Utilization and control of ecological interactions in polymicrobial infections and community-based microbial cell factories

Wigneswaran, Vinoth; Amador Hierro, Cristina Isabel; Jelsbak, Lotte; Sternberg, Claus; Jelsbak, Lars

Published in:
F1000Research

Link to article, DOI:
10.12688/f1000research.7876.1

Publication date:
2016

Document Version
Publisher's PDF, also known as Version of record

Link back to DTU Orbit

Citation (APA):
Wigneswaran, V., Amador Hierro, C. I., Jelsbak, L., Sternberg, C., & Jelsbak, L. (2016). Utilization and control of ecological interactions in polymicrobial infections and community-based microbial cell factories. F1000Research, 5, [421]. DOI: 10.12688/f1000research.7876.1
Utilization and control of ecological interactions in polymicrobial infections and community-based microbial cell factories [version 1; referees: 3 approved]

Vinoth Wigneswaran¹, Cristina Isabel Amador¹, Lotte Jelsbak², Claus Sternberg¹, Lars Jelsbak¹

¹Department of Systems Biology, Technical University of Denmark, Kgs. Lyngby, Denmark
²Department of Science and Environment, Roskilde University, Roskilde, Denmark

Abstract

Microbial activities are most often shaped by interactions between co-existing microbes within mixed-species communities. Dissection of the molecular mechanisms of species interactions within communities is a central issue in microbial ecology, and our ability to engineer and control microbial communities depends, to a large extent, on our knowledge of these interactions. This review highlights the recent advances regarding molecular characterization of microbe-microbe interactions that modulate community structure, activity, and stability, and aims to illustrate how these findings have helped us reach an engineering-level understanding of microbial communities in relation to both human health and industrial biotechnology.
Introduction
Most microbial species are embedded within ecological communities containing many species that interact with one another and their physical environment. Virtually all important microbial activities are shaped by interactions between co-existing microbes within mixed-species communities. These interactions (e.g. in the form of physical, chemical, and genetic signals such as cell-cell contact, metabolite exchange, and horizontal gene transfer) control synergistic, antagonistic, or neutral relationships among the interacting partners and are thus responsible for overall community properties such as species composition and function. In addition, microbial interactions may be dynamic and dependent on environmental context, and microbial communities can have different spatial interactive distributions ranging from metabolic interactions between unassociated planktonic cells in the ocean and long-distance electrical signaling within microbial communities to local cell-cell interactions occurring within surface-attached biofilms. Furthermore, a series of recent studies have shown that microbes and microbe-host interactions can also be mediated by small, air-transmittable molecules.

Given this complexity among microbial interactive processes, it remains a central challenge to improve our understanding of the molecular mechanisms underlying these interaction processes, their combinatorial effects, and how these interactions ultimately modulate the diversity, behaviors, and activities of the individual species within complex microbial communities.

Dissection of the molecular mechanisms of species interactions within communities is an important issue in microbial ecology. Recently, studies of a diverse range of microbial ecosystems have provided new insight into this area by combining omics methods with classical microbiology cultivation techniques. These systems include multispecies microbial communities formed during the production of fermented food, microbial communities in acid mine drainages and other polluted habitats, the commensal microbiota of corals, as well as several other ecosystems. In this review, we focus primarily on studies of microbe-microbe interactions in host-associated microbial communities and with respect to the engineering of mixed-species microbial cell factories. We use these two examples to broadly illustrate and discuss how knowledge of species interactions is of importance in relation to our ability to control and utilize microbial systems.

