High rhamnolipids production correlates with a high intracellular *R*- to *S*-specific enoyl-CoA hydratase activity

Ahmad M. Abdel-Mawgoud* and Eric Déziel

INRS - Institut Armand-Frappier, Laval, Québec, Canada, H7V 1B7

ahmad.saleh@iaf.inrs.ca

Abstract

Rhamnolipids (RLs) are multipurpose surface-active molecules produced from rhamnose and *R*-3-hydroxyalkanoate ($C_{10\pm2}$) precursors by the pathogenic bacterium *Pseudomonas aeruginosa.*

Interestingly, 3-hydroxyalkanoate precursors of RLs are exclusively in the *R*-form and we recently reported that they originate from enoyl-CoA ($C_{10\pm2}$) intermediates of β-oxidation diverted by the action of *R*-specific enoyl-CoA hydratase (*R*-ECH), called RhlY/Z.

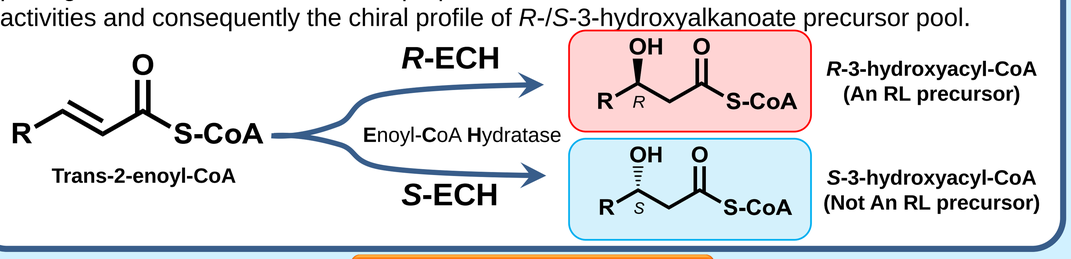
S- and *R*-ECH catalyze the stereospecific hydration of 2-enoyl-CoA into the corresponding *S*- and *R*-3-hydroxyacyl-CoA, respectively.

In this study, we investigated the correlation between the net *R*-/*S*-ECH activity of *P. aeruginosa* PA14 in comparison with different bacteria under various culture conditions using our recently developed chiral HPLC method coupled with tandem mass spectrometry (MS) for the stereospecific and quantitative analysis of *S*-/*R*-3-hydroxyalkanoates.

