

Introduction



In the last decades, the problem of diffusion of new multidrug-resistant (MDR) bacterial pathogens is alarming due to the increase morbidity and mortality of the infections caused by them.



Ramoplanin (Rmpl) is lipoglycopeptide whose mode of action is considered promising for the treatment of infections caused by Gram-positive MDR pathogens. Rmpl inhibits cell wall biosynthesis by blocking the conversion of lipid intermediate II into lipid II, a step specifically catalyzed by N-acetylglucosaminyl transferase [1].



The clinical development of Rmpl was initially prevented because of its low local tolerability when injected intravenously. Despite this, there are many compounds from the group of lipoglycopeptides that can potentially be used as antimicrobial agents in clinical practice [2].



Rmpl is produced by a soil-dwelling "rare" actinobacterium *Actinoplanes ramoplaninifer* ATCC 33076 [3]. Many biosynthetic and regulatory aspects of its production remain unclear, partially due to the challenges with cultivation and gene-engineering manipulations of the producer strain.

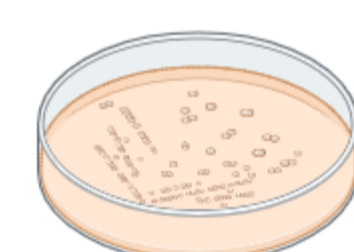
The aim of this work:

- to investigate the Rmpl production under laboratory conditions;
- to explore the morphological properties of the wild-type strain.

Methods

1. Investigation of cultural properties of *A. ramoplaninifer* while cultivated on different solid media.

Table 1. List of solid nutrient media, used for analysis



ISP2	ISP3	ISP4	ISP5	ISP6	ISP7	MMGT
R2	SFM	SG	SMMS	TM1	TSA	YMPG

2. Cultivation under the Rmpl production conditions [4].

3. The Rmpl extraction method was established. It includes changing the pH of the culture using HCl 10N to pH 3.5 and extraction with a mixture of methanol and isopropanol (5:1).

4. Quantitative Rmpl analysis by HPLC. The method for HPLC analysis was also established following the literature [4] and introducing some modifications.

