Biofilm transplantation in the deep sea

Irene Wagner-Döbler

Helmholz-Centre for Infection Research (HZI), Inhoffenstr. 7, 39124 Braunschweig, Germany

Phone +49-531-6181-3080. Email Irene.Wagner-Doebler@helmholtz-hzi.de

A gold rush is currently going on in microbial ecology which is powered by the possibility to determine the full complexity of microbial communities through next generation sequencing. Accordingly, enormous efforts are underway to describe microbiomes worldwide, in humans, animals, plants, soil, air, and the ocean. While much can be learned from these studies, only experiments will finally unravel mechanisms. One of the key questions is how a microbial community is assembled from a pool of bacteria in the environment, and how it responds to change – be it the increase in CO_2 concentration in the ocean, or antibiotic treatment of the gut microbiome. The study by Zhang *et al*. (Zhang *et al.* 2015) in this issue is one of the very few that approaches this problem experimentally in the natural environment. The authors selected a habitat which is both extremely interesting and difficult to access. They studied the Thuwall Seep in the Red Sea at 850 m depth, and used a remotely operated vehicle (ROV) to place a steel frame carrying substrata for biofilm growth into the brine pool and into the adjacent normal bottom water (NBW). Biofilms were allowed to develop for three days, and then those that had been growing in the brine pool were transported to normal bottom water and stayed there for another three days, and vice versa. The "switched" biofilms were then compared with their source communities by metagenome sequencing. Strikingly, both "switched" biofilms were now dominated by the same two species. These species were able to cope with conditions in both source ecosystems, as shown by assembly of their genomes and detection of expression of key genes. The biofilms had adapted to environmental change, rather than to brine pools or NBW. The study shows both the resilience and adaptability of biofilm communities and has implications for microbial ecology in general and even for therapeutic approaches such as transplantation of fecal microbiomes.

Seeps and hydrothermal vents are regions in the ocean where fluids from deeper layers of the Earth`s crust reach the seabed due to pressure gradients or tectonic activity, often at continental margins. While the water at hydrothermal vents can be above 100°C, cold seeps do not exhibit temperature anomalies. Both are islands of diversity and productivity in an otherwise oligotrophic deep sea environment, because the expelled fluids contain high concentrations of metals that are transformed into biomass by chemoautotrophic bacteria. Those bacteria, which form intimate symbioses with metazoans, are the basis of the food-chain, resulting in highly specialized and diverse metazoan vent communities (Dubilier *et al.* 2008). Deep sea vents are the only habitats on Earth which are powered by chemosynthesis rather than photosynthesis (Jannasch *et al.* 1985).

The Thuwal Seeps have been discovered during a cruise of the research vessel *Aegaeo* in 2011 (Batang *et al.* 2012). They are located on the continental margin of the Red Sea at a depth of 840 – 850 m (Fig. 1A). The brine pool covers an area of 2.2 km^2. It does not exhibit a significant thermal anomaly (<0.3%) and is the coldest (21.7°C) and least saline (74%) among brine pools in the Red Sea. It has a rich metazoan fauna and outlet rims of the site are densely covered with bacterial mats.

The authors studied the response of biofilms to sudden environmental change. Biofilms are communities of microorganisms that develop attached to a solid substratum (Dang *et al.* 2016) (Fig. 1B). In a biofilm, the

bacteria are embedded in extracellular polymeric substances (EPS) that they excrete and that are commonly known as slime. Biofilms protect bacteria from adverse influences like antibiotics. They have been described as “cities of microorganisms” because of the complexity of interactions, which include cross-feeding between different species and cell-cell communication through quorum sensing (Sztajer *et al.* 2014).

The deep sea biofilms studied here were grown in two contrasting environments: The brine pond had 74% salinity and more than 10x higher concentrations of metals than the NBW. In the NBW salinity was normal and the concentration of organic carbon was 100x higher than in the brine pond. Those two environments formed a meta-community, i.e. it was possible for bacteria to migrate from one to the other. After three days of growth on six different types of substrate, the authors transplanted (switched) biofilms from the brine pond to the normal bottom water (S-NBW) and from the normal bottom water to the brine pond (S-brine) (Figure 1C and D).

