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Supplementary material

Supplementary Materials

Innate and adaptive immune proteins in the preen gland secretions of male house sparrows

Background

In order to support the identification of lysozyme as the antimicrobial peptide underlying the antibacterial activity of preen oil, we compared the lytic activity of our samples before and after applying a heat-inactivation treatment designed to denature and eliminate the enzymatic activity of lysozyme.

Methods

We identified a subset of the preen oil samples with sufficient sample volume (> 3µl) remaining after the initial assays (n = 10). This sample volume provided sufficient sample for the lysoplate assay, taking into account the potential loss of sample volume during application of the heat treatment and pipetting. For heat denaturation, we treated samples for 20 minutes at 100°C in a water bath (Masschalck et al. 2001; Vilcacundo et al. 2018). Following heat treatment, the assay was performed as described in the main manuscript. We also applied the heat treatment to the chicken egg white lysozyme (L6876, Sigma) standards (0.5, 0.8, 1, 2, 4, 8, 10, 20 and 40 µg/ml), and compared untreated and treated standards, to confirm inactivation of a known lysozyme under our assay conditions.

Results

Heat treatment resulted in the complete elimination of enzymatic activities of all preen oil samples (Table S1). Heat treatment also eliminated or reduced enzymatic activity in the lysozyme controls in a dose-dependent manner (Table S2), which is consistent with previous findings (Masschalck et al. 2001). Taken together, these findings support the identification of lysozyme or lysozyme-like proteins as being responsible for bacterial clearing in our assays.

Table S1. Lysozyme concentration before and after the heat inactivation and the percentage decrease in lytic activity in the 10 preen oil samples collected from male house sparrows.

Sample ID	Original lysozyme concentration (µg/ml)	Lysozyme concentration (µg/ml) after heat inactivation	% decrease in lysozyme activity
ED49455	0.774	below detection levels (no clearing zone)	100
ED49450	1.208	below detection levels (no clearing zone)	100
ED49454	1.909	below detection levels (no clearing zone)	100
ED49438	2.294	below detection levels (no clearing zone)	100
ED49419	3.331	below detection levels (no clearing zone)	100
ED49441	3.470	below detection levels (no clearing zone)	100
ED49442	3.776	below detection levels (no clearing zone)	100
ED49409	4.339	below detection levels (no clearing zone)	100
ED49428	5.178	below detection levels (no clearing zone)	100
ED49413	16.421	below detection levels (no clearing zone)	100

Table S2. The diameter of the clearing zone before and after the heat inactivation and the percentage decrease in lytic activity in chicken egg white lysozyme (L6876, Sigma) standard curve (0.5, 0.8, 1, 2, 4, 8, 10, 20 and 40 µg/ml).

Lysozyme standard (µg/ml)	Diameter of clearing zone (cm)	Diameter of clearing zone (cm) after heat inactivation	% decrease in lysozyme activity
40	1.166667	0.992	14.97145
20	1.029667	0.762	25.99549
10	0.783333	0.427333	45.44683
8	0.741333	0	100
4	0.540667	0	100
2	0.375333	0	100
1	0.347667	0	100
0.8	0.322	0	100
0.5	0	0	-

NB. In this plate, our lower detection limit was 0.8 µg/ml, as no clearing zone was observed for the 0.5 µg/ml standard.

References

Masschalck, B., Van Houdt, R., Van Haver, E. G. R. And Michiels, C W. 2001. Inactivation of Gram-Negative Bacteria by Lysozyme, Denatured Lysozyme, and Lysozyme-Derived Peptides under High Hydrostatic Pressure. - Applied and Environmental Microbiology 67: 339-344.

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