

Insect-symbiont gene expression in the midgut bacteriocytes of a blood-sucking parasite

1 Sheep ked *Melophagus ovinus* is a species of wingless, blood-sucking insect that is permanently as-
2 sociated with vertebrate host and transmitted via host interactions. Its primary bacterial symbiont
3 *Arsenophonus melophagi* lives intracellularly in bacteriocytes that assemble into special structure (bacte-
4 riome) in midgut.

5 Sheep ked interacts with its symbiont nutritionally. Transcription analysis shows symbiont high ex-
6 pression of a pathway that converts proline to L-glutamate through the PutA enzyme (EC 1.5.5.2/1.2.1.88)
7 and its subsequent conversion to D -glutamate by the MurI enzyme (EC 5.1.1.3). Proline is almost al-
8 ways the most common amino acid in insect hemolymph (Arrese and Soulages 2010). In insects, proline
9 is generally reserved for energy-demanding activity such as flight. In wingless sheep ked, proline storage
10 might be used for bacterial symbiont for energy metabolism and peptidoglycan synthesis. However, the
11 symbiont expression of B-vitamin synthesis is low, except that of lipoic acid. It is possible that B vitamins
12 are not essential for sheep ked, or are only needed during particular life stage. It is also possible that
13 sheep ked does not rely on symbiont for B vitamins.

14 Symbiont *Arsenophonus* might be dependent on host for metal ions. High expression of zinc trans-
15 porters in bacteriocytes and symbionts, together with low expression of zinc protease in bacteriocytes,
16 indicate that symbiont *Arsenophonus* is demanding on zinc. Symbiont zinc-dependent proteins might be
17 protease, or a putative metallo-beta-lactamase. Beta-lactamases are enzymes that provide bacteria with
18 resistance to beta-lactam antibiotics such as penicillin, ampicillin. Metallo-beta-lactamases in particular
19 are well-known for their resistance to a broad spectrum of beta-lactam antibiotics and beta-lactamase
20 inhibitors (Bradford 2001; Drawz and Bonomo 2010). The sheep from which sheep keds are collected
21 are often treated with beta-lactam antibiotics. Besides, ferritin is highly expressed among whole gut,
22 and transferrin is down-regulated in bacteriocytes. Ferritin sequesters iron from a blood meal and blocks
23 iron ions intracellularly, while transferrin mediates transport of iron through blood plasma. Transfer-
24 rin can also act as an antimicrobial protein sequestering iron from pathogens (Yoshiga et al. 2001).
25 Down-regulation of transferrin in bacteriome might be a sort of immune privilege.

26 There is potential immune compromise in sheep ked bacteriome. Immune response genes such as
27 attacin (antimicrobial peptide) and two lysozymes, are down-regulated in bacteriocytes. PGRP-LB, an
28 amidase that degrades peptidoglycan to inhibit immune responses, is highly expressed along whole gut,
29 while GGBP, a pattern recognition receptor, is lowly expressed along gut. Furthermore, sheep ked lacks
30 peritrophic matrix, a physical barrier to protect against pathogens. It is consist with the lack of chitin
31 synthesis in sheep ked gut.