5. Co-cultivation of two morphotypes of *A. ramoplaninifer*.

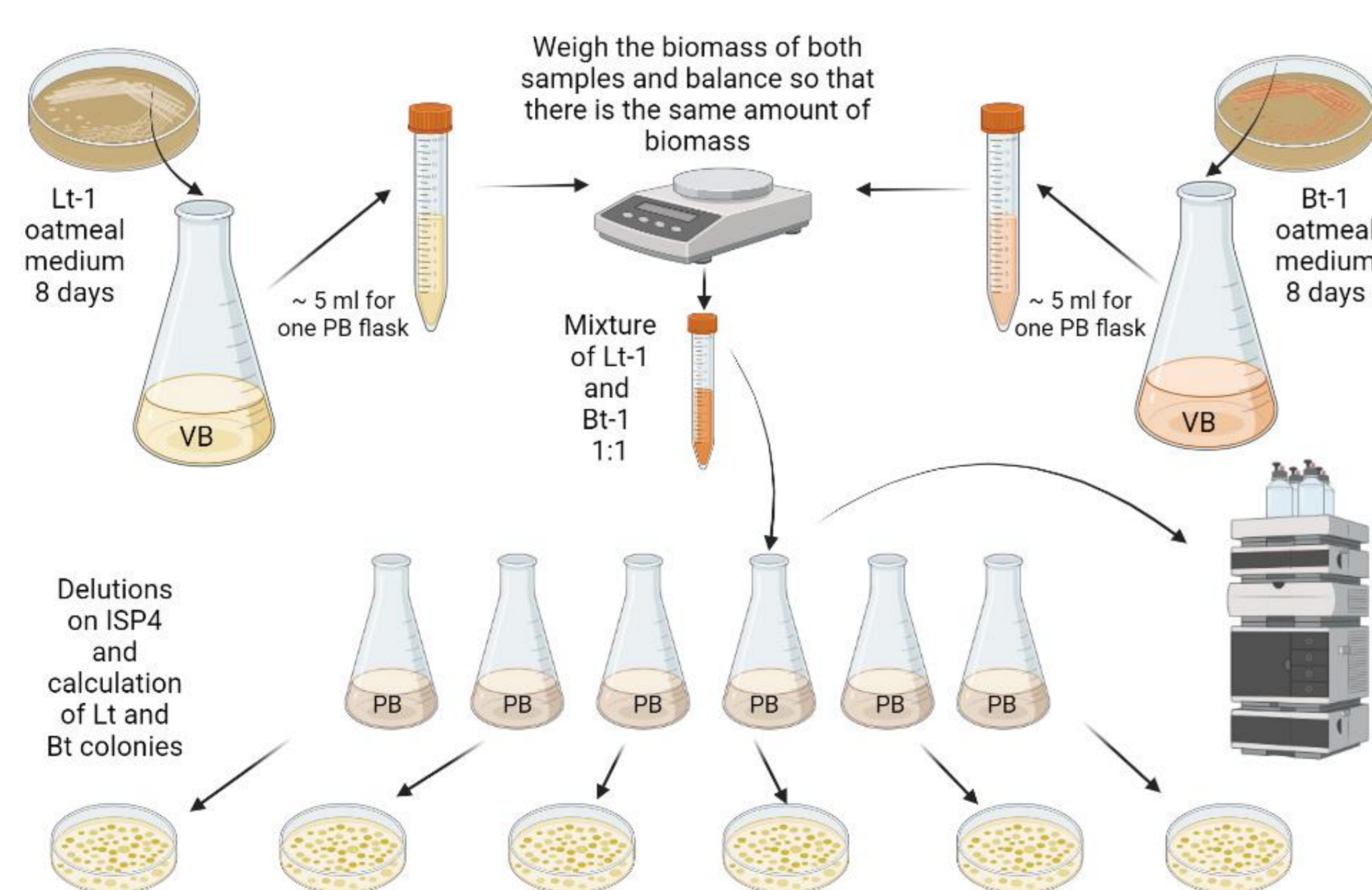


Figure 1. Scheme of conducting an experiment on co-cultivation of two morphotypes found in *A. ramoplaninifer* culture

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Results

- We have revealed that especially on ISP4 agar the culture exhibited heterogeneity, which led to the purification of **two morphotypes**, further denoted as Lt (a light orange colony color), and Bt (a bright orange colony color)

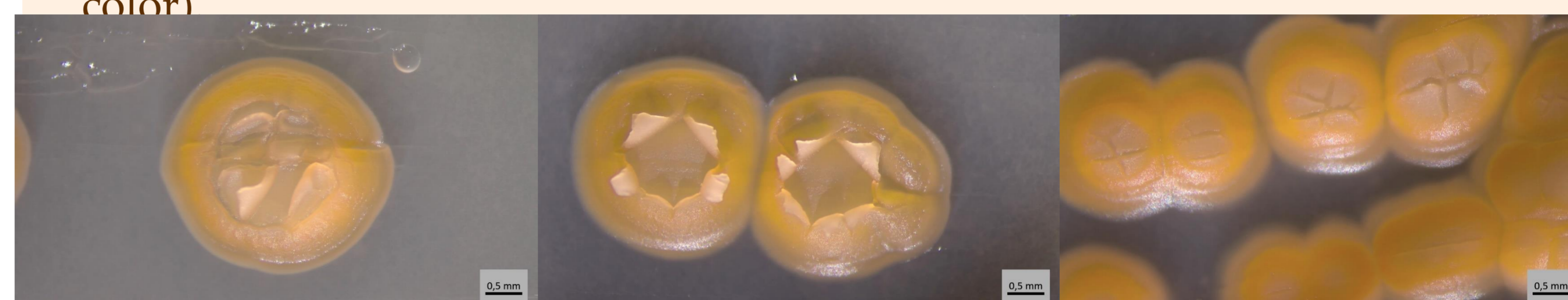


Figure 2. Photos of colonies of *A. ramoplaninifer* Bt using a stereoscope



Figure 3. Photos of colonies of *A. ramoplaninifer* Lt using a stereoscope

- During **separate cultivation** under the Rmpl production conditions in liquid media, Lt exhibited notably higher productivity of Rmpl when compared to Bt morphotype grown under the same conditions.

