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The *Pseudomonas aeruginosa* RhlA enzyme is involved in rhamnolipid and polyhydroxyalkanoate production

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Abstract *Pseudomonas aeruginosa* produces the biosurfactant rhamnolipid, which has several potential biotechnological applications. The synthesis of this surfactant is catalyzed by rhamnosyltransferase 1, composed of the proteins RhlA and RhlB. Here we report that RhlA plays a role not only in surfactant synthesis, but also in the production of polyhydroxyalkanoates, polymers that can be used for the synthesis of biodegradable plastics.

Keywords *Pseudomonas aeruginosa* · Rhamnolipid · Polyhydroxyalkanoates

Introduction

Pseudomonas aeruginosa is an environmental bacterium that can be isolated from many different habitants, but is also an opportunistic human pathogen causing serious nosocomial infections [4]. The secretion of numerous toxic compounds and hydrolytic enzymes is correlated with pathogenicity [16, 19]. These exoproducts include proteases, phospholipase C, exotoxin A, pyocyanin and rhamnolipids [16, 19].

The precise role that rhamnolipids play in *P. aeruginosa* life cycle and pathogenicity is not known. Due to their tenso-active properties, these compounds have several potential industrial and environmental applications [7, 9]. These uses include the production of fine chemicals, characterization of surfaces and surface coatings, use as additives for environmental remediation and use as biological control agents.

P. aeruginosa cell-free extracts were found to produce rhamnolipids by two sequential reactions [1]. The first reaction is catalyzed by rhamnosyltransferase 1 (Rt1) and uses dTDP-L-rhamnose and a medium-chain β-hydroxy fatty acid as precursors, yielding mono-rhamnolipid (rhamnosyl-β-hydroxydecanoyl-β-hydroxydecanoic acid), which in turn is the substrate, together with dTDP-L-rhamnose, of rhamnosyltransferase 2 (Rt2) that produces di-rhamnolipid (rhamnosyl-rhamnosyl-β-hydroxydecanoyl-β-hydroxydecanoate). Rt1 is composed of two polypeptides, RhlA and RhlB [10]. The latter protein is the catalytic subunit [10] and seems to be an inner membrane protein [13]. We found recently that the RhlA role in rhamnolipid production seems to be the synthesis of its β-hydroxy fatty acid dimer moiety (Cabrera and Soberón-Chávez, unpublished). It was also reported that RhlA β-hydroxy-fatty acid-dimerizing activity is responsible for the synthesis of the surfactant 3-(3hydroxyalkanoyloxy)-alkanoic acids (HAAs [5]). Rt2 is composed of a single protein called RhlC [13].

We recently determined that *Escherichia coli* expressing the *rhlAB* operon, together with the genes involved in dTDP-L-rhamnose synthesis (*rmlBDAC* operon) produces mono-rhamnolipid, when grown either with glucose or with oleic acid as carbon source (Cabrera and Soberón-Chávez, unpublished). These results suggest that Rt1 can use either ACP- or CoA-fatty acids as substrate (Cabrera and Soberón-Chávez, unpublished).

Poly-hydroxyalkanoates (PHA) are bacterial storage compounds which are synthesized by the polymerization of β-hydroxy-acids by poly-hydroxyalkanoate synthases (PhaC), using CoA-linked fatty acids as substrate [17, 18]. *P. aeruginosa* mainly produces PHA consisting of medium-chain-length polymers, mainly poly-β-hydroxydecanoate [17]. PHAs are of biotechnological importance since they can be used to produce "biodegradable plastics" [8, 12].

It was previously discussed by Campos-García et al. [2] and Rehm et al. [15] that rhamnolipids and PHA synthesis

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in *P. aeruginosa* have a close metabolic relation since fatty acid derivatives are used as substrates in both pathways. However, it was shown that PhaC can only used CoA-fatty acids as substrates and thus the activity of a transacylase, encoded by *phaG*, is required for PHA production when bacteria are grown with a sugar as carbon source [14]. As mentioned, we recently determined that Rt1 expressed in *E. coli* catalyzes the synthesis of rhamnolipid when bacteria are grown on a medium with glucose or oleic acid as carbon source (Cabrera and Soberón-Chávez, unpublished). These results suggest that Rt1 can use both ACP-fatty and CoA-fatty acids as substrates for mono-rhamnolipid production (Cabrera and Soberón-Chávez, unpublished).

Materials and methods

The total rhamnolipid concentration was determined from cell culture supernatants by measuring rhamnose concentration after acid hydrolysis using the orcinol method [3]. PHA was measured as described previously [2]. The *P. aeruginosa* cultures used to measure rhamnolipid and PHA production were grown on PPGAS medium [20] at 29°C for 24 h.

Results and discussion

Considering the reported relation between rhamnolipids and PHA synthesis in *P. aeruginosa*, we made the hypothesis that Rt1 enzymatic activity competes with enzymes involved in PHA production for the consumption of fatty acid precursors. It was reported that the enhanced expression of the *rhlAB* operon in the wild-type strain PG201 resulted in an increased rhamnolipid production [11]. We introduced plasmid pUO58 [10] coding for this operon into the PAO1 strain [6] and, as reported [11], found that it produced increased amounts of rhamnolipids (Table 1). According to our hypothesis, PAO1/pUO58 produced a reduced amount of PHA (Table 1), presumably due to the drainage of PHA fatty acid precursors for rhamnolipid synthesis.

