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Multispecies microbial communities. Part II: Principles of molecular communications

Wielogatunkowe systemy mikrobiologiczne. Część II: Zasady komunikacji molekularnej

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ABSTRACT Multispecies microbial consortia are a major form of life that includes examples of medical significance such as the gut flora, opportunistic pathogens living in hospital environments, bacterial-fungal consortia present in dental cavities etc. The stability of such consortia is poorly understood and is generally discussed in terms of species-specific mechanisms. On the other hand, there is a growing body of evidence that there are general stability criteria for polymicrobial consortia. This essay gives an overview of the underlying concepts and presents some of the general experimental and theoretical analysis tools applicable to multispecies consortia. Recent results indicate that some species are capable of stable, long-term collaboration while non-cooperating cheat mutants can cause a local collapse of the community. These simple mechanisms provide a protection against unwanted mutations and environmental challenges so they may serve as guidelines for developing defense strategies against mixed microbial infections.

KEY WORDS: bacterial consortia, communication, cooperation, quorum sensing, *Pseudomonas aeruginosa*

STRESZCZENIE Wielogatunkowe systemy mikrobiologiczne są jedną z głównych form życia. Przykłady takich systemów obejmujące florę jelitową, oportunistyczne patogeny szpitalne, czy też bakteryjno-grzybicze systemy istniejące w zębach próchniczych mają istotne znaczenie medyczne. Mechanizmy stabilizacji tych systemów są słabo poznane i często rozważane w kontekście specyficznych cech gatunkowych. Z drugiej strony coraz więcej danych świadczy o istnieniu ogólnych kryteriów, które muszą być spełnione w istniejących systemach wielogatunkowych. Poniższa praca daje przegląd podstawowych pojęć i prezentuje eksperymentalne oraz teoretyczne metody stosowane w badaniach nad systemami wielogatunkowymi. Ostatnie doniesienia wskazują, że niektóre gatunki są zdolne do trwałej, długoczasowej współpracy z innymi, ale ich mutacje mogą zniszczyć system. Ten prosty mechanizm chroni przed niepożądanymi mutacjami i może być wskazówką pozwalającą rozwijać metody obrony przed infekcjami mieszanymi.

SŁOWA KLUCZOWE: konsorcja bakteryjne, komunikacja, kooperacja, *quorum sensing*, *Pseudomonas aeruginosa*

Introduction

Many microbes live in large, multispecies communities in which the participants jointly exploit the resources. Multispecies microbial consortia constitute a major form of life that is found in environments ranging from 10 km above sea level to more than 20 km below the surface of the oceans, and have always been among the most important members and maintainers of the planet's ecosystem. Microbial consortia often contain hundreds of different species that share secreted materials in a densely packed environment. Currently there is no sufficient experimental evidence to explain why such consortia can be stable against environmental challenges or against the emergence of non-cooperating cheater mutants. This theoretical problem is also important for future practical applications, since gut microflora, the rhizosphere, opportunistic pathogens living in hospital environments, bacterial-fungal consortia present in dental cavities or biofilms damaging underwater metal surfaces are all such consortia.

Many prokaryotes possess inter-cellular signaling systems which allow species to colonise new habitats, to invade hosts and to spread over surfaces (1-3) A typical example is quorum sensing (QS) which enables bacteria to switch from low activity to high activity regimes using signaling molecules as well as „public goods" (*e.g.* surfactants, enzymes, siderophores) that facilitate movement, nutrient uptake amongst other things (3, 4). Signaling molecules are believed in most cases to be transferred by diffusion in the medium surrounding the bacterial populations so their local concentration can vary according to local cell density, positional and/or spatial constraints – for a recent review see (5). It is also known that a large part of sequenced bacterial species contain sensors for exogenous signals produced by other bacteria or potential host organisms (6, 7). QS regulates many cellular mechanisms, one of these is the well detectable swarming movement (8, 9). Swarming bacteria cooperate by sharing signals and public goods, but on the other hand, they also compete with each other for space and resources. Bacterial communities must therefore rely on an apparent equilibrium between co-operation and competition.

