Charles University
Faculty of Science
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Theoretical and evolutionary biology

Summary of the Doctoral thesis

Morphogenesis of the bacterial colonies and their mutually influencing

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Abstract

This thesis follows previous works of our group (Rieger T. *et al*., 2008; Cepl J. *et al*., 2010 and Patkova I. e*t al*., 2012), where we focused on the morphology of the bacterial colonies *Serratia marcescens* and its variety caused by changing of the inoculation conditions on nutrient agar. When bacterial colonies *S. marcescens* are grown on nutrient agar enriched with glucose isolated enough from other colonies in its living space, it can form coloured structured colonies, which we named morphotype „fountain" (**F**). This morpotype becomes ideal for following studies of mutual influencing of the bacterial colonies, because of its ability of pigmentation change or structure loss caused by altering surrounding inoculation conditions.

We noticed in normal sowed agar plates, that bacterial colonies, which grows in the close distance with other colonies develop their pigmentation sooner, than colonies, that grows more isolated. We studied how is this influencing happening and what are the necessary conditions for it. We proved, that different species of bacterial macrocolonies (*S. marcescens* – morphotype (M), *S. rubidea* and *E. coli*) emits into the nutrient agar informative signal, which makes the recipient colonies *S. marcescens* reacts on this signal with the same manner (X structure). It looks, that this is kind of universal reaction on some compounds emitted by diferent species of Enterobacteria.

Growth of the **F** morphotype on minimal agar is conditional on its induction by growing hetospecific or conspecific macrocolony nearby. In the work on signal molecule identification we found out, that growing macrocolony of *S. rubidea* (**R**) emits to distant agar (up to few centimetres) protein, which we identified as hypothetical protein (35KDa) of *Serratia marcescens* „WP_025304701.1" with 100 % identity. Nevertheless filtration of the functional minimal media obtained from growing fluid culture of **R** morfotype through the mebrane with cut-off 3500Da proved, that the induction of the growth of **F** colony on minimal agar plate is not made by this protein.

Medium obtained from the growing fluid culture of the **R** morphotype, when is deprived from the bacterial cells, induce growth of **F** colony when is added on the minimal agar plate. In the next part of this thesis we tried to identify signal molecule that is contained in the minimal medium obtained from the fluid culture of **R** morphotype by modern biology analytic methods. We detected taht possible siganl molecule can be short thermostable peptide of approximate mass 3284 Da.

We believe, that this thesis can act as a solid base for further study, that can lead, with optimalization of purging methods, to identification of the signal molecules emitted and received by different species of Enterobacteria and thus contribute to our knowledge of intra – and inter-species communiaction of microorganisms.

Content

1. Introduction

If we want to understand some word, we have to look for its origin. Word communication originate from latin word communicare, which means „collectively share something, make something common".

Communication incorporates not only change of informations, but also participation on the final impact and context of the information, which can alter whole meaning of this process. In this meaning, we can understand the word communication more as synonym of the word interaction, process of mutual influencing and effecting (Vybiral Z., 2005).

All organisms lives in constantly changing environment. Ability of adaptation to this changes is crucial for success of every living being, and in long-term meaning for success of every species.

Facing the fact, that there is constant flow of substances, energy and informations between all organisms and the surrounding environment, the capability of communication between single organisms is crucial for their surviving. Constant changes of the surrounding environment generates pressure on the individual organisms or whole comunities and urge them to develop new strategies for survival. These new strategies often involves new communication possibilities of developing organisms.

If we want to investigate in laboratory influence of the communication with the environment on the final phenotype of the studied organism, we need to think about whole broadness of possible information inputs, which can affect the final phenotype as well. That's why we need to carefully choose our experimental setups, cause we need to have whole system in the constant conditions and always alter one parametr only. Then we can study precisely, how does this paramentr affect the final phenotype of the multicellular body.

In the past times bacteria was considered as primitive single cell organisms, which are living separately or accumulated in simple structured colonies , where all of the bacterias are identical passive particules. In recent times it becomes obvious, that we can think about bacterial colonies as a multicellular communities (everyone with 10^9 – 10^{12} cells), which have advanced possibilities how to survive in environment, via tasks dividing, gene express regulation, cell diferenciation and even by developing special work groups (bacteria with specific genetical abilities) (Ben-Jacb E. *et al.*, 2004).