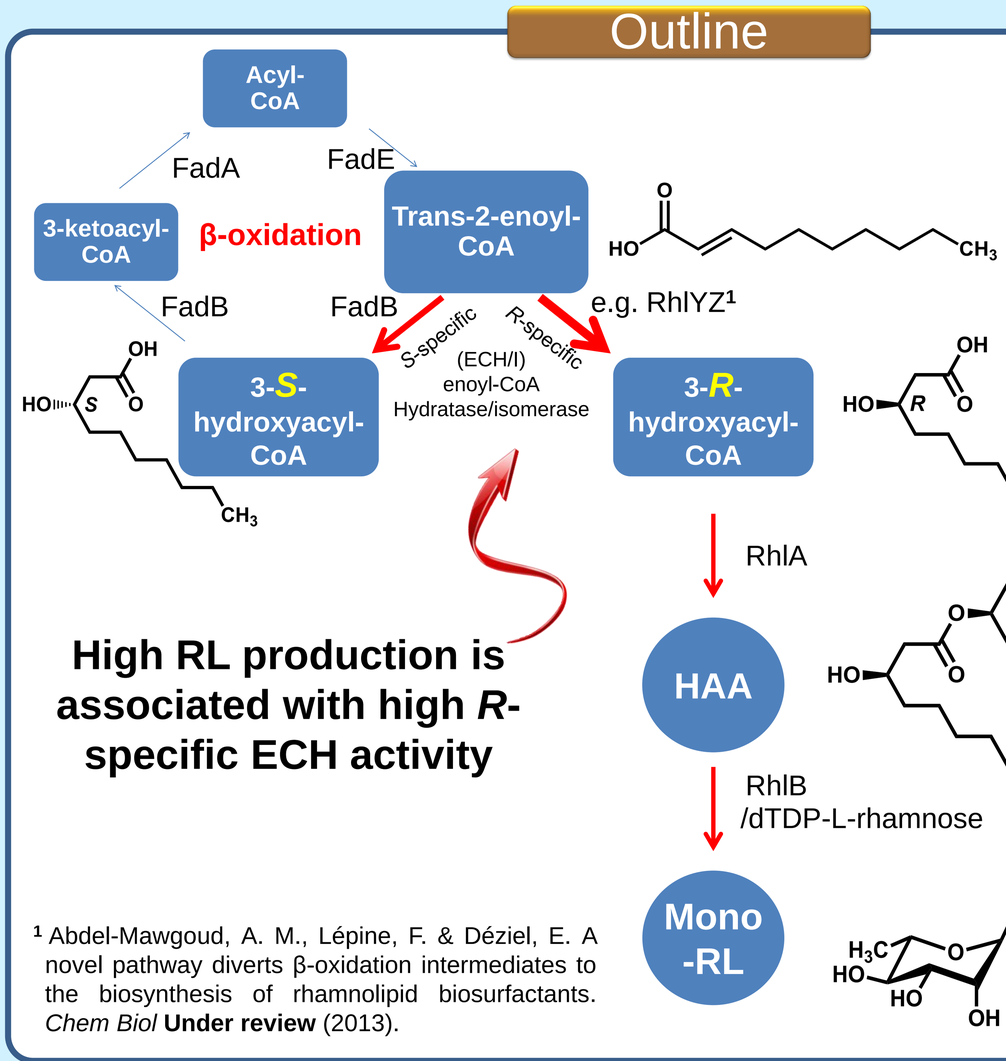
We show that, in mineral salts medium designed for high RLs production, the net ECH activity of *P. aeruginosa* is almost exclusively of the *R*-specific type (*R*-ECH). On the other hand, in rich medium conditions characterized by low RLs production, a nearly equal *S*- to *R*-ECH activities was observed.

We also evaluated the net ECH activity in two other bacteria that are candidates for the heterologous expression of rhamnolipids genes, namely, *Escherichia coli* and *Pseudomonas putida,* and found that the former is of mainly *S*-ECH type whether in rich or minimal media, while the latter, like *P. aeruginosa,* is of equal *R*-/*S*-ECH in rich medium and of *R*-ECH in mineral medium.

These observations could explain the reported failures to express RLs production in *E. coli* and its success in *P. putida*. This study reveals an important analytical tool for evaluation of the candidacy of different hosts for the heterologous expression of RLs, preferred in non-pathogenic hosts for commercial purposes, based on the nature of the net *R*-/*S*-ECH activities and consequently the chiral profile of *R*-/*S*-3-hydroxyalkanoate precursor pool.



Outline



In the minimal medium condition, designed for high rhamnolipids production, *Pseudomonas aeruginsoa* showed high ***R***-specific ECH activity

High RL production is associated with high *R*-specific ECH activity

[1] Abdel-Mawgoud, A. M., Lépine, F. & Déziel, E. A novel pathway diverts β-oxidation intermediates to the biosynthesis of rhamnolipid biosurfactants. *Chem Biol* **Under review** (2013).

Methodology

HPLC/MS-MS method for estimation of *R*-/*S*-ECH activities [2]

***In vitro* ECH reaction using clarified total cell lysate and derivatization of ECH reaction products**

Trans-2-decenoyl-CoA (Reaction substrate)

Total cell lysate (with *R*-/*S*-ECH activity)

***R*-/*S*-3-hydroxydecanoyl-CoA**

1) Alk. hydrolysis of the CoA group
2) Derivation with 3,5-dimethylphenylisocyanate

