxylase and/or malic enzyme in combination with PEP synthetase. As in *C. glutamicum* (Sauer and Eikmanns 2005), PEP carboxykinase (RHA1_RS25350) is probably the only enzyme responsible for PEP synthesis from TCA cycle intermediates in *R. jostii* RHA1, since the gene coding a PEP synthetase is lacking in its genome. Nevertheless, some PEP syntheses are present in other *Rhodococcus* species such us *R. equi* (REQ_RS07445), *R. pyridinivorans* (Y013_RS04990), and *R. aetherivorans* (AAT18_RS13085). In *M. tuberculosis*, the role of Pck enzyme in gluconeogenesis has been demonstrated in presence of lipid substrates, being essential for intracellular growth and survival in macrophages during infection (Basu et al. 2018). On the other hand, pyruvate can be provided

from malate by the action of some malic enzymes (ME). The ME enzymes have been associated also with the production of reducing equivalents for anabolic processes in several organisms (Spaans et al. 2015). In RHA1 strain as well as other *Rhodococcus* species, there are several genes coding for these kinds of enzymes, and some of them seem to be associated also with the generation of NADPH for lipid biosynthesis in oleaginous species (Hernández and Alvarez, unpublished results) (Fig. 1).

Under glycolytic conditions, the final products of glycolysis, PEP and pyruvate through the pyruvate kinase and pyruvate dehydrogenase complex, feed acetyl-CoA into the TCA cycle (Fig. 1 and Table 1). Anaplerotic reactions (C3-carboxylation) must replenish TCA cycle intermediates that were bled off for anabolic processes. This function is accomplished in most bacteria by PEP carboxylase and/or pyruvate carboxylase, which convert PEP and pyruvate, respectively, to oxaloacetate (Sauer and Eikmanns 2005). In *M. tuberculosis* H37Rv, it has been demonstrated that these anaplerotic functions involve not only pyruvate carboxylase (PCA) but also Pck and ME enzymes. The anaplerotic node was analyzed through different variant mutants of these genes, showing they are also essential for intracellular growth of *M. tuberculosis* strain. Particularly it was demonstrated that PCA is required for growth on pyruvate and that the phenotype could be complemented by addition of aspartate (as proxy for oxaloacetate) or by growing cultures in presence of 5% CO_2. These results showed the anaplerotic role of PCA replenishing oxaloacetate into TCA cycle and that this function could be complemented by CO_2 addition probably by action of either ME or Pck (Basu et al. 2018).

R. jostii RHA1 seems to possess PEP carboxylase and pyruvate carboxylase in its genome as anaplerotic enzymes (Fig. 1 and Table 1). However, as occurs in *M. tuberculosis H37Rv*, Pck enzyme of *R. jostii* RHA1 would be operating in the carboxylating direction (anaplerotic) or in gluconeogenic direction (Basu et al. 2018). It is known that pyruvate carboxylase plays a major anaplerotic role in mammals, yeast, and in a few prokaryotes such as *Rhodopseudomonas spheroids*, *Arthrobacter globiformis*, and *Mycobacterium smegmatis*. Many other bacteria such as *Pseudomonas citronellolis*, *Azotobacter vinelandii*, or *C. glutamicum* are able to use both PEP carboxylase and pyruvate carboxylase as anaplerotic enzymes (Sauer and Eikmanns 2005).

As occurs in *C. glutamicum* (Sauer and Eikmanns 2005), putative PEP carboxylase gene (*ppc*) of RHA1 strain is clustered with the genes glyceraldehyde-3-phosphate dehydrogenase (*gap*2), phosphoglycerate kinase (*pgk*), and triose-phosphate isomerase (*tpi*A) of the glycolytic pathway.