Cell-to-cell communication enables unicellular organisms to sense and manipulate their local environment in a way single cells can not. They can invade new habitats, colonize hosts, as well as react to chemical and geometric factors of the environment. For example, bacterial colonies can track clues (exogenous signals)

emitted by hosts or can avoid other bacterial colonies . Interestingly, we know many details about the two extremes: genetic regulatory mechanisms on the one hand, and population dynamics and colony patterns on the other, but the link between these two levels is currently at large missing. Our focus is this missing link, this essay gives overview of current results on how colony behavior emerges from cell-to-cell communication, as well as underlying concepts and some of the new experimental and theoretical analysis tools.

The jungle of terminology: interactions, signals, cues

Microorganisms uptake and secrete materials, hence they both react to as well as modify the environment. Coexisting microorganisms are thought to interact if the population of one species is different in the absence and in the presence of a second one. Interactions can be defined in the evolutionary context of populations as well as at the level of cellular /molecular processes.

From the evolutionary perspective, if populations of two species interact, the effects can be defined in terms of the absolute or relative growth rates (or fitness) of the respective populations. Social interactions can be generally classified based on the positive and negative evolutionary effects of the interactions (tab, I) (10-12). By distinguishing more than two types of interactions and allowing more types of social behaviours, one can arrive to the known, biologically relevant concepts such as commensalism, synergism, parasitism, syntrophy etc.

If we refer to sequestered molecules, it is useful to note whether or not the evolutionary effect is beneficial (+) or costly (-) for the sender as well as for the receiver (13, 14). This allows one to determine if a molecule can be considered a true signal that is evolved to transmit information or just a cue indicating an environmental effect (tab, II).

In this essay we are not directly concerned with the evolutionary fate of the cells, we simply speak about nutrients, signals, toxins, public goods, wastes. At the molecular level, cells are thought to compete for space and nutrients and communicate via sequestered molecules. While the population-centered view is linked to evolutionary scenarios, the molecular approach views interactions as ad hoc metabolic exchanges mediated by ingested and/or sequestered molecules. Our overview is limited in many respects, for instance it will ignore the exchange of plasmids and other genetic materials.

Since sequestered molecules are diverse, Monds and O'Toole proposed practical guidelines for defining signal molecules (15, 16), these are: i) the signal is secreted and has been identified; ii) mechanisms exist to sense and respond specifically to the signal; iii) the concentration of the signal required to elicit the response is not toxic to the cell; iv) the response evoked is separable from the primary metabolism of the signal; v) the purified signal molecule can reproduce the biological response at a physiologically relevant concentration; and vi) the signal network is adaptive at the level of the community (17).

One can further classify signals according to the underlying mechanisms. Here we will concentrate on those events where an external signal or cue *alters gene expression*, such as, for instance, in QS.

Finally, we can define the directionality of signaling. Signals can act on the cells emitting them and/or directed only towards another species. Signals acting on their own production in a positive feedback loop are called autoinducers, and most QS regulating signals fall into this category. Between two coexisting species, one-way and two-way signaling exists.

One of the aims of this brief overview it was to make clear that molecular communications between cells of two communicating species can be regarded as an extremely rich and complex network of interactions, even if each kind of cells emit only few types of substances. The take home lesson from this theoretical overview is therefore simple: all interspecies interactions are different, and must be analyzed separately.

Table I: A classification of social behaviour (13, 14)
Tabela I: Klasyfikacja zachowań społecznych (13, 14)

Microbial interaction *Interakcje mikrobiologiczne*	**Effect on sender** *Wpływ na oddziałującego*	**Effect on receiver** *Wpływ na poddanego oddziaływaniu*
Mutualism / *Mutualizm*	+	+
Altruism / *Altruizm*	-	+
Selfishness / *Egoizm*	+	-
Spite / *Złośliwość*	-	-

Table II: Communication types defined according to their fitness consequences (14)
Tabela II: Rodzaje komunikatów w zależności od ich konsekwencji (14)

	Evolved effect on sender *Wpływ na oddziałującego*	**Evolved effect on receiver** *Wpływ na poddanego oddziaływaniu*
Signal	+	+
Cue	-	+
Coercion	+	-

Recent theoretical insights

The study of bacterial consortia has been facilitated by a number of recent theoretical insights that place the commonalities of the diverse microbial communities into a new perspective.

