SYNTHESIS AND FUNCTIONS OF BACTERIAL LIPID A FOR SAFE VACCINE ADJUVANT DEVELOPMENT

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