Feisthauer et al. (2008) reported the dependence of externally provided CO_2 for growth in *R. opacus* 1CP in comparison with *Pseudomonas knackmussii* B13, which was able to grow in the absence of external CO_2 under similar culture conditions. Using $^{13}CO_2$, the authors demonstrated that during growth on glucose, *R. opacus* 1CP showed lower C yield than *P. knackmussii* B13. In addition, fatty acids (principally the odd-numbered fraction) and amino acids (principally the aspartate family) contained in *R. opacus* 1CP were much higher enriched in ^{13}C than those in strain B13 (Feisthauer et al. 2008). The authors concluded that *R. opacus* 1CP possesses an essential dependence on heterotrophic CO_2 fixation by anaplerotic

reactions. The odd-numbered fatty acids are usually produced by *Rhodococcus* species using propionyl-CoA as precursor for biosynthesis, as has been reported previously (Alvarez et al. 1997). Propionyl-CoA is produced through the methyl malonyl-CoA pathway using TCA cycle intermediates as precursors (Fig. 1). These intermediates can be formed by direct carboxylation of PEP or pyruvate through anaplerotic reactions, as has been reported for *R. ruber* (Anderson et al. 1995), *R. opacus* PD630 (Alvarez et al. 1997), and *R. opacus* 1CP (Feisthauer et al. 2008). The use of TCA cycle intermediates for the biosynthesis of odd-numbered fatty acids, which may account up to 20–30% of the total fatty acids in many *Rhodococcus* strains, is probably in detriment of the energy gain by cells and may explain the lower C yields of *Rhodococcus* in comparison with other Gram-negative bacteria (Feisthauer et al. 2008). Altogether, these results emphasize the role of the PEP carboxylase and pyruvate carboxylase enzymes in *R. jostii* RHA1 metabolism, a very close species to *R. opacus*. The occurrence of additional anaplerotic enzymes as Pck enzyme (C3 carboxylation) in *R. jostii* and *R. opacus* genomes should be investigated in the future.

Pyruvate kinase and the pyruvate dehydrogenase complex, which are responsible for the production of acetyl-CoA from PEP, are also implicated in the PEP-pyruvate-oxaloacetate node (Fig. 1 and Table 1). The acetyl-CoA fuels the TCA cycle to produce energy and anabolic precursors. The pyruvate:quinine oxidoreductase enzyme, which is present in *C. glutamicum*, seems not to occur in RHA1 genome; thus, the bypass of the pyruvate dehydrogenase reaction via pyruvate:quinine oxidoreductase, in combination with AskA and Pta enzymes, may be not functional in *R. jostii*. However, the *askA* and *pta* genes encoding a putative acetate kinase and a phosphotransacetylase, respectively, are present in the RHA1 genome. When acetyl-CoA is in excess and the C flux exceeds the amphibolic capability of the central metabolism, acetyl-CoA can be converted to acetate and ATP by the AK-PTA pathway (Yasuda et al. 2007). The acetyl-CoA synthetase enzymes can reactivate back acetate to acetyl-CoA when the metabolic situation changes (Fig. 1). The occurrence of the AK-PTA pathway in *R. jostii* RHA1 and probably in other members of the genus may contribute with the control of C fluxes and the maintenance of the intracellular acetyl-CoA pools during fluctuating nutritional conditions, as found in the environment. In this context, *askA* and *pta* genes were downregulated by strain RHA1 during cultivation of cells under nitrogen-limiting conditions, which promote a high flux of acetyl-CoA toward the fatty acid biosynthesis pathway for the accumulation of triacylglycerols. The *askA* and *pta* genes are also present in *R. opacus* B4 and *R. erythropolis* PR4 genomes.

In general, the demand of acetyl-CoA in rhodococci is probably high, considering the high content of different lipid species found in their cellular structures, such as the cell envelope and the storage lipids as triacylglycerols. As *Mycobacterium tuberculosis*, the genome of *R. jostii* RHA1 possesses a multiplicity of genes involved in lipid metabolism (Cole et al. 1998; Hernández et al. 2008). The synthesis of acetyl-CoA from PEP via pyruvate kinase and pyruvate dehydrogenase complex may be the main source for acetyl-CoA production in rhodococci. Otherwise, other reactions could contribute with the intracellular acetyl-CoA pool, such as the

reaction catalyzed by citrate lyase enzyme, which converts citric acid into acetyl-CoA and oxaloacetate and the eventual activation of acetate to acetyl-CoA by an acetyl-CoA synthetase. Transcriptomic analysis of *R. jostii* RHA1 under conditions that lead to TAG accumulation revealed a partial repression of pyruvate dehydrogenase enzyme to generate acetyl-CoA; however, it was determined the induction of several enzymes acetyl-CoA synthetases (Juarez et al. 2017).