Cross-talk and solos. There is ample evidence of cross-talk between various AHL QS sytems inside a single cell (18). Second, genomic studies revealed that many proteobacteria possess QS solo LuxR-family proteins for which there is no obvious cognate LuxI synthase within the same genome (7). These findings suggest that within a microbial community, bacteria are able to respond not only to their own signals but to a wide range of substances released by other species. An equilibrium of signaling may therefore be a prerequisite for community stability.

Cheat mutants All forms of cooperation in nature are exploitable by cheats. Two kinds of QS cheat mutants have been used in recent studies. SN (signal negative) mutants can be obtained by inactivating the AHL synthase gene(s). SN mutants do not synthesize the signal but are able respond to it and therefore produce the public goods. SB (signal blind) mutants can be prepared by inactivating the QS Receptor gene. SB cells are capable to produce low levels of AHL signals but cannot produce public goods. Such mutants are supposed to be metabolically more efficient than the wild type and are known to persistently emerge in vivo, in environments such as in the lungs of cystic fibrosis patients. It is not yet not clear why these avidly growing mutants do not take over a community.

Recently it was found that QS mutants can produce two interesting types of transitions in microbial consortia. SB mutants of *P. aeruginosa* can collapse a colony of WT *P. aeruginosa* cells, but the col-

lapse is local. On the other hand, SN type mutants are capable of stably co-swarming communities with *P. aeruginosa* cells (19) (fig. 1). These results could be accommodated by a model of local communications that pictures microbial consortia as locally recruited and locally communicating communities composed of continuously emerging microcommunities (20). Such complex communities can localize and eliminate an unwanted mutants by local collapse, which provides a protection against unwanted mutations.

New experimental techniques

Measuring the composition and growth rate of synthetic consortia in well defined culture conditions is a standard method for evaluating cheaters. The strategy consists in constructing synthetic consortia of QS-active and QS deficient cells, and growing them in conditions where only QS cells can survive. One of the problems is that the predicted behavior of consortia is different in well mixed, closed cultures as compared to natural habitats, such as an open surface. The underlying reasons for this are plausible: for example, a relatively small number of WT cells can produce enough signals or exoenzymes to keep a large number of mutant cells active in a well mixed culture. The so-called swarming agar plates provide a good model for surface-bound communities. Swarming plates are minimal media semisolid plates on which swarming bacterial motility may be detected by the formation of migration patterns (21). Experiments of swarming plates provides results which are different from those obtained in close liquid cultures: For instance, SB mutants that grow very well in mixed SB-WT cultures swiftly collapse the same community on swarming agar plates (19).

Microfluidics One of the problems associated with the study of bacterial communities is the dependence of the behavior on the topology of the microenvironments. Microfluidics provide unique possibilities for constructing controlled environments in which the behavior of cellular microcommunities can be observed, as shown by an impressive series of articles in leading scientific journals (22-26). Flow chambers of various geometries, with characteristic sizes ranging from cm to μm can be constructed and the flow-through properties can be regulated so that the community can be studied either in a completely constrained, or in an essentially open environment constantly refilled with fresh medium. One can add various substances (signaling molecules, bacteriostatic agents etc.) to the flow at precisely controlled manner so the exact kinetics of the response can be described in quantitative terms. These techniques allow one to directly observe the synchronous activation of small cell-communities (26) as well as the behavior of single cells.

