Supplementary Materials and Methods

Isolation and MALDI-MS Analysis of Lipid A:

1.5 L cultures of *Y. pseudotuberculosis* were grown at 26°C or 37°C in LB broth **with 180 rpm shaking** until an OD_{600} of ~1.5 was reached. Cells were harvested by centrifugation, washed with PBS, and stored at -20°C until lipid A extraction. Previously described modifications to the methods of Caroff and Raetz were used to chemically isolate LPS, followed by the liberation of lipid A from attached polysaccharide by mild-acid hydrolysis and finally lipid A was chemically extracted using using the method of Bligh and Dyer (1-4).

Isolated lipid A was further purified over a DEAE column to improve the quality of spectra obtained by MALDI-MS. Briefly, dried lipid A suspended in 2:3:1 (v/v) chlorofom:methanol:water was applied to pre-equilibrated column, column was washed with 10 column volumes of 2:3:1 C:M:W, and total lipid A species were eluted using 10 column volumes of 2:3:1 C:M:480 mM ammonium acetate. An additional two-phase Bligh-Dyer extraction was performed on the eluate to remove ammonium acetate. Isolated lipid A was dried under nitrogen and stored at -20°C until MALDI-MS analysis. Lipid A was resuspended in 50 µl chloroform-methanol (4:1). An empirically determined amount of lipid A, varied per sample, was mixed with 0.5 µl of matrix (saturated 6-aza-2-thiothymine in 50% acetonitrile: saturated tribasic ammonium citrate (20:1, v/v)) and spotted on a 100 well MALDI plate. Spectra obtained represent the average of >300 shots.

Supplementary Figure legends

Figure. 1. Mass spectrometry analysis of lipid A from *Y. pseudotuberculosis* PB1+, χ10052 and χ10055 grown at 26°C or 37°C. (A) *Y. pseudotuberculosis* PB1+ cultured at 26°C and 37°C; (B) χ10052 cultured at 26°C and 37°C; (C) χ10055 cultured at 26°C and 37°C. At 26°C, the three strains synthesized tetra-acylated lipid A (*m*/*z*: 1403.84, 1411.76, 1426.84 and 1534.91); penta-acylated lipid A (*m*/*z*: 1557.99, 1593.93, 1649.99, 1689.05, 1724.99, 1773.00, 1781.05 and 1829.06); hexa-acylated lipid A (*m*/*z*: 1796.22, 1871.22, 1887.2, and 1927.28); and hepta-acylated lipid A (*m*/*z*: 2109.44 and 2165.51). At 37°C, the three strains synthesized penta-acylated lipid A (*m*/*z*: 1557.99, 1637.04, 1689.05); hexa-acylated lipid A (*m*/*z*: 1796.22 1887.2, 1927.28, 1983.34, 2109.44 and 2165.51); and hepta-acylated lipid A (*m*/*z*: 2109.44 and 2165.51). *m/z* values in green represent putative lauroyl (C12) containing species present in WT *Y. pseudotuberculosis* PB1+, that are absent in χ10052.

Figure. 2. Proposed chemical structures of *Y. pseudotuberculosis* lipid A species at 26°C based on MALDI-MS data. Proposed structures include tetra- (A), penta- (B), hexa- (C) and hepta-acylated (D) lipid A species. Structural assignments are based on the presence of putative homologs to lipid A modification genes present in the *Y. pseudotuberculosis* genome, and are consistent with evidence obtained in other studies (**Rebeil et al.**, 2004; **Krasikova et al**., 1978. See comments in main text file).

Figure. 3. Proposed chemical structures of *Y. pseudotuberculosis* lipid A species at 37^{o}C based on MALDI-MS data. Assignments are based on the presence of putative homologs to lipid A modification genes present in the *Y. pseudotuberculosis* genome, and are consistent with evidence obtained in other studies (**Rebeil et al.**, 2004; **Krasikova et al**., 1978. See comments in main text file).

Figure. 4. Antigen-specific IL-4 cytokine in the culture supernatants produced from splenic cells and the ratio of INF-γ with IL-4. Splenocytes isolated from Swiss Webster mice vaccinated orally with strains χ10057(pYA3332, vector control), χ10057(pYA5199, *yopE*$_{Nt138}$-*lcrV*) or BSG at 21 days after initial immunization were stimulated in vitro with 4 **μ**g/ml of either a purified LcrV or YpL. The mitogen Concanavalin A (ConA: 1 **μ**g/ml) served as positive controls and RPMI 1640 media used as negative controls. (A) Antigen-specific IL-4 cytokine in the culture supernatants produced from splenic cells after 3 days stimulation was measured by Bioplex assays. (B) The ratio of INF-γ with IL-4. The mean ± SE was composed of 8 mice for each experiment (two experiments were pooled together). **: $P < 0.001$, ns: not significant.

Supplementary References

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