For this purpose, bacteria develops wide range of biochemical communication instruments, as a simple molecules, AHL's for example (Bassler B.L., a Losick R., 2006), outer membrane vesicules (OMV) (Schwechheimer C. a Kuehn M.J., 2015), peptide signal molecules and bacteriocins (Dirix G. *et al.*, 2004) and even whole aggregates of this agents (plasmid, viruses) (Miller R.V., 1998).

With this tools horizontal genes and informations in a colony or between different organisms (plaque, biofilms) is arranged.

Bacteria uses for intra – and inter-species communication different physical, chemical and biological prosesses.

Study of this signal pathways strikes on many difficulties, which can't be sometimes surpassed by using of modernmicrobiology or molecular biology methods

Recipient organism can recognise signal molecules in very low concentrations, that can be under limits of used identification methods.

2. Aims of the study

This thesis follows previous works of our group, where we studied morphogenesis, variability and mutual communication of bacterial colonies for many years. We focused on morphogenesis of colonies of bacteria Serratia marcescens. We studied, how can alter younger and final phenotype (**F** pattern) of the colony according to changes of surrounding environment during the cultivation.

Aim of this study was to decribe closely effect of close growing colonies. Then we focused on physical and biochemical nature of this influencing. During this study, we described effect of growth induction of the **F** colonies on the minmal agar (**F** phenotype doesn't grow on minimal agar in normal circumstances) in the presence of growing macrocolonies (**M, R**, *E. coli*) in the same living space.

After this observations, next aim of this study was:

1: Identify the mechanism of transmission of this informative agens (air, agar)
2: Identification of this signal molecule

3. Material and methods

Bacterial species: *Serratia rubidaea (***R***), Serratia marcescens (***F, M***), E. coli*

Bacterial culture cultivation: Methods of OD measurement, types od sowing of bacterial colonies,incubation conditions and documentation was decribed in attached publication (Rieger T., et al, 2008).

SDS PAGE electroforesis

MALDI TOF

Protein fingerprint

Gel chromatografy

Chromatografy (reverse phase)

NMR spektroskopy

Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR)

4. Results and discussion

In clasical sowing we recorded faster developing of **F** colonies, when they were growing closer to the other colonies, than the colonies which grows more isolated (Fig.1)

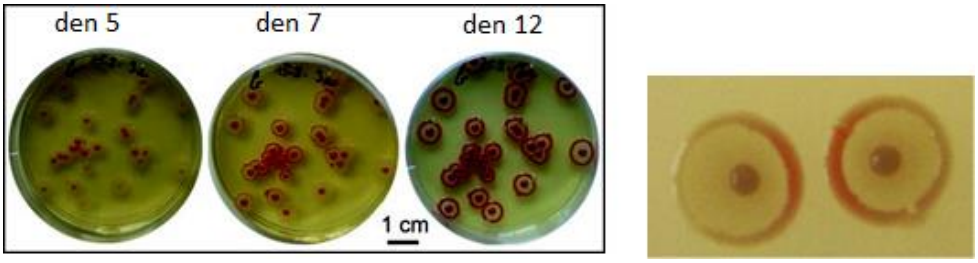
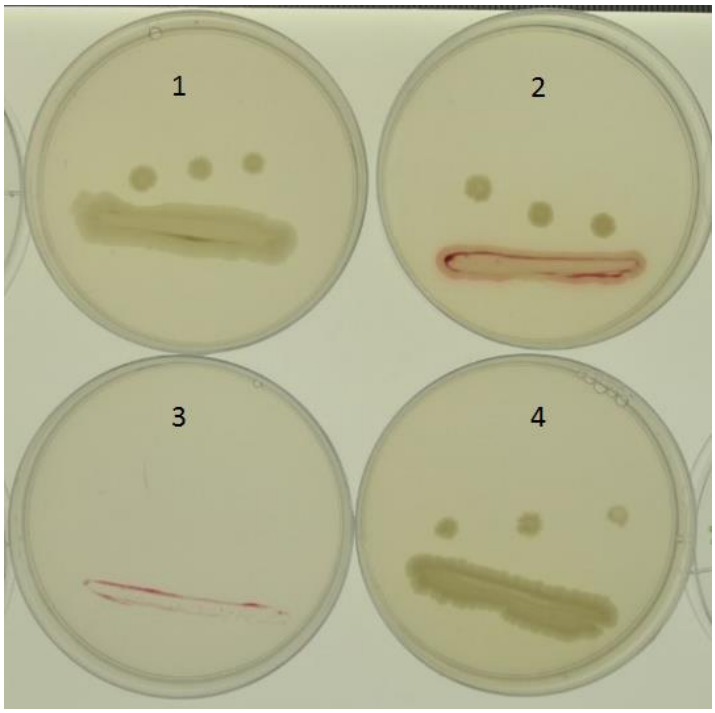


Fig. 1: Faster colouring of F colonies sowed in close proximity; Left – time progress of growth colonies on the Petri dish; right – detail of faster colouring of F colony (Patkova I. *et al.*, 2012)

We detect, that this faster development is caused by some agens difunded into the agar by other colonies.