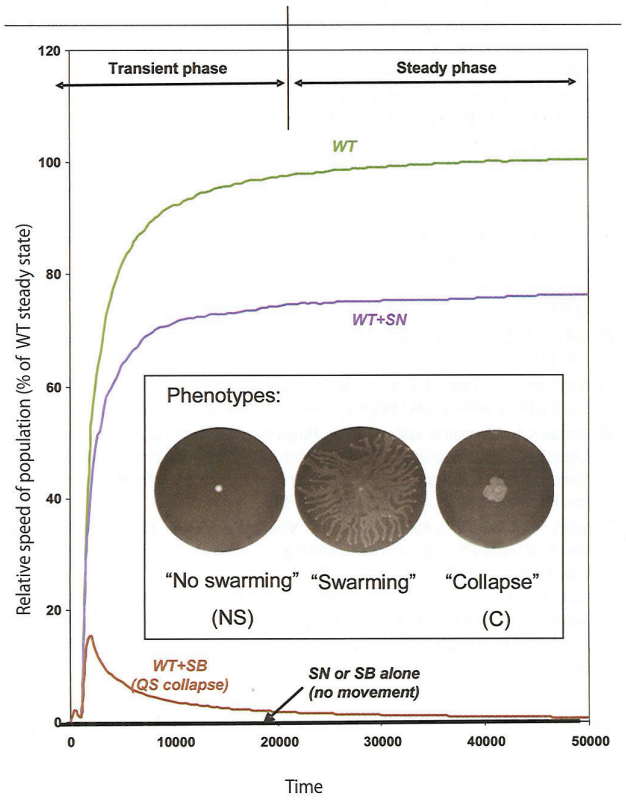

Fig. 1. Modelling the behaviour of a synthetic *P. aeruginosa* community on agar plates (insets) and by computer simulations. The communities consist of WT *P. aeruginosa*, SN mutants that can respond to QS signals produced by the WT but are incapable of producing it, and SB mutants that neither produce, nor respond to the signal. Both on agar plates and in computer simulations, SN mutants co-swarm with WT, but SB mutants collapse the community.
The simulation time is in arbitrary unit. The plates are "swarming agar plaes" (see text), pictures taken at 24 hours. After ref. (19)

Ryc. 1. Modelowanie zachowań sztucznej populacji *P. aeruginosa* na płytkach agarowych i za pomocą symulacji komputerowej. Populacje zawierają WT *P. aeruginosa*, SN (signal negative) mutanty, które reagują na QS sygnały wytwarzane przez WT, ale są niezdolne do ich produkcji, oraz SB (signal blind) mutanty, które zarówno nie wytwarzają, jak i nie reagują na sygnały. Tak na płytkach agarowych, jak i w symulacji komputerowej mutanty SM namnażają się wraz z WT, podczas gdy SB mutanty niszczą populacje

Generic computational models for bacterial consortia

In silico models allow us to interpret the observed signaling dynamics and to identify the underlying factors. Modeling of bacterial growth on 2D surfaces has a long history (27), studying the correlation of colony growth and the underlying genetic regulatory mechanisms is a relatively new approach. Recently it was shown that the salient features of bacterial communities, like density-dependent activation, tracking of signals, swarming of QS- mutants, are the direct consequences of the QS regulatory mechanism (28). It was also shown by computer simulations that a model of a swarming bacterial community can undergo local collapse if attacked by the same types of cheater mutants that cause collapse in agar plate experiments (19, 20). These theoretical results are important because they indicate that some, seemingly complex properties of microbial consortia do not necessarily rely on specific attack or defense mechanisms, so there may be some hope to find common strategies against them.

Conclusions and potential medical applications

Microbial cells release and ingest a variety of molecules that spread in the environment via diffusion and/or evaporation. Species that are compatible in terms of sequestered products and resource requirements can form stable communities that combine the skills of the participants, and as a consequence, microbial consortia can be more stable – and by extension, more pathogenic – than their constituent members. On the other hand, the recruitment of viable microbial consortia seems to be an *ad hoc* event in which competition can be pictured as warfare of opposing parties. When probiotic bacteria, such as *Lactobacilli* are used to populate the surface of certain target cells, we use their competition abilities to fence off

pathogens. Complete victories in this war are rare, and there is a continuing threat that the infection will rise again – a fact that most practitioners are painfully aware of. There have been recent suggestions to use QS mutants in the medical practice. QS deficient mutants of *P. aeruginosa* sensitive to a selected antibiotic were suggested to compete out WT strains of *P. aeruginosa* in the lung of cystic fibrosis patients in such a manner that the surviving mutants could be then eradicated by antibiotic treatment (29). While this scenario seems correct in the theoretical sense, there is a danger that the QS deficient mutant may be more pathogenic than the original pathogen itself, especially since QS deficient mutants are abundant in lethal cases of cystic fibrosis (30-32).