3,5-Dimethylphenylurethane-derivative of *R*-/*S*-3-hydroxydecanoic acid

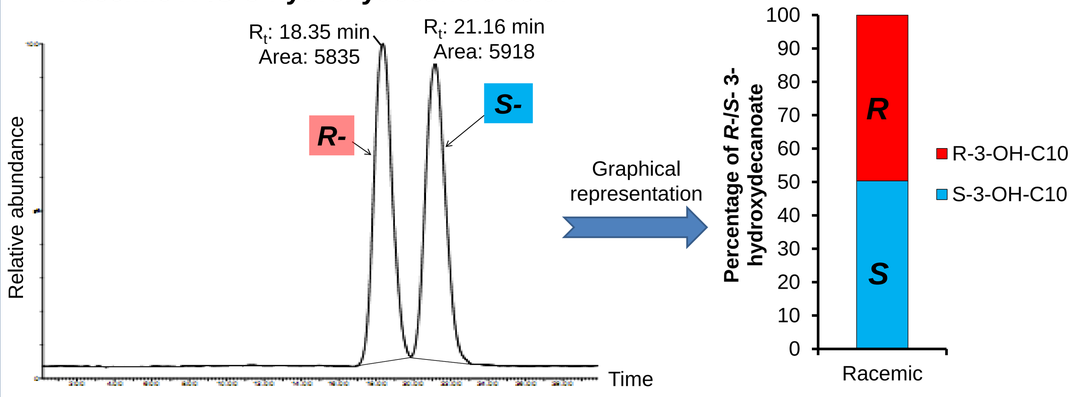
Chiral chromatographic separation and tandem MS detection of derivatized ECH reaction product

1) Chiral chromatographic separation

Lux Cellulose-1 column: Cellulose tris(3,5-dimethylphenylcarbamate)

2) Tandem MS detection of free fatty acid fragment

Racemic *R*-/*S*-3-hydroxydecanoic acid:



[2] Abdel-Mawgoud, A. M., Lépine, F. & Déziel, E. A chiral high-performance liquid chromatography–tandem mass spectrometry method for the stereospecific analysis of enoyl-coenzyme A hydratases/isomerases. *Journal of Chromatography A* ***1306, 37-43 (2013).***

Results-1

R-specific ECH activity is in synchrony with RL production level by *P. aeruginosa* in different culture conditions

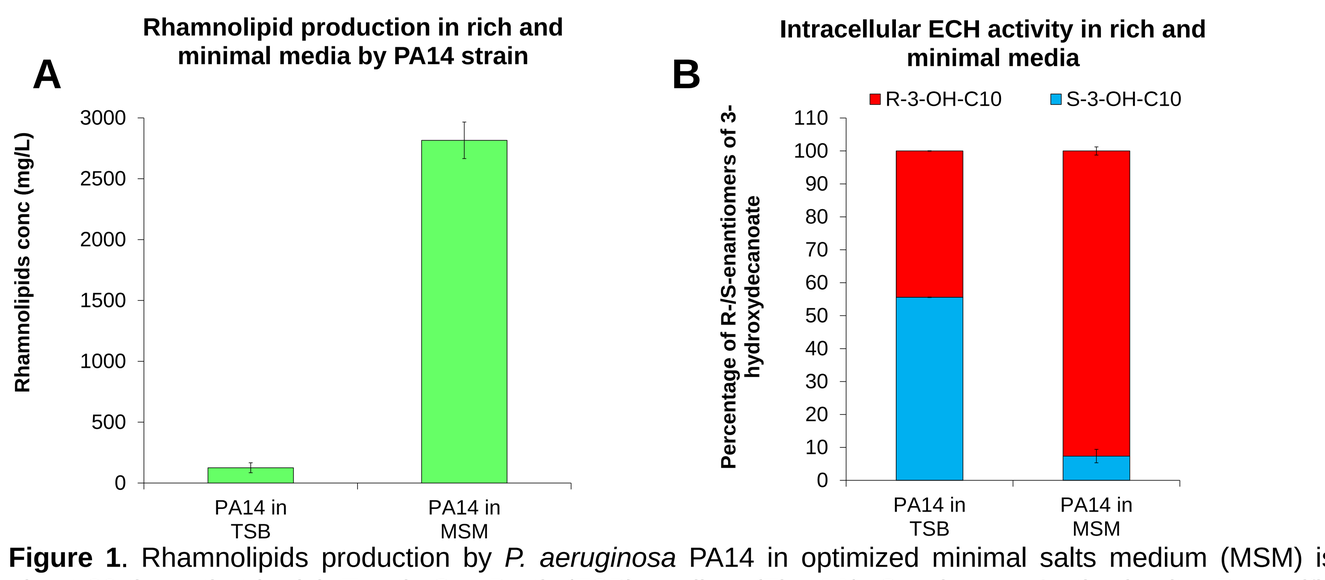


Figure 1. Rhamnolipids production by *P. aeruginosa* PA14 in optimized minimal salts medium (MSM) is about 20 times that in rich Tryptic Soy Broth (TSB) medium (Fig. **1A**). Synchronously, the *in vitro* *R*-specific ECH activity (supplying *R*-3-hydroxyacyl-CoA precursors of RL) in cells of *P. aeruginosa* PA14 cultivated in MSM is two times higher than that when cultivated in rich medium (Fig. **1B**)