Advances in studies of pathogen-microbiota interactions
In relation to infectious diseases, it is becoming increasingly clear that interactions between bacterial pathogens and other microbial species present at the infection site (for example, co-infecting pathogens or commensal bacteria) can influence disease phenotype or clinical outcome. One example of the importance of such pathogen-microbiota interactions is the well-established role of the intestinal commensal microbiota regarding the prevention of colonization of invading microorganisms including bacterial pathogens in a process known as colonization resistance. The ability to characterize microbial community structures using 16S ribosomal RNA (rRNA)-based phylogenies or full metagenomic sequencing has now resulted in a much deeper understanding of the interplay between the human microbiome and bacterial pathogens with respect to infectious disease development. For example, studies of the microbial communities in certain chronic infections such as cystic fibrosis (CF) have revealed clear correlations between loss of community diversity and disease progression. CF patients are predisposed to airway infections from a number of bacterial opportunistic pathogens, among which Pseudomonas aeruginosa, Staphylococcus aureus, Haemophilus influenzae, and Burkholderia cepacia complex (BCC) have been directly associated with CF lung disease. However, recent studies based on culture-independent methods have demonstrated the presence of many additional bacterial species previously undetected by culture and have revealed a greater microbial diversity in CF airways than previously recognized. CF airways clearly represent a complex and diverse polymicrobial ecosystem, and, as the disease symptoms become more severe, the CF lung microbiota becomes dominated by the primary pathogen (which most often is the opportunistic pathogen P. aeruginosa). These results are suggestive of a wider role of the respiratory microbiota and highlight the importance of interactions between the primary pathogen and the microbiota in relation to disease progression.

There are several recent and parallel examples of interactions between the commensal microbiota and possible pathogens which are responsible for limiting colonization and infections by bacterial pathogens such as Staphylococcus aureus in the nasal cavity and enteropathogenic Escherichia coli and Vibrio cholera in the gut. Despite these exciting observations, we are still far from being able to efficiently harness the protective capability of the commensal microbiota against pathogens. Nevertheless, these and related findings clearly point toward chemical and/or biological interference with microbial interaction networks within diseased hosts as alternative treatment strategies against pathogens.

The findings mentioned above highlight the importance of research aimed at systematic mapping of interspecies interactions regarding different types of bacterial infections in combination with the identification and molecular characterization of these interactions. In other words, it is now critical to move beyond correlative research and studies focused on generating microbiome “parts” lists and to instead begin to focus on causality and function at the molecular level. Indeed, a few pioneering studies have recently illustrated these points very clearly, and there are now clear examples of identified microbe-microbe interactions mediated by bacterial metabolites and gene products that function either to limit pathogen colonization or to potentiate pathogen expansion or virulence. Although it is obviously challenging to identify and characterize microbial interspecies interactions in infected hosts, interdisciplinary approaches that combine classical microbiological in vitro cultivation techniques with advancing technologies such as three-dimensional (3D) printing, imaging mass spectrometry, and development of realistic and controllable in vitro model systems now make it possible.
to begin systematically teasing apart the interactions among cultivated key community members and to determine how these interactions modify pathogen behaviors.

**Engineering synthetic multispecies communities for bioproduction purposes**

In nature, microbes form interacting mixed-species communities to accomplish complex chemical conversions through division of labor among the individual organisms. We have successfully harnessed the power of such natural microbial communities in food and other industries for decades\(^{32,33}\), and this has logically led to the emerging concept of community-based cell factories in which synthetic microbial communities are rationally designed and engineered to produce valuable chemicals. Recent studies have indeed demonstrated the potential value of such engineered mixed-species communities as production platforms. In one recent example, a synthetic mixed-species community of *E. coli* and *Saccharomyces cerevisiae* was engineered to produce complex pharmaceutical molecules including precursors of the anti-cancer drug paclitaxel\(^{34}\). By engineering the two organisms to host specific portions of the biosynthetic pathways, it was possible to construct a co-culture system in which an intermediate metabolite was first produced by *E. coli* and then further functionalized by *S. cerevisiae* to give the final product. This study is the first demonstration of the segregation of long and complex biosynthetic pathways into separate organisms each carrying portions of the pathway, which not only enables parallel optimization of the independent pathway modules but also makes it possible to use the best match between particular pathway modules and specific hosts. In another recent study, a fungal-bacterial community was engineered to convert lignocellulosic biomass into biofuels\(^{35}\). Here, the community contained the fungus *Trichoderma reesei*, which can hydrolyze lignocellulosic biomass into soluble saccharides, and the bacterium *E. coli*, which can metabolize these saccharides into isopropanol. In this example, one species provided the carbon source for the second species, which in turn was able to produce the final product on its own.

![Image](https://via.placeholder.com/150)

It is clear from these and other studies that successful engineering of community-based microbial cell factories relies greatly on our molecular understanding of microbe-microbe interactions and how these influence community assembly, stability, and activity.