Interestingly, the switched communities were different to both source communities. The S-brine community and the S-NBW community adapted to the new environment, but they also kept some characteristics of the previous biofilm composition. Even more interestingly, both switched communities were now dominated by the same two species that had not been extremely dominant in the source communities. Those two strains were apparently able to cope with conditions in both systems. Their genomes were assembled from the metagenomes. *Marinobacter* sp. was a heterotroph that had many genes for heavy metal transport and resistance to oxidative stress. *Oleispira* sp. was also a heterotroph and had the potential to use nitrate as an electron acceptor.

The analysis of genomes can only show the potential of a microorganism, but not what it actually does. To demonstrate that in fact those pathways were operating in the biofilms under the respective conditions in the deep sea, the authors investigated changes in the expression of key genes using q-RT-PCR (quantitative PCR of reverse transcribed mRNA). They applied primers designed specifically for *Marinobacter* sp. and *Oleispira* sp. and observed an up-regulation of amino-acid metabolism related genes in the S-NBW biofilms for both microorganisms, while they found up-regulation of oxidative stress related genes in the S-brine biofilms, again for both species.

What does it all mean? The biofilms responded to the transplantation by modifying their composition in a process termed species sorting. Species sorting occurs when bacteria form a community by selection of a subset of species from a pool of species according to local abiotic and biotic conditions. It is a new term for the old paradigm “everything is everywhere – but: The environment selects (Baas Becking 1934). The pool of species might theoretically be all bacteria that are on the planet (Gibbons *et al.* 2013) or, more conservatively, all species that are forming the meta-community. But how is the selection of species controlled, which rules can be recognized for species sorting? The striking observation in the experiments reported by Zhang *et al.* is that biofilms that were subject to sudden environmental change selected for generalists that were able to cope with both environments. The biofilm communities adapted not so much to brine or NBW, but to the problem of fluctuating conditions. It will be most interesting to determine if this resilience is a specific characteristic of biofilm communities or if it is also observed in non-sessile microbial communities. Moreover, it would be interesting to investigate how it is dependent on the frequency and amplitude of change. In such a way the experiment conducted in the depths of the Red Sea provided results relevant for the whole of microbial ecology.

Figure Legend

Thuwall Seeps and experimental design. A Thuwall Seeps. The location of the brine pools in the Red Sea axial zone is shown by blue shade. Seeps are shown in red. Thuwall Seeps I and II and Thuwal Seeps brine pool are shown in insets on the right superimposed on depth contours. (Adapted from Batang *et al.* 2012, with permission). The work by Zhang *et al.* was performed in Thuwall seeps II.

B Holder with carousels carrying substrates for biofilm growth. Left, side view, right, view from above. (From Zhang *et al.* 2014, with permission.)

C Remotely operated vehicle (ROV) in the process of transporting the holder to the Thuwall Seep at 850 m depth in the Red Sea. (Image kindly provided by Weipeng Zhang).

D Interface between brine (br) and seawater (sw) on the eastern part of the brine pool. (Adapted from Batang *et al.* 2012, with permission).

Reference List

Baas Becking LGM. Geobiologie of inleiding tot de milieukunde. The Hague, the Netherlands: W.P. Van Stockum & Zoon. 1934.

Batang ZB, Papathanassiou E, l-Suwailem A *et al.* (2012) First discovery of a cold seep on the continental margin of the central Red Sea. *J Mar Sys*, **94**, 247-253.

Dang H, Lovell CR (2016) Microbial Surface Colonization and Biofilm Development in Marine Environments. *Microbiol.Mol.Biol.Rev.*, **80**, 91-138.

Dubilier N, Bergin C, Lott C (2008) Symbiotic diversity in marine animals: the art of harnessing chemosynthesis. *Nat.Rev.Microbiol.*, **6**, 725-740.

Gibbons SM, Caporaso JG, Pirrung M *et al.* (2013) Evidence for a persistent microbial seed bank throughout the global ocean. *Proc.Natl.Acad.Sci.U.S.A.*, **110**, 4651-4655.

Jannasch HW, Mottl MJ (1985) Geomicrobiology of deep-sea hydrothermal vents. *Science.*, **229**, 717-725.

Sztajer H, Szafranski SP, Tomasch J *et al.* (2014) Cross-feeding and interkingdom communication in dual-species biofilms of *Streptococcus mutans* and *Candida albicans*. *ISME J*, **8**, 2256-2271.

Zhang W, Tian R, Yang B *et al.* (2015) Environmental switching during biofilm development in a cold seep system and functional determinants of species sorting. *Mol.Ecol.*, **XX**, XXXX.