Results-2

Chiral lipid profiling as a tool for identification of candidate heterologous hosts for RL expression: e.g. *P. putida* KT2440

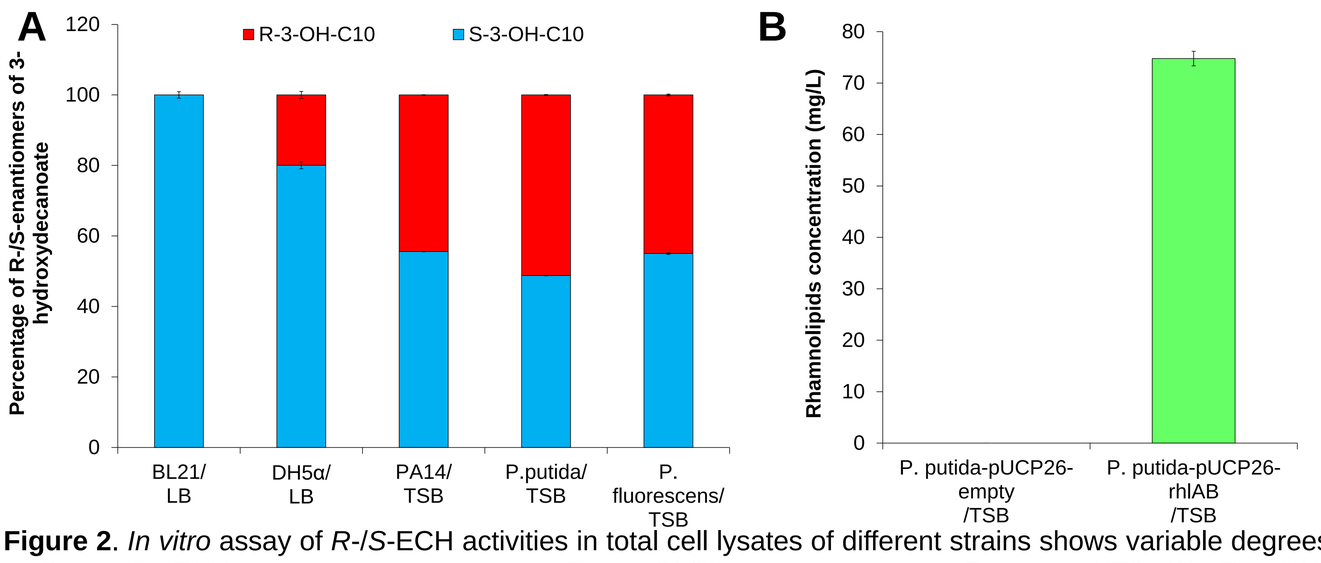


Figure 2. *In vitro* assay of *R*-/*S*-ECH activities in total cell lysates of different strains shows variable degrees of *R*-specific ECH activities which is null in *E. coli* BL21 and detectable in *P. putida* (KT2440) (Fig. **2A**). Accordingly, the latter strain is rather suggested to be supplying lipid precursors of RL and hence a probable candidate host for RL expression as proved experimentally in Fig. **2B.**

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Conclusions

- We demonstrate an efficient tool of chiral HPLC-tandem MS to estimate the net ECH activity in cell lysates and determine its type (*R*-/*S*-specificity).
- For maximum and/or heterologous expression of rhamnolipids, it is a prerequisite that the host is naturally supplying the two precursors of Rhamnolipids (RL); L-rhamnose and *R*-3-hydroxyalkanoate.
- The provided tool helps in the identification of the physiological conditions with maximal supply of the latter precursor, *R*-3-hydroxyalkanoate, for maximum production of RL.
- Finally, the method is useful in the identification of potential candidate hosts for heterologous expression of RL based on the nature of their *R*-specific ECH activity.