**Controlling the stability of community-based cell factories**

Unlike their natural counterparts, synthetic communities are often unstable. For example, different growth rates among the constituent organisms and secretion of toxic metabolites during growth can influence the stability of the community and will often lead to single-species domination or extinction of the community\(^{36}\). This general instability of synthetic communities limits their translation into real-world applications in industrial biotechnology, and achieving long-term maintenance of synthetic communities is a significant challenge that must be solved.

Although we still have an incomplete understanding of the multiple competitive and cooperative interactions that control microbial community assembly and activity, many different strategies have been successfully employed to increase the stability of synthetic communities. In the first example described above, Zhou *et al.*\(^{34}\) used knowledge of the metabolic capacities of the constituent organisms to construct a specific environment that favored community stability: *E. coli* can use xylose as a carbon source, but when grown on this carbon source, *E. coli* excretes acetate, which is inhibitory to its own growth. On the other hand, *S. cerevisiae* can use acetate as a carbon source but not xylose. The use of a specific carbon source (in this case xylose) thus created a mutualistic interaction between the two organisms, which in turn stabilized the community.

In the other mixed-species community (containing *T. reesei* and *E. coli*) described in the previous section, Minty *et al.*\(^{35}\) took advantage of the particular co-operator/cheater relationship that existed in their engineered fungal-bacterial community and used ecological theory to establish specific conditions (in terms of population sizes) that could stabilize this interaction.

However, community-stabilizing culture conditions—similar to the ones described in these two examples—may be difficult to design and construct for other synthetic communities. Most likely, it is reasonable to expect that alternative approaches will be required in most other situations. These alternative methods may include the construction of synthetic interactions by genetic engineering of the participating species to enforce their interaction. For example, genetic construction of pairs of auxotrophs that cross-feed and support the growth of one another when co-cultured has been shown to be an effective approach for improving community maintenance\(^{37,38}\). Other strategies have relied on programming specific mutualistic interactions by means of synthetic intercellular signaling circuits\(^{39,41}\). However, such synthetic interactions are of course also targets of evolutionary process and the long-term stability of these genetic modifications is currently not well understood.

**Form and function in microbial communities**

A fundamental principle in biology is that structure (form) and function are inseparable elements. For example, spatial separation of cells that are then subsequently linked together through controlled proximity is an organizational theme frequently observed at all levels in biology\(^{42}\). In relation to natural microbial communities, it is well established that spatial organization of the component species has significant impact on the function and activity of the system\(^{43,45}\). Interestingly, such structure/function considerations are often not included in the design of synthetic microbial communities or considered in relation to human infections where the spatial and dynamic distribution of bacteria (including pathogens) and their activities within the human host have been found to be more complex than previously realized\(^{46-48}\).

Regarding the construction of community-based cell factories, it is certainly a possibility that alternative community-stabilizing methods should build on knowledge of structure/function relationships. Indeed, it has been shown that spatial separation and artificial positioning of cells within synthetic microbial communities improve community function and stability\(^{36}\). Recent advances in fluidics-based bacterial cultivation chambers\(^{45}\), 3D printing methods\(^{50}\), and other micro-patterning techniques\(^{51}\) represent exciting areas in this direction that may advance our ability to efficiently design and control the spatial organization of cells within microbial communities.
Summary

As either members of infection communities within colonized hosts or part of synthetic communities for sustainable bioproduction, both pathogenic and industrially relevant bacteria are placed in polymicrobial environments in which interactions and spatial position modulate their activity. In both areas, there is a clear need to move beyond the current sequenced-based technologies often used to characterize complex microbial communities and to begin to identify and characterize the function of microbial interactions and the role of spatial organization. The examples shown here illustrate that such knowledge can provide new strategies for better control of bacterial infection and optimized utilization of community-based microbial cell factories. Finally, we emphasize that although our discussion is focused on examples of multispecies bacterial systems in relation to disease and biosynthesis, we believe these are indeed representative examples of an awakening field within microbial ecology focused on understanding species interactions in many types of polymicrobial ecosystems.

Competing interests

The authors declare that they have no competing interests.

