Highlight

Pseudomonas aeruginosa: the making of a pathogen

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Comment on Hilker *et al.* (2015) *Interclonal gradient of virulence in the Pseudomonas aeruginosa pangenome from disease and environment*

What makes a bacterial pathogen be a pathogen? Simple as it sounds, this question is not at all trivial to tackle as it is plagued with man-made pre-conceptions and often erroneous interpretative frameworks. One can intuitively grasp the term *pathogen* as something that does wrong e.g. causes infectious diseases to humans and by extension, to plants and animals. But let us face it: defining a fundamental biological phenomenon on the sole basis of how much it affects us, human beings (and animals and plants of importance to us), does not look like a sound scientific criterium. Only the extremes are well delineated. At one of the ends we could consider clearly beneficial microbes with little or no reputation of having ever caused damage to us, e.g. *Lactobacillus acidophilus* or *Bifidobacterium*. At the other end we have genuinely virulent species such as *Salmonella typhimurium*, *Shigella flexneri*, *Yersinia pestis*, etc., -not to mention enteroinvasive, enteropathogenic or enterohemorrhagic *E. coli*, that make their living by doing harm to their potential hosts. But there is an ample grey zone between the extremes of the scale where the frontier between being virulent or not is more fuzzy. This is the territory of the so-called *opportunistic* pathogens –commensals by default that at some point adopt an aggressive interplay with their hosts because of endogenous or exogenous signals and circumstances.

The frequent textbook view of opportunistic virulence is that practically all microbes with ecological compatibility with a given host (for instance able to grow at body temperature) have the potential to become pathogens. The immune system would thus be the only barrier for them not to deploy a hostile behaviour (Fauci and Morens, 2012). This would account for why otherwise inoffensive microorganisms that reside in diverse body niches may become pathogenic in immune-compromised

1 or otherwise stressed individuals. Also, some pathogenic species that are trapped within a complex
2 net of interactions within a commensal consortium can take over upon e.g. antibiotic treatment. But
3 this common wisdom has some pitfalls. For instance, we co-exist intimately with Archaea (Cavicchioli,
4 2011) but they seem to be innocuous to us. They are found as a major component of our normal
5 microbial flora, being present in typical locations e.g. the gut and dental plaque. But despite our
6 closeness to them and ample evolutionary opportunities, there is no conclusive evidence that Archaea
7 can cause infections (Cavicchioli *et al.*, 2003; Perez-Chaparro *et al.*, 2014). A number of explanations
8 have been proposed, some of them related to our biochemical incompatibility with them (Sadhukhan
9 and Raghunathan, 2014), an issue that I will return to later.

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11 It is common to blame bacteria with a pathogenic lifestyle because they possess specific traits
12 allowing penetration in a target tissue to the detriment of the whole afflicted organism. But what are
13 such traits and how we can recognize them? The onset of easy DNA sequence has placed the
14 emphasis on identification of the so-called *virulence factors*. These are gene products that can be
15 discerned as specifically evolved to benefit from a host by escaping its defences and submitting the
16 targets's resources to the pathogen's exploitation. Since the pioneering work of S. Falkow in the early
17 1980s (Finlay and Falkow, 1989) a large number of such factors (mostly proteins) have been identified
18 that improve colonization of a new niche in the host (e.g. attachment), enable escape (or active
19 suppression) of the immune response, allow penetration of the bacteria (or proteins thereof) in the
20 target cells, and other amazingly intricate mechanisms. Screening for genes that determine virulence
21 with *in vivo* expression technologies (IVET) or other methods often yield a large number of metabolic
22 genes, but these are largely ignored and attention is focused instead on the conspicuous molecular
23 weaponry represented by such factors (Handfield *et al.*, 2000). Their presence in a given genome
24 often means instant classification as a virulent strain or species – with all the clinical and even
25 regulatory consequences that such a label may have. The prevailing tenet is thus that one species or
26 strain is virulent because it has virulence factors - and virulence factors are thus to be found in virulent
27 strains. But are genes of such factors the actual ID of pathogens?

