Abstract

Eukaryotes have evolved the very complex ubiquitin system, which is involved in regulating a plethora of pathways including proteasomal degradation, receptor signalling, DNA damage response and xenophagy. The small protein ubiquitin is attached to substrates by E3 ligases, which can not only conjugate a single ubiquitin moiety, but a variety of different chain types. These chain types function determine the fate of the substrate. Ubiquitin modifications can be cleaved by deubiquitinases (DUBs). Therefore, DUBs are able to reverse and fine-tune the ubiquitin signals.
Bacteria on the other hand do not have a ubiquitin system. Interestingly, distinct intracellular bacteria have evolved proteins that function as E3 ligases or DUBs, which are secreted as effectors into the host cell and subvert the host ubiquitin system. This is especially important, because in xenophagy, the host cell ubiquitinates invading bacteria, leading to their lysosomal degradation. In order to prevent this process, bacterial secreted DUBs reverse the ubiquitin signal.

Classically, bacterial DUBs are found in a few gram-negative intracellular pathogens. These bacteria encode one or two DUBs, which are linkage promiscuous or prefer K63 chains. While eukaryotic DUBs can be divided into eight subfamilies (JAMM, UCH, USP, OTU, Josephin, MINDY, ZUP1, VTD), some bacterial DUBs have similarities to the eukaryotic OTU family, but the majority is more similar to CE clan proteases, which in eukaryotes cleave SUMO or NEDD8, but not ubiquitin.
For a long time, *Legionella* was the only bacterium harbouring a complex repertoire of 10 DUBs, most of them from CE clan, but also an OTU-type DUB with two DUB domains, one of them being specific for K6-linked ubiquitin chains and a DUB uniquely found in this bacterium, which displays M1 specificity. The knowledge about the role of Legionella DUBs during infection increased significantly in the last years. However, it is still unknown why Legionella so extensively manipulates the host ubiquitin system, while other bacteria get along with only one or two secreted DUBs.

In this study, I analysed the DUB repertoire of two unrelated bacteria, *Simkania negevensis* and *Amoebophilus asiaticus*. *Simkania* harbours eight active DUBs of five different classes (USP, Josephin, VTD, OTU, CE clan), three of them rarely/never reported for bacteria so far. Two *Simkania* DUBs show M1 and K6 specificities like Legionella, but have evolved from different origin. In *Amoebophilus*, the astonishing amount of 14 DUB domains were shown to be active and two new OTU subfamilies were identified. Moreover, a domain specific for K48 chains was found, which is a common activity for eukaryotic DUBs, but has never been reported for bacteria before.
In summary, these results show that the bacterial subversion of the host ubiquitin system was largely underestimated due to observation bias.