Grant information

The Villum Foundation provided funding for this study to Lars Jelsbak (Grant number VKR023113). Lars Jelsbak acknowledges additional funding from the Novo Nordisk Foundation and the Lundbeck Foundation.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

1. Dubey GP, Ben-Yehuda S: Intercellular nanotubes mediate bacterial communication. Cell. 2011; 144(4):590–600. PubMed Abstract | Publisher Full Text | F1000 Recommendation

2. Møller S, Sternberg C, Andersen JB, et al.: In situ gene expression in mixed-culture biofilms: evidence of metabolic interactions between community members. Appl Environ Microbiol. 1998; 64(2):721–732. PubMed Abstract | Free Full Text

3. Stecher B, Densler R, Maier L, et al.: Gut inflammation can boost horizontal gene transfer between pathogenic and commensal Enterobacteriaceae. Proc Natl Acad Sci U S A. 2012; 109(4):1269–1274. PubMed Abstract | Publisher Full Text | Free Full Text

4. McCarron J, Becker JW, Repepe DJ, et al.: Microbial community transcriptomes reveal microbes and metabolic pathways associated with dissolved organic matter turnover in the sea. Proc Natl Acad Sci U S A. 2010; 107(38):16426–16427. PubMed Abstract | Publisher Full Text | Free Full Text

5. Prindle A, Liu J, Asayl M, et al.: Ion channels enable electrical communication in bacterial communities. Nature. 2015; 527(7576):59–63. PubMed Abstract | Publisher Full Text | F1000 Recommendation

6. Pfeffer C, Larsen S, Song J, et al.: Filamentous bacteria transport electrons over centimetre distances. Nature. 2012; 491(7423):218–221. PubMed Abstract | Publisher Full Text | F1000 Recommendation

7. Jelsbak L, Seggaard-Andersen L: Pattern formation by a cell surface-associated morphogen in Myxococcus xanthus. Proc Natl Acad Sci U S A. 2002; 99(4):2032–2037. PubMed Abstract | Publisher Full Text | Free Full Text

8. Léotoff S, Audrain B, Bernier SP, et al.: Aerial exposure to the bacterial volatile compound trimethylamine modifies antibiotic resistance of physically separated bacteria by raising culture medium pH. MBio. 2014; 5(1):e00344–13. PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

9. Niu Q, Huang X, Zhang L, et al.: A Trojan horse mechanism of bacterial pathogenesis against nematodes. Proc Natl Acad Sci U S A. 2010; 107(38):16631–16636. PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

10. Chen Y, Gozzi K, Yan F, et al.: Acetic Acid Acts as a Volatile Signal To Stimulate Bacterial Biofilm Formation. MBio. 2015; 6(3):e00392. PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

11. Wolfe BE, Button JE, Santarelli M, et al.: Cheese rind communities provide tractable systems for in situ and in vitro studies of microbial diversity. Cell. 2014; 158(2):422–433. PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

12. Suesserts R, de Bok FA, Huigenholt J, et al.: Unraveling microbial interactions in food fermentations: from classical to genomics approaches. Appl Environ Microbiol. 2008; 74(16):4997–5007. PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

13. Méndez-Garcia C, Mesa V, Sprenger RR, et al.: Microbial stratification in low pH oxic and suboxic macroscopic growths along an acid mine drainage. ISME J. 2014; 8(6):1259–1274. PubMed Abstract | Publisher Full Text | Free Full Text

14. Meyer JL, Gunasekera SP, Scott RM, et al.: Microbiome shifts and the inhibition of quorum sensing by Black Band Disease cyanobacteria. ISME J. 2015. PubMed Abstract | Publisher Full Text | F1000 Recommendation

15. Bohnhoff M, Miller CP: Enhanced susceptibility to Salmonella infection in streptomycin-treated mice. J Infect Dis. 1962; 111(2):117–127. PubMed Abstract | Publisher Full Text

16. Zhao J, Schloss PD, Kalikin LM, et al.: Decade-long bacterial community dynamics in cystic fibrosis airways. Proc Natl Acad Sci U S A. 2012; 109(15):5809–5814. PubMed Abstract | Publisher Full Text | Free Full Text

17. Cox MJ, Altgaier M, Taylor B, et al.: Airway microbiota and pathogen abundance in age-stratified cystic fibrosis patients. PLoS One. 2010; 5(6):e11044. PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

18. Blainey PC, Milia CE, Cornfield DN, et al.: Quantitative analysis of the human airway microbial ecology reveals a pervasive signature for cystic fibrosis. Sci Transl Med. 2012; 4(153):152ra130. PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