The significant induction of a gene coding for a putative citrate lyase enzyme by cells of strain RHA1 has been observed during their cultivation under nitrogen starvation conditions.

5 The Tricarboxylic Acid Cycle (TCA)

In aerobic bacteria like *Rhodococcus*, the TCA cycle in the oxidative direction oxidizes acetate to CO_2 and provides reducing power (NADH and $FADH_2$) for energy production and metabolic intermediates for use in biosynthesis pathways. *R. jostii* RHA1 seems to be able to drive the entire TCA cycle oxidatively, since it possesses all necessary genes/enzymes (Table 1 and Fig. 1). The reducing power generated under aerobic conditions via the TCA cycle, in addition to the glycolysis or β-oxidation pathway, could be reoxidized through the respiratory electron transport chain. As mentioned above, the TCA cycle in *Rhodococcus* members serves as a source for the production of propionyl-CoA, via methyl malonyl-CoA pathway, which is used for the biosynthesis of odd-numbered fatty acids (Anderson et al. 1995; Alvarez et al. 1997; Feisthauer et al. 2008) (Fig. 1).

Interestingly, genomic data revealed the presence of the key enzymes of the reductive TCA cycle in the RHA1 genome, including genes encoding citrate lyase and 2-oxoglutarate synthase, in addition to the rest of the TCA cycle enzymes, which may act reversible (Fig. 1 and Table 1). The occurrence of those enzymes in the RHA1 genome suggests that this strain should be able to drive the entire reductive TCA cycle. Citrate lyase converts citric acid into acetyl-CoA and oxaloacetate, whereas the 2-oxoglutarate synthase produces 2-oxoglutarate from succinyl-CoA. This allows the metabolism to incorporate CO_2 and to serve as an engine for synthesis instead of energy production (Srinivasan and Morowitz 2006). CO_2 fixation also occurs in *R. erythropolis* N9T-4 which possess an alternative TCA oxidative cycle. In Yano et al. (2015), the authors propose a bypass of oxoglutarate to succinate through succinic semialdehyde instead of the classic succinyl-CoA in this strain. They also demonstrate a shortcut to prevent CO_2 losses that occur within the TCA cycle. The possible autotrophic metabolism in *Rhodococcus* will be exposed deeper in a specific chapter of this book by Yoshida et al. The switch from oxidative to reductive TCA cycle may both facilitate carbon fixation and restore the balance of oxidative and reductive reactions during environmental fluctuations (Srinivasan and Morowitz 2006). The ability to drive the TCA cycle in both directions, either oxidative or reductive, has been also reported for other actinomycetes, such as *Mycobacterium tuberculosis* and *Streptomyces coelicolor* (Srinivasan

and Morowitz 2006). In addition, the genes coding for key enzymes of the reductive TCA cycle seem also to be present in *R. opacus* B4 and *R. erythropolis* PR4 genomes. A partial or fully functional oxidative or reductive TCA cycle may be used by these microorganisms to balance metabolism and to adapt to diverse environmental conditions, for example, (a) starvation as demonstrated for RHA1 that showed a decrease in seven enzymes involved in the oxidative and reductive TCA cycle due to the shutdown of energy production or carbon-containing compounds (Patrauchan et al. 2012) or (b) pollutant stress as shown in a rhodococcal strain isolated from lithium-mining areas that overexpress TCA enzymes involved in the synthesis of intermediates of the TCA cycle (Belfiore et al. 2017). The authors postulate that these metabolites act as scavengers of ROS. A similar behavior was observed during degradation of tetrahydrofuran by *Rhodococcus* sp.YYL (He et al. 2014).