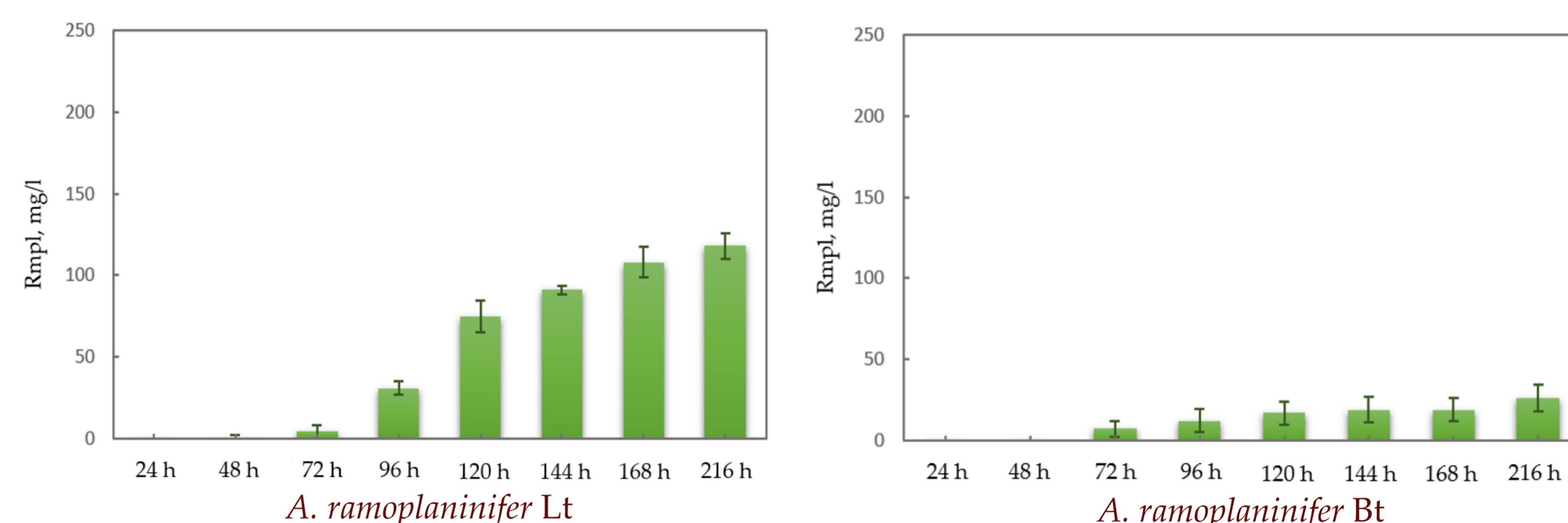


Figure 4. Results of quantitative assessment of Rmpl by HPLC in bacterial cultures of two morphotypes. Cultures were grown for 192 h in liquid production medium PB with the addition of L-leucine in the amount of 5 g/l.

- During **co-cultivation** of Lt and Bt in productive liquid media, Bt gradually outcompeted Lt over time, resulting in the dominance of Bt as the sole morphotype present in the culture. These results correlated with the decreased level of Rmpl synthesis in co-culture, corresponding to the reduced abundance of the Lt morphotype in it.

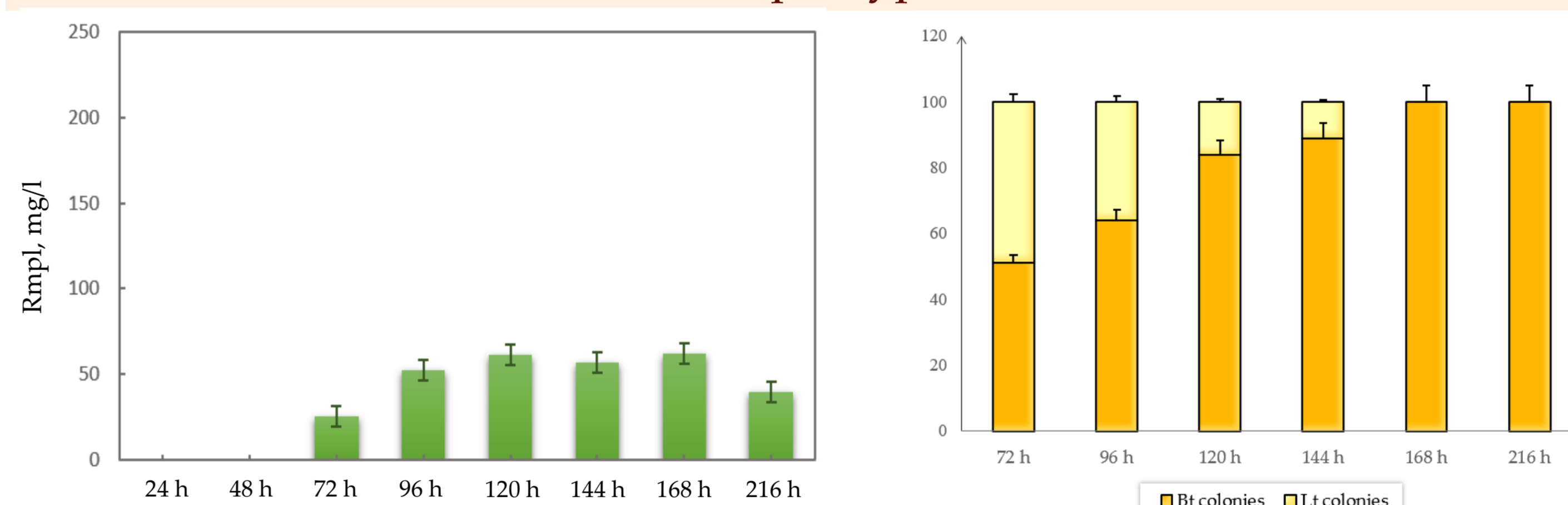


Figure 5. Results of quantitative assessment of Rmpl by HPLC in mixed bacterial cultures of two morphotypes. Cultures were grown for 192 h in liquid production medium PB with the addition of L-leucine in the amount of 5 g/l.

- As we can see from the obtained results, the adjacent cultivation of two morphotypes in a 1:1 wet biomass weight ratio does not improve the level of Rmpl synthesis. The question of cultivation and the optimal percentage ratio between Lt and Bt remains open for further research.
- The work to correlate morphological differences with the genomic properties of both strains by sequencing and comparing the genomes of Lt and Bt is underway in our laboratories.

Conclusion

- On ISP4 agar the culture exhibited heterogeneity, which led to the purification of two morphotypes;
- We established to separate cultures for Lt and Bt morphotypes presented in wild-type strain, and conducted a thorough examination of their respective characteristics and properties;
- In liquid media Lt exhibited notably higher productivity of Rmpl when compared to Bt morphotype grown under the same conditions;
- Moreover, when Lt and Bt were co-cultivated, the bright morphotype over time replaced the light one, becoming completely dominant in the culture after 144 hours of growth.

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