Polymicrobial consortia display a large variety of phenotypes that obscure their commonalities. The examples reviewed here suggest that microbial consortia can combine the skills of the constituent species. Some species are capable of stable, long-term collaboration while non-cooperating cheat mutants can cause a local collapse of the community. These simple mechanisms provide a protection against unwanted mutations and can serve as guidelines for developing defense strategies against mixed microbial infections.

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References

1. Bassler B.L.: *Small talk. Cell-to-cell communication in bacteria.* Cell, 2002, 109, 421-424.
2. Camilli A., Bassler B.L.: *Bacterial small-molecule signaling pathways.* Science, 2006, 311, 1113--1116.
3. Fuqua C., Parsek M.R., Greenberg E.P.: *Regulation of gene expression by cell-to-cell communication: acyl-homoserine lactone quorum sensing.* Annu. Rev. Genet., 2001, 35, 439-468.
4. Fuqua C., Greenberg E.P.: *Listening in on bacteria: acyl-homoserine lactone signalling.* Nat. Rev. Mol. Cell Biol., 2002, 3, 685-695.
5. Alberghini S., Polone E., Corich V., Carlot M., Seno F., Trovato A., Squartini A.: *Consequences of relative cellular positioning on quorum sensing and bacterial cell-to-cell communication.* FEMS Microbiol. Lett., 2009, 292, 149-161.
6. Ryan R.P., Dow J.M.: *Diffusible signals and interspecies communication in bacteria.* Microbiology, 2008, 154, 1845-1858.
7. Subramoni S., Venturi V.: *LuxR-family 'solos': bachelor sensors/regulators of signalling molecules.* Microbiology, 2009, 155, 1377-1385.
8. Daniels R., Vanderleyden J., Michiels J.: *Quorum sensing and swarming migration in bacteria.* FEMS Microbiol. Rev., 2004, 28, 261-289.
9. Fraser G.M., Hughes C.: *Swarming motility.* Curr. Opin. Microbiol., 1999, 2, 630-635.
10. Hamilton W.D.: *The genetical evolution of social behaviour. I.* J. Theor. Biol., 1964, 7, 1-16.
11. Hamilton W.D.: *The genetical evolution of social behaviour. II.* J. Theor. Biol., 1964, 7, 17-52.
12. Hamilton W.D.: *Selfish and spiteful behaviour in an evolutionary model.* Nature, 1970, 228, 1218--1220.
13. Diggle S.P., Gardner A., West S.A., Griffin A.S.: *Evolutionary theory of bacterial quorum sensing: when is a signal not a signal?* Philos. Trans. R. Soc. Lond. B. Biol. Sci., 2007, 362, 1241-1249.
14. Maynard Smith J., Harper D.: *Animal signals.* New York, Oxford University Press, 2003.
15. Monds R, O'Toole G. [in:] *Chemical Communication Among Bacteria.* eds S. Winans, B.L Bassler. Washington, DC: ASM Press, 2008.
16. Winzer K., Hardie K.R., Williams P.: *Bacterial cell-to-cell communication: sorry, can't talk now – gone to lunch!* Curr. Opin. Microbiol., 2002, 5, 216-222.
17. De Sordi L., Muhlschlegel F.A.: *Quorum sensing and fungal-bacterial interactions in Candida albicans: a communicative network regulating microbial coexistence and virulence.* FEMS Yeast Res., 2009, 9, 990-999.
18. Straight P.D, Kolter R.: *Interspecies chemical communication in bacterial development.* Annu. Rev. Microbiol., 2009, 63, 99-118.
