MALDI-TOF analysis of rhamnolipids produced by *Pseudomonas aeruginosa* NCAIM (P) B001380

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This study represents MALDI-TOF analysis of rhamnolipids (RLs) produced by strain *P. aeruginosa* NCAIM (P) B001380. Strain was grown on Lurie-Bertani (LB) medium. Composition of rhamnolipid (RL) mixture on Lurie-Bertani medium was monitored for seven days. Total number of RL congeners produced from the first to the seventh day was: 6 (5 mono-RLs, 1 di-RLs), 7 (5 mono-RLs, 2 di-RLs), 6 (5 mono-RLs, 1 di-RLs), 4 (3 mono-RLs, 1 di-RLs), 2 (1 mono-RLs, 1 di-RLs), 2 (2 mono-RLs, 0 di-RLs), 4 (3 mono-RLs, 1 di-RLs). Total number of mono-RL congeners rapidly declined after the third day of fermentation. Number of di-RLs practically didn't change, but different structure of RL congeners were present, depending on day of growth. RL profile significantly varied during fermentation on LB medium. The first three days of fermentation mono-RLs contribute to diversity of RLs mixture, after that number of different congeners decline till the end of fermentation.

Introduction

Rhamnolipids are low-molecular-mass biosurfactants. The principal RLs are mono-rhamno-di-lipidic and di-rhamno-di-lipidic congeners, while mono-rhamno-mono-lipidic and di-rhamno-mono-lipidic congeners are biosynthesized only under certain cultivation conditions (Thazbi *et al.*, 2004). RLs are microbial secondary metabolites (Van Hamme *et al.*, 2006) and their production is possible from most carbon sources supporting bacterial growth. (Tahzibi *et al.*, 2004, Soberón-Chávez *et al.*, 2005, Chayabutra *et al.*, 2001).

During the last a few decades, increasing interest in biological surfactants led to an intensification of research for their cost-efficient production. Also, search for alternative rhamnolipid-producing strains makes a structure analysis and constant adaptation of the existing analytical methods necessary. A great success of Mass Spectrometry (MS) in biological sciences and an intensive development of lipidomics give rise to development of MS analytical platform for RL high throughput investigation.

Dynamic of RL production by *P. aeruginosa* NCAIM (P) B001380 was analyzed using a recently reported MALDI-TOF based method. MALDI-TOF-MS has been developed as a novel and convenient technique for rapid high-throughput screening of RL-containing samples and RL-producing *Pseudomonas spp.* MALDI-TOF-MS assignments have been successfully verified by GC/MS, HPLC, and NMR spectroscopy, confirming the validity of the MALDI-TOF mass spectrometry method (Hayd *et al.*, 2008, Price *et al.*, 2009).

Material and methods

Strain *P. aeruginosa* NCAIM (P) B001380 was isolated from industrial mineral metal-cutting oil (Karadzic *et al.*, 2004).

The strain was activated on nutrient agar at 30 °C for 24 h and fermentation was carried in LB medium, at 30 oC for seven days, at 250 cycles/min (Karadzic *et al.*, 2004).

Crude preparation of RL was obtained by acidic precipitation, followed by extraction of chloroform: methanol (2:1) (Heyd *et al.*, 2008).

Concentration of RL (Crl) was determined spectrophotometrically at 421 nm, with orcinol reaction using rhamnose as a standard (Wilhelm *et al.*, 2007).

Isolated RL mixture was analyzed by MALDI-TOF. Mass spectra were acquired on a Voyager Biospectrometry DE Pro Workstation (Perseptive Biosystems, Framingham, MA, USA), using matrix solution of 2,5-dihydroxybenzoic acid, concentration of 0.5 M in methanol containing 0.1% trifluoroacetic acid (TFA) (Schiller *et al.*, 2004).

Results

Composition of RL mixture on LB medium was monitored for seven days. Total number of RL congeners was: 6 (5 mono-RLs, 1 di-RLs), 7 (5 mono-RLs, 2 di-RLs), 6 (5 mono-RLs, 1 di-RLs), 4 (3 mono-RLs, 1 di-RLs), 2 (1 mono-RLs, 1 di-RLs), 2 (2 mono-RLs, 0 di-RLs), 4 (3 mono-RLs, 1 di-RLs), from the first to the seventh day, respectively. Total number of mono-RL congeners rapidly declined after the third day of fermentation. The first three days of fermentation mono-RLs that dominated were Rha-C8-C10:1, Rha-C10-C12:1, Rha-C10-C14:1 and Rha-C10-C14, while after that period Rha-C8-C10:1 and Rha-C16-C16 were detected. Rha-C8-C8, Rha-C10-C10 and Rha-C10-C10-CH3 were sporadically occurring. Number of di-RLs practically didn't change, but different structure of RL congeners were present, depending on day of growth.