We have to switch our cultivation media from undefined to defined **(MMA)** because of the better chance to identify this signal agens. We found out, that F colony doesn't grow (only survives) on this **MMA** agar in normal circumstances.

We recorded ability of conspecific (**M**) or heterospecific (*E. coli*, **R**) macrocolonies to induce growth of **F** colonies on **MMA**. (Fig. 2). This fact aims our next study on this phenomenon



Obr. 2: Doted F colonies above different macrokolonies on MMA. 1 - **F** colony doted above growing macrocolony *E. coli*; 2 - **F** colony doted above growing macrocolony **R**; 3 - **F** colony (not growing) doted above not growing macrocolony **F**; 4 - **F** colony doted above growing macrocolony **M.**

We proved that growing macrocolony R produce during its growth protein (100 % match with hypothetical protein "WP_025304701.1" with size 35 KDa) into the surrounding agar, that can have signal function. In our case it wasn't our searched agens, which we proved by filtration of active conditioned medium through membrane with cut off 3500 Da (it still works after filtration).

We striked on limitations of optimalization separative methods. We were unable to clear and concentrate our signal molecule enough for the modern biology methods to investigate it properly.

Nevertheless we showed, that this growth induction of **F** colony on minimal agar can be done by small thermostable peptide of approximate weight 3284 Da (about 21 aminoacids), which difuse through agar (Fig. 3).

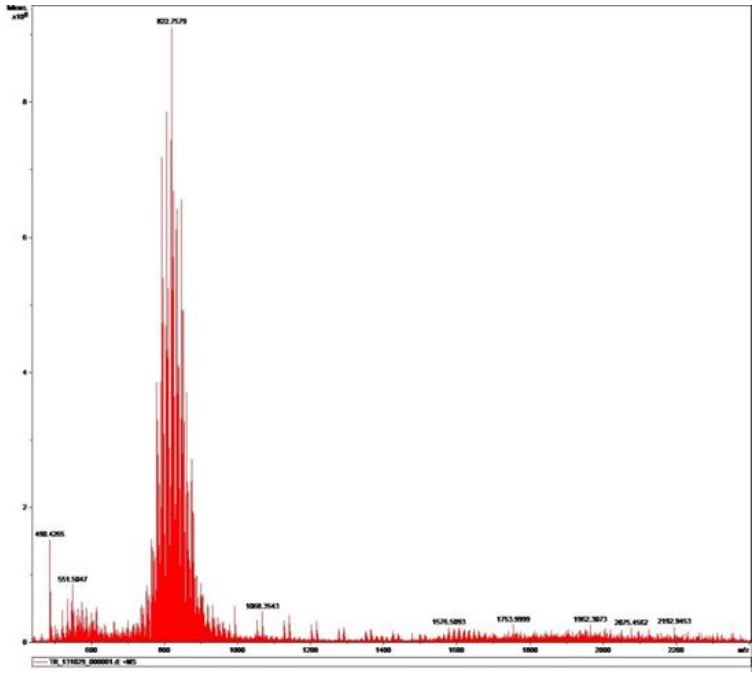


Fig. 3: FT-ICR spektrometry of functional agens; X axis- m/z (matter/charge); Y axis – intensity of signal

5. Conclusions

We defined, that inductor of helper agens can be conspecif or heterospecif growing macrocolony.

We specified, that helper agens is spread through agar or liquid medium, but not air.

We exclude nutrition condicioned growth of **F** colonies on minimal agar.

We proved, that growing macrocolony R produce during its growth protein (100 % match with hypothetical protein “WP_025304701.1” with size 35 KDa).

We defined, tah tour signal molecule is smaller than 3500 Da.

Thanks to FT-ICR mass spektrometry we found molecule with strong intensity and weight aproximately 3284 Da.

NMR analysis showed, that our signal molecule can be short peptide.

6. References

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7. List of publications

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