6 The Glyoxylate Pathway

Strain RHA1 possesses both enzymes involved in the glyoxylate pathway: isocitrate lyase and malate synthase (Table 1). This pathway serves as a mechanism to replenish oxaloacetate during growth on acetate and fatty acids (Fig. 1). The glyoxylate pathway is relevant not only for *R. jostii* RHA1 but also for other triacylglycerol-accumulating *Rhodococcus* strains, such as *R. opacus* PD630, because it links the gluconeogenesis with the oxidation of acetyl residues obtained by the β-oxidation of fatty acids during mobilization of the stored triacylglycerols. Members of the genus *Rhodococcus* are able to accumulate variable amounts of triacylglycerols, which are degraded during carbon starvation (Alvarez et al. 2000; Alvarez 2006). The upregulation of isocitrate lyase by cells of strain RHA1 suggested the activation of the glyoxylate shunt under C-starvation, which correlated with the use of intracellular fatty acids as carbon and energy source (Patrauchan et al. 2012). The synthesis of triacylglycerols seems to be an important metabolic pathway in some species of rhodococci for the maintenance of energy homeostasis. Triacylglycerols are synthesized in these bacteria when a carbon source is available and then degraded to provide carbon and energy during C-starvation via the successive operation of β-oxidation, the glyoxylate cycle, the partial TCA, and gluconeogenesis. Thus, the glyoxylate cycle may be very active in rhodococci due to their dependence on the degradation of stored triacylglycerols under nutritional fluctuating conditions. In this context, the glyoxylate cycle plays a pivotal role in the persistence of *Mycobacterium tuberculosis* in mice by sustaining intracellular infection in inflammatory macrophages (Eisenreich et al. 2010). This pathway enables mycobacteria to utilize carbon sources when TCA cycle is shut down during O_2 and nutrient limitation (Boshoff and Barry 2005; Tang et al. 2009). Furthermore, it was recently described the importance of malate synthase for the *M. tuberculosis* growth and survival on even fatty acids by detoxification of glyoxylate, arising from acetyl-CoA metabolism, by assimilation of a second molecule of acetyl-CoA (Puckett et al.

2017). This function is also important in *R. fascians*, the only phytopathogen described so far, to avoid the toxicity of the glyoxylate produced when bacteria catabolize compounds from plants as nutrient factors during issues of infection (Vereecke et al. 2002). On the other hand, Yano et al. (2015) described the relevance of gluconeogenesis and glyoxylate cycle to oligotrophic growth and CO_2 fixation in strain *R. erythropolis* N9T-4; this pathway avoids the loss of carbon as CO_2 molecules and preserves CoA units in the TCA cycle.

7 Litoautotrophic Processes in *Rhodococcus*

Rhodococcus bacteria are usually considered chemoheterotrophic microorganisms, which use organic compounds as sources of carbon and energy. However, there is bioinformatic and experimental evidence that members of this genus are rather facultative chemolithoautotrophs. Lithotrophy is the use of an inorganic compound as a source of energy. Some aerobic bacteria are able to remove electrons from a substrate and put them through an electron transport system that will produce ATP by electron transport phosphorylation. When lithotrophs take their carbon from CO_2, they are autotrophs. In this context, Grzeszik et al. (1997) reported the capability of *R. opacus* strain MR11, which is very close to strain RHA1, for growing on CO_2 and gaseous H_2 as the sole carbon and energy sources. Aragno and Schlegel (1992) previously identified a soluble hydrogenase system in strain MR11, which was localized in the cytoplasm and catalyzed the transfer of electrons directly to NAD. On the other hand, *R. equi* was able to consume H_2 in the late exponential and stationary phase (Meredith et al. 2014). The examination of genomic data revealed that *R. jostii* RHA1 possesses a gene cluster (*RHA1_RS22440* to *RHA1_RS22555*) encoding a putative hydrogenase system. Hydrogenase genes also occur in the genome of *R. opacus* B4 and *R. erythropolis* PR4. Altogether, these results indicated that *Rhodococcus* members are not only able to use a wide range of organic compounds as carbon and energy sources but also to oxidize H_2 as an additional energy source. Moreover, analysis of the genome database of *R. jostii* RHA1 revealed the occurrence of additional litoautotrophic pathways, including genes coding for putative carbon monoxide dehydrogenases (CODH) and a thiocyanate hydrolase enzyme. Thiocyanate hydrolase, which usually occurs in obligate as well as in facultative chemolithotrophs, is a cobalt(III)-containing enzyme catalyzing the degradation of thiocyanate to carbonyl sulfide and ammonia (Yamasaki et al. 2002). The genes coding for the three subunits of the enzyme (α, β, and γ) are localized into an operon (*RHA1_RS21555-RHA1_RS21565*) in the RHA1 genome. The homologous of these RHA1 genes can be identified in *R. opacus* B4 genome with the same locus organization. Curiously, thiocyanate hydrolase genes are missing in *R. erythropolis* PR4 genome. On the other hand, the genomes of *R. jostii* RHA1 and *R. opacus* B4 contain three clusters including genes coding for putative CO dehydrogenases (CODH). Patrauchan et al. (2012) reported a 250-fold increase in CO dehydrogenase concurrent with a 130-fold increase in CODH activity during