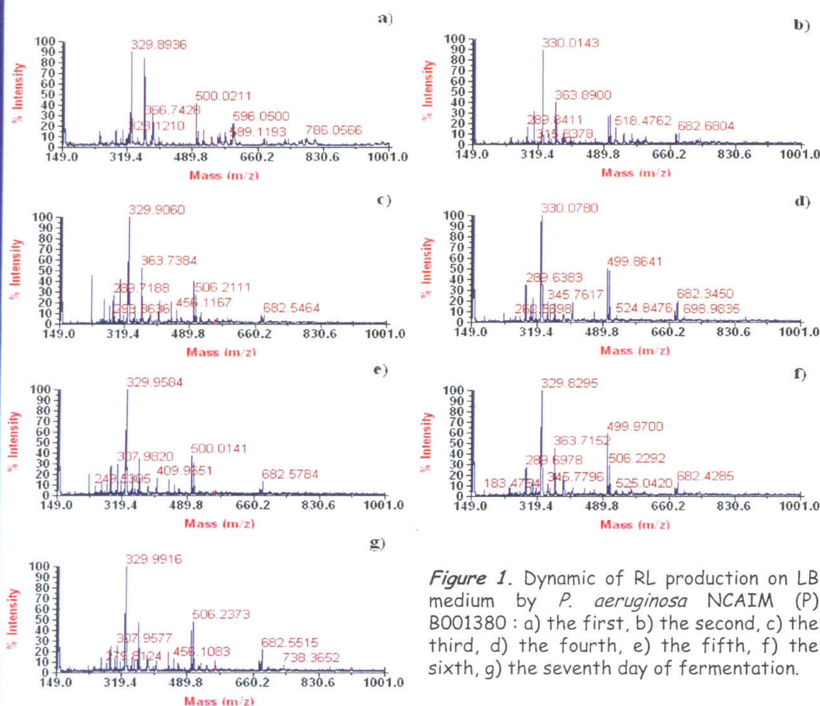

Figure 1. Dynamic of RL production on LB medium by *P. aeruginosa* NCAIM (P) B001380 : a) the first, b) the second, c) the third, d) the fourth, e) the fifth, f) the sixth, g) the seventh day of fermentation.

Table 1. Dynamic of rhamnolipid production of *P. aeruginosa* NCAIM (P) B001380 on LB medium: profile of RLs and concentrations (Crl)

Day	Mono-RL congener Rha-	Di-RL congener Rha-Rha-	Crl mg/l
1	C8-C10:1/C10:1-C8, C10-C12:1/C12:1-C10, C10-C14/C14-C10//C12-C12, C10-C14:1/C14:1-C10//C12-C12:1/C12:1-C12, C10-C10-CH3	C14-C14	51
2	C10-C10, C10-C12/C12-C10, C10-C12:1/C12:1, C10-C14/C14-C10//C12-C12, C10-C14:1/C14:1-C10//C12-C12:1/C12:1-C12,	C12-C12, C12-C14/C14-C12	53
3	C8-C8, C8-C10:1/C10:1-C8, C10-C14/C14-C10//C12-C12, C10-C14:1/C14:1-C10//C12-C12:1/C12:1-C12, C16-C16	C12-C12	74
4	C8-C10:1/C10:1-C8, C14-C16/C16-C14, C16-C16	C10-C12:1/C12:1-C10	57
5	C8-C10:1/C10:1-C8	C10-C12:1/C12:1-C10	80
6	C10-C10, C10-C10-CH3	/	96
7	C8-C8, C8-C10:1/C10:1-C8, C16-C16	C10-C12:1/C12:1-C10	53

During fermentation Crl of RLs was varying in a range from 50 to 100 mg/l. Two maxima of rhamnolipid production were obtained (Table 1): the first maximum at the third day (74 mg/l) and the second maximum between the fifth and the sixth day (80-96 mg/l). Days with the highest production of RLs showed different diversity of RL mixture (3th, 5th and 6th day had 6, 2, 2 different RL congeners, respectively).

Conclusion

RL profile significantly varied during growth of *P. aeruginosa* NCAIM (P) B001380 on LB medium.

For the first three days of bacterial growth mono-RLs significantly contributed to diversity of RLs mixture, after that their number declined till the end of fermentation. Number of di-RLs was low and practically didn't change, albeit different structure of RL congeners were present, depending on the phase of growth. The highest diversity of RL mixture was at the second day of fermentation (7 different congeners). The highest Crl were obtained after 72 h (74 mg/l) and between fifth and sixth day of fermentation (80-96 mg/l).

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