19. Holby N: Epidemiological investigations of the respiratory tract bacteriology in patients with cystic fibrosis. Acta Pathol Microbiol Scand B Microbiol Immunol. 1974; 82(4):541–550. PubMed Abstract | Publisher Full Text

20. Harrison F: Microbial ecology of the cystic fibrosis lung. Microbiology. 2007; 153(Pt 4):917–923. PubMed Abstract | Publisher Full Text

21. Yang L, Jelsbak L, Molin S: Microbial ecology and adaptation in cystic fibrosis airways. Environ Microbiol. 2011; 13(7):1682–1689. PubMed Abstract | Publisher Full Text

22. Isewe T, Uehara Y, Shinji H, et al.: Staphylococcus epidermidis Esp inhibits Staphylococcus aureus biofilm formation and nasal colonization. Nature. 2010; 465(7296):346–349. PubMed Abstract | Publisher Full Text | F1000 Recommendation

23. Fukuda S, Toh H, Hase K, et al.: Bilateral bacteria can protect from enteropathogenic infection through production of acetate. Nature. 2011; 469(7311):543–547. PubMed Abstract | Publisher Full Text | F1000 Recommendation

24. Heiso A, Ahmed AM, Subramanian S, et al.: Members of the human gut microbiota involved in recovery from Vibrio cholerae infection. Nature. 2014; 515(7527):423–426. PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

25. Buffie CG, Buzzo V, Stein RR, et al.: Precision microbiome reconstitution restores bile acid mediated resistance to Clostridium difficile. Nature. 2015; 517(7533):205–208. PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

26. Stacy A, Everett J, Jorth P, et al.: Bacterial fight-and-flight responses enhance virulence in a polymicrobial infection. Proc Natl Acad Sci U S A. 2014; 111(21):7819–7824. PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

27. Korgoskar A, Trived U, Rumbaugh KP, et al.: Community surveillance enhances Pseudomonas aeruginosa virulence during polymicrobial infection.
28. Frydenlund Michelsen C, Hossein Khademi SM, Krogh Johansen H, et al.: Evolution of metabolic divergence in Pseudomonas aeruginosa during long-term infection facilitates a proto-cooperative interspecies interaction. ISME J. 2015. PubMed Abstract | Publisher Full Text

29. Connell JL, Kim J, Shear JB, et al.: Real-time monitoring of quorum sensing in 3D-printed bacterial aggregates using scanning electrochemical microscopy. Proc Natl Acad Sci U S A. 2014; 111(51): 18255–18260. PubMed Abstract | Publisher Full Text | Free Full Text

30. Price KE, Naimie AA, Griffin EF, et al.: Microbial consortium enhances production of natural products. Proc Natl Acad Sci U S A. 2015; 112(34): 10851–10856. PubMed Abstract | Publisher Full Text | Free Full Text

31. Shou W, Ram S, Vilar JM, et al.: Synthetic cooperation in engineered yeast populations. Proc Natl Acad Sci U S A. 2011; 108(10): 3655–3660. PubMed Abstract | Publisher Full Text | Free Full Text

32. Zhu A, Guo J, Ni BJ, et al.: Interkingdom metabolic transformations captured by microbial imaging mass spectrometry. Proc Natl Acad Sci U S A. 2012; 109(34): 13811–13816. PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

33. Price KE, Naimie AA, Griffin EF, et al.: Microbial consortium enhances production of natural products. Proc Natl Acad Sci U S A. 2015; 112(34): 10851–10856. PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

34. Zhou K, Qiao K, Edgar S, et al.: Distributing a metabolic pathway among a microbial consortium enhances production of natural products. Nat Biotechnol. 2010; 28(6): 577–581. PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

35. Minty JJ, Singer ME, Schols SA, et al.: Design and characterization of synthetic fungal-bacterial consortia for direct production of isobutanol from cellulosic biomass. Proc Natl Acad Sci U S A. 2013; 110(36): 14592–14597. PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

36. Kim HJ, Boodicker JQ, Choi JW, et al.: Defined spatial structure stabilizes a synthetic multispecies bacterial community. Proc Natl Acad Sci U S A. 2008; 105(47): 18188–18193. PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

37. Kerner A, Park J, Williams A, et al.: A programmable Escherichia coli consortium via tunable symbiosis. PLoS One. 2012; 7(3): e34002. PubMed Abstract | Publisher Full Text | Free Full Text