cultivation of RHA1 strain under carbon starvation conditions, suggesting a switch from heterotrophic to autotrophic metabolism. The authors suggested that CODH enables assimilation of CO_2 or CO into cellular building blocks under these environmental conditions. Autotrophic growth has also been demonstrated in *R. erythropolis* N9T-4, a bacterial strain isolated from stored crude oil (Ohhata et al. 2007). This strain was able to grow on a basal medium without any additional carbon, nitrogen, sulfur, and energy sources but required CO_2 for oligotrophic growth. The authors suggested that N9T-4 possesses a novel CO_2 fixation pathway linked to the formaldehyde metabolism (Yoshida et al. 2011). Strain N9T-4 can utilize carbon, nitrogen, and sulfur compounds, directly from the atmosphere. It has been suggested that N9T-4 can utilize traces amounts of atmospheric ammonia as a nitrogen source through the strong expression of an ammonium transporter gene (*amtB*) (Yoshida et al. 2014).

Altogether, these studies confirmed the existence of diverse litoautotrophic pathways in rhodococci that allow them to survive and thrive in oligotrophic environments. CODH, hydrogenase, thiocyanate hydrolase, and other still unknown metabolic systems may avoid the release of carbon as CO_2 by rhodococcal metabolism and may serve as auxiliary mechanisms for energy metabolism during nutritional starvation conditions.

8 Concluding Remarks

Rhodococcus bacteria are endowed with a robust, flexible, and versatile central metabolism, which is necessary for metabolically adapting to environments with fluctuating nutritional conditions. The central metabolism must provide all necessary intermediates for the biosynthesis of a wide diversity of molecules and complex macromolecules for assembling cellular structures like the cellular envelope or secondary metabolites. These bacteria exert a very efficient management of their nutritional resources, based on the flexibility of their metabolism and the diversity of metabolic reactions. The partitioning of carbon through the central metabolism of the substrates to either energy production or synthesis of compounds depends on the metabolic demands of cells. Rhodococci seem to possess the ability to conserve useful energy during catabolism, distributing the carbon flux of the substrate between the energy production and the biosynthesis of essential molecules, reserving a part of the carbon into diverse storage compounds, such as triacylglycerols, polyhydroxyalkanoates, and glycogen. Thus, cells may resign energetic productivity and growth yield, which may be compensated to some extent with additional processes providing energy and carbon intermediates from inorganic compounds, such as H_2, CO_2, or thiocyanate, among others. This feature may be environmentally favorable for growth and survival in environments with low-energy fluxes, such as in soil or marine sediments. The flexibility of the central metabolism is another essential feature of rhodococci. These microorganisms seem to have the potential to use alternatively different glycolytic pathways, such as EMP or ED pathways, the

PPP pathway, and a partial or fully functional TCA in both, oxidative or reductive directions, according to the conditions. Moreover, the central metabolism of rhodococci possesses some mechanisms that probably permit cells to respond rapidly to changes in nutritional state and to balance metabolism. One of them may be the glycogen biosynthesis, which may deal with the sugar excess during exponential growth phase, and probably serve as a pool of sugars for using when necessary. Other point may be the AK-PTA pathway, which may contribute with the control of C fluxes and the maintenance of the intracellular acetyl-CoA pools during fluctuating conditions. The management of acetyl-CoA pool may be a key point for rhodococci metabolism, since they usually synthesize many different lipid species, which perform important function in their interactions with the environment. In this context, the PEP-pyruvate-oxaloacetate node may play a key role in the C flux distribution within the overall cell metabolism.

Despite the numerous *Rhodococcus* genomes now available, the biochemistry and the molecular biology of the central metabolism of the *Rhodococcus* genus are still poorly known. More studies involving the regulation of metabolic genes and enzymes, the kinetic characterization of enzymes, or the analysis of carbon fluxes through the metabolism should help to define a clearer picture of the functionality and regulation of the central metabolism within the cellular context in rhodococci.

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