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29 It is within this context of assumptions on what to expect in a bacterial pathogen that the paper of
30 Hilker *et al.* (2015) deploys its whole value. In this very interesting contribution, B. Tümmler's team in
31 Hannover rolled its sleeves up and made a systematic comparative analysis of the actual virulence of

a large number of *Pseudomonas aeruginosa* strains isolated from either patients infected with the clones at stake (lungs, catheters, keratitis), or from the environment (soil, seawater, freshwater, plants), the latter with no previous record of having infected anything. All strains were then tested for virulence in 3 different assays: infection of lettuce leaves, infection of larvae (*Galleria mellonella*) and murine airway infection. The virulence of each strain in every test system was then ranked and the genomes of the bacteria inspected for the presence of pathogenicity determinants. And here came the surprises.

First, the origin and previous lives of the strains under study made little difference as a predictor of how they would do in the next infection rounds. Some strains isolated from badly damaged patients did poorly in the standardized virulence tests, while others from the environment and unlikely to have been recently infecting any eukaryotic target, turned out to be quite aggressive in the same assays. The second revelation of Hilker *et al.* (2015) was that the degree of virulence among strains varied enormously depending on the test system. This indicated that the constellation of traits that made *P. aeruginosa* to be a pathogen for one host might be irrelevant for another. But the most striking result of this work was that the genome of the strain that more consistently qualified as *virulent* in all assays (the clone called F469) lacked any of the genes that would normally be expected in a pathogen.

The take home lesson of this work is that there is more to virulence than the possession or not of a panel of virulence factors. Taking for granted that they necessarily do harm (and that their absence certify safety) could be in fact misleading. Consider for instance the strain *P. fluorescens* SBW25. This is a growth-promoting plant colonizer that actively expresses a Type-III secretion system (T3SS) during its interplay with the host (Jackson *et al.*, 2005). While T3SSs are regarded as archetypal virulence factors, it could well happen that the effectors injected by *P. fluorescens* SBW25 to the plant cells modulate the host metabolism for the better, not for the worse (other plant-associated *Pseudomonas* e.g. *P. syringae* do inject detrimental effectors; Lindeberg *et al.*, 2012). Also, shocking as it may look in first sight, *P. aeruginosa* infections have even been suggested to have a role in defending the human host against cancer (Bernardes *et al.*, 2013; Chakrabarty, 2014). Can we then make a clear divide between good and bad bacteria on the only basis of their taxonomic ID and the recognizable determinants encoded in their genome?

No question that virulence factors participate in the infection process, but are they the only players? The clear answer of Hilker *et al.* (2015) is no. I have argued before (de Lorenzo, 2014) that focusing on such factors to understand virulence is like being mesmerized by the technological details of weaponry in a war – ignoring the economy, energy, food, water and other background problems where many conflicts emerge. Metabolism is the economy of bacteria (and living systems in general) and it is naïve to think that we can understand pathogenesis without paying attention to the metabolic transactions, most often unnoticed, that happen between the host and the infectious agents. In an unusual but very welcome twist, Hilker *et al.* (2015) also examine what they call *metabolic competence* of the *P. aeruginosa* strains under examination i.e. the contents and ratios of the various pools of NAD(P) and NAD(P)H. Interestingly, these figures vary tremendously among isolates and do depart from textbook values. But it was not possible in this work to correlate such metabolic descriptors with virulence profiles. This is not surprising, because the measures of NAD(P)/H were made on cells growing in flasks on tryptic soy broth, which surely causes a physiological regime quite different of the biochemical and nutritional conditions that bacteria experience in target tissues.

The poorly studied links between central metabolic functions of microorganisms and their ability to become pathogens should definitely receive more attention. Biochemical compatibility between the infectious agent and the target (see e.g. the case of Archaea above) might be key. It is possible also that metabolic adaptation of environmental bacteria to the endogenous or exogenous oxidative stress that frame their existence outdoors becomes an asset to endure the action of macrophages (Yuk *et al.*, 2012) when entering a new host. By the same token, evolving resistance to the host defences can facilitate a superior capacity of pathogens to expand their metabolism towards new compounds (de Lorenzo, 2014). It cannot be casual that potentially virulent strains of *P. aeruginosa* (Alonso *et al.*, 1999; Marin *et al.*, 2003) or *Mycobacteria* (Cerniglia, 1993) are often found among the best biodegraders of environmental pollutants. The connections between chemical contamination and the emergence of new pathogenic capacities in bacteria that inhabit such niches certainly need to be explored in more detail. In the meantime, Hilker *et al.* (2015) truly puts a finger on an uneasy puzzle that oblige us to rethink prefixed ideas on what virulence is.

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