19. Venturi V., Bertani I., Kerényi A., Netotea S., Pongor S.: *Co-swarming and local collapse: quorum sensing conveys resilience to bacterial communities by localizing cheater mutants in Pseudomonas aeruginosa.* PLoS One, 2010, 5, e9998.
20. Venturi V., Kerényi A., Reiz B., Bihary D., Pongor S.: *Locality versus globality in bacterial signalling: can local communication stabilize bacterial communities?* Biology Direct, 2010, in press.
21. Murray T.S., Kazmierczak B.I.: *FlhF is required for swimming and swarming in Pseudomonas aeruginosa.* J. Bacteriol., 2006, 188, 6995-7004.
22. Kim H.J., Boedicker J.Q., Choi J.W., Ismagilov R.F.: *Defined spatial structure stabilizes a synthetic multispecies bacterial community.* Proc. Natl. Acad. Sci. USA, 2008, 105, 18188-18193.
23. Kastrup C.J., Boedicker J.Q., Pomerantsev A.P., Moayeri M., Bian Y., Pompano R.R., Kline T.R., Sylvestre P., Shen F., Leppla S.H., Tang W.J., Ismagilov R.F.: *Spatial localization of bacteria controls coagulation of human blood by 'quorum acting'.* Nat. Chem. Biol., 2008, 4, 742-750.
24. Boedicker J.Q., Vincent M.E., Ismagilov R.F.: *Microfluidic confinement of single cells of bacteria in small volumes initiates high-density behavior of quorum sensing and growth and reveals its variability.* Angew. Chem. Int. Ed. Engl., 2009, 48, 5908-5911.
25. Carnes E.C., Lopez D.M., Donegan N.P., Cheung A., Gresham H., Timmins G.S., Brinker C.J.: *Confinement-induced quorum sensing of individual Staphylococcus aureus bacteria.* Nat. Chem. Biol., 2010, 6, 41-45.
26. Danino T., Mondragon-Palomino O., Tsimring L., Hasty J.: *A synchronized quorum of genetic clocks.* Nature, 2010, 463, 326-330.
27. Ben-Jacob E, Cohen I, Gutnick DL. *Cooperative organization of bacterial colonies: from genotype to morphotype.* Annu Rev Microbiol, 1998, 52, 779-806.
28. Netotea S., Pongor S.: *Evolution of robust and efficient system topologies.* Cell. Immunol., 2006, 244, 80-83.
29. Rumbaugh K.P., Diggle S.P., Watters C.M., Ross-Gillespie A., Griffin A.S., West S.A.: *Quorum sensing and the social evolution of bacterial virulence.* Curr. Biol., 2009, 19, 341-345.
30. Mathee K., Narasimhan G., Valdes C., Qiu X., Matewish J.M., Koehrsen M., Rokas A., Yandava C.N., Engels R., Zeng E., Olavarietta R., Doud M., Smith R.S., Montgomery P., White J.R., Godfrey P.A., Kodira C., Birren B., Galagan J.E., Lory S.: *Dynamics of Pseudomonas aeruginosa genome evolution.* Proc. Natl. Acad. Sci. USA, 2008, 105, 3100-3105.
31. Sibley C.D., Parkins M.D., Rabin H.R., Duan K., Norgaard J.C., Surette M.G.: *A polymicrobial perspective of pulmonary infections exposes an enigmatic pathogen in cystic fibrosis patients.* Proc. Natl. Acad. Sci. USA, 2008, 105, 15070-15075.
32. Smith E.E., Buckley D.G., Wu Z., Saenphimmachak C., Hoffman L.R., D'Argenio D.A., Miller S.I., Ramsey B.W., Speert D.P., Moskowitz S.M., Burns J.L., Kaul R., Olson M.V.: *Genetic adaptation by Pseudomonas aeruginosa to the airways of cystic fibrosis patients.* Proc. Natl. Acad. Sci. USA, 2006, 103, 8487-8492.

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