38. Shou W, Ram S, Vilar JM: Synthetic cooperation in engineered yeast populations. Proc Natl Acad Sci U S A. 2007; 104(6): 1877–1882. PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

39. Brenner K, Arnold FH: Self-organization, layered structure, and aggregation enhance persistence of a synthetic biofilm consortium. PLoS One. 2011; 6(2): e16791. PubMed Abstract | Publisher Full Text | Free Full Text

40. Brenner K, Karig DK, Weiss R, et al.: Engineered bidirectional communication mediates a consensus in a microbial biofilm consortium. Proc Natl Acad Sci U S A. 2007; 104(44): 17300–17304. PubMed Abstract | Publisher Full Text | Free Full Text

41. Hong SH, Hegde M, Kim J, et al.: Synthetic quorum-sensing circuit to control consortial biofilm formation and dispersal in a microfluidic device. Nat Commun. 2012; 3: 613. PubMed Abstract | Publisher Full Text | Free Full Text

42. Gijzen HJ, Barugahare M: Contribution of anaerobic protozoa and methanogens to hindgut metabolic activities of the American cockroach, Periplaneta americana. Appl Environ Microbiol. 1992; 58(8): 2565–2570. PubMed Abstract | Publisher Full Text | Free Full Text

43. Schramm A, Lansen LH, Revsbech NP, et al.: Structure and function of a nitrifying biofilm as determined by in situ hybridization and the use of microelectrodes. Appl Environ Microbiol. 1996; 62(12): 4641–4647. PubMed Abstract | Publisher Full Text | Free Full Text

44. MacLeod FA, Guest SR, Costerton JW: Layered structure of bacterial aggregates produced in an upflow anaerobic sludge bed and filter reactor. Appl Environ Microbiol. 1990; 56(6): 1598–1607. PubMed Abstract | Publisher Full Text | Free Full Text

45. Raynaud X, Numan N: Spatial ecology of bacteria at the microscale in soil. PLoS One. 2014; 9(1): e87217. PubMed Abstract | Publisher Full Text | Free Full Text

46. Markussen T, Marvig RL, Gómez-Lozano M, et al.: Environmental heterogeneity drives within-host diversification and evolution of Pseudomonas aeruginosa. MBio. 2014; 5(5): e01592–14. PubMed Abstract | Publisher Full Text | Free Full Text

47. Yan M, Pamp SJ, Fukuyama J, et al.: Nasal microenvironments and interspecific interactions influence nasal microbiota complexity and S. aureus carriage. Cell Host Microbe. 2013; 14(6): 631–640. PubMed Abstract | Publisher Full Text | Free Full Text

48. Lee SM, Donaldson GP, Mikulski Z, et al.: Bacterial colonization factors control specificity and stability of the gut microbiota. Nature. 2013; 501(7467): 436–439. PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

49. Tolker-Nielsen T, Sternberg C: Methods for studying biofilm formation: flow cells and confocal laser scanning microscopy. Methods Mol Biol. 2014; 1149: 615–629. PubMed Abstract | Publisher Full Text | Free Full Text

50. Yaguchi T, Dwidar M, Byun CK, et al.: Aqueous two-phase system-derived biofilms for bacterial interaction studies. Biomacromolecules. 2012; 13(9): 2665–2669. PubMed Abstract | Publisher Full Text
Open Peer Review

Current Referee Status: ✓ ✓ ✓

Editorial Note on the Review Process
F1000 Faculty Reviews are commissioned from members of the prestigious F1000 Faculty and are edited as a service to readers. In order to make these reviews as comprehensive and accessible as possible, the referees provide input before publication and only the final, revised version is published. The referees who approved the final version are listed with their names and affiliations but without their reports on earlier versions (any comments will already have been addressed in the published version).

The referees who approved this article are:

1 Robert J Palmer, Oral Infection & Immunity Branch, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD, USA
   Competing Interests: No competing interests were disclosed.

2 Willem M de Vos, Laboratory of Microbiology, Wageningen University, Wageningen, Netherlands
   Competing Interests: No competing interests were disclosed.

3 Juan Luis Ramos, Abengoa, Palmas Altas, Seville, Spain
   Competing Interests: No competing interests were disclosed.