

Gut microbiota and phenoloxidasas from *Rhodnius prolixus*: effect of antibiotics on temporal patterns of expression

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Gut microbiota has already been implicated in several physiological processes in insects, as synthesis of vitamins, essential aminoacids, pheromones and digestive enzymes, detoxification of plant allelochemicals and breakdown of lignin. In spite of that, the factors controlling its diversity and development are poorly understood. We recently described an intestinal phenoloxidase (PO) activity in *R. prolixus* 5<sup>th</sup> instar nymphs. In this work, we tried to establish if there is any relationship between the development of microorganisms and activity of gut PO in *R. prolixus* 1<sup>th</sup> instar nymphs. Growing inhibition of total cultivatable gut microbes by the antibiotics tetracycline (20µg/µL), gentamycin (0.3%,w/v), erythromycin (5µg/µL), nystatin (10,000U/mL) were respectively 99.6%, 97%, 0% and 0%. Total inhibition was obtained only using tetracycline, gentamycin and nystatin together (concentrations above). Feeding *R. prolixus* 1<sup>th</sup> instar nymphs with blood plus antibiotics (tetracycline 50µg/mL; gentamycin 0.05%,w/v; nystatin 1,000U/mL) resulted in strong inhibition of PO specific activities in anterior midgut (ranging from 87% 2 days after feeding to 26% at day 7). Prophenoloxidase (PPO) is affected only at day 5, with 50% reduction. At day 7, PPO in antibiotic-treated insects did not change, while in controls PPO expression is reduced to negligible levels. Anterior midgut PPO per animal did not change with antibiotics. PPO percentual activation in the anterior midgut at day 2 dramatically changes with treatment from 100% (control) to 40% (antibiotics). In the posterior midgut, PO/PPO specific activity is affected by the antibiotic treatment only 2 days after feeding, with 50% up-regulation. These results strongly suggest that PPO activation, PO activity and PPO expression are regulated by the development of gut microbiota.

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