To further confirm this hypothesis, we determined the amount of PHA produced by a *P. aeruginosarhlA* mutant [13] (Table 1), compared to the wild-type strain PAO1. In accordance with the proposed relation of rhamnolipid and PHA synthesis this mutant, which lacks rhamnolipid production, had an increased PHA production (Table 1), suggesting that, in the absence of rhamnolipid synthesis, fatty acid precursors are more available for PHA synthesis.

Unexpectedly, however, when this *P. aeruginosa* PAO1 *rhlA* mutant was complemented *in trans* by plasmid pUO58, PHA production was further increased, while rhamnolipid production was only partially complemented (Table 1). These results suggest that RhlA (due to its ability to catalize the formation of fatty acid dimers (Cabrera and Soberón-Chávez, unpublished; [6]), besides being involved in mono-rhamnolipid synthesis, is also involved in the production of a PHA intermediary, and that when this protein is hyper-produced, in a *rhlA* mutant background, fatty acid intermediaries are preferentially used to produce PHA. As shown previously [14] and in this paper (Table 1), RhlA activity is not indispensable for PHA synthesis.

The results obtained clearly show the inverse relation between rhamnolipids and PHA synthesis, presumably due to competition for fatty acid precursors, and the involvement of RhlA in the synthesis of a common precursor of these compounds, presumably the fatty acid dimer (Fig. 1). However, it is difficult to explain the different effect of pUO58 expression in the wild-type PAO1 strain and in its *rhlA* mutant derivative. A pos-

Table 1 Production (μg/ml) of rhamnolipids and PHA by different *P. aeruginosa* strains. *ND* Not detected. Standard deviations were always less than 15% of the reported values

Strain	Rhamnolipid (%)	PHA (%)
PAO1	454.19 (100)	135. 51 (100)
PAO1/pUO58	655.54 (144.5)	20.55 (16.6)
PAO1 *rhlA*	ND	271.51 (200)
PAO1 *rhlA*/pUO58	40.61 (8.9)	361.85 (267)

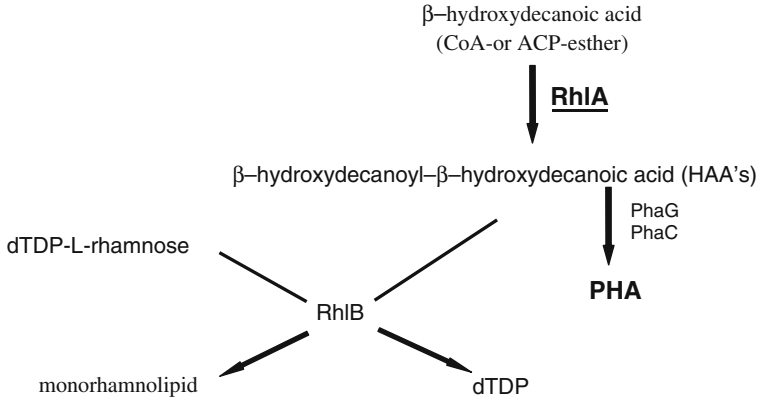

Fig. 1 Schematic representation of the proposed role of RhlA in rhamnolipid and PHA production and its reported activity in the synthesis of surfactants, HAAs [5]

sible explanation is that the relation between RhlA and RhlB is different in strains PAO1/pUO58 and PAO1 *rhlA*/pUO58 and that, in the *rhlA* mutant background, the imbalance of this ratio favors the metabolic flux to go in the direction of PHA synthesis, instead to the synthesis of rhamnolipids. This hypothesis remains to be experimentally validated.

The different ratio of RhlA and RhlB in the PAO1 wild-type strain and in its *rhlA* mutant derivative suggest that there is a point of differential regulation of the expression of these proteins, either at the transcriptional or post-transcriptional level. It was reported that *rhlA* and *rhlB* constitute an operon, but that the latter gene contains a weak promoter [10]. RhlB is an inner membrane protein [13], but the regulation of its folding and membrane insertion has not been studied.

As stated above and based on mono-rhamnolipid production in *E. coli* (Cabrera and Soberón-Chávez, unpublished), we proposed that RhlA has the catalytic activity to form ACP-β-hydroxy- or CoA-β-hydroxy-fatty acid dimers that can be used as substrates by RhlB to produce mono-rhanolipid. The results presented here (Table 1) further suggest that the β-hydroxy-fatty acid dimers produced by this Rt1 component can be used as substrates by enzymes involved in PHA synthesis (PhaG or PhaC) to produce PHA (Fig. 1).

Here we found that expression of the *rhlAB* operon on a plasmid could lead either to rhamnolipid or to PHA hyperproduction, depending on the strain used (Table 1). Taken together, these results suggest that the flow of the reactions leading to the three *P. aeruginosa* products depending on RhlA activity (rhamnolipids, PHA, HAAs; Fig. 1) is determined by the activity of the enzymes using ACP-β-hydroxy- or CoA-β-hydroxy-fatty acid dimers as substrates and on the relative concentration of these metabolites.

The results presented herein permit a better understanding of *P. aeruginosa* rhamnolipids and PHA biosynthetic pathways and the complex relationship between them. Both of these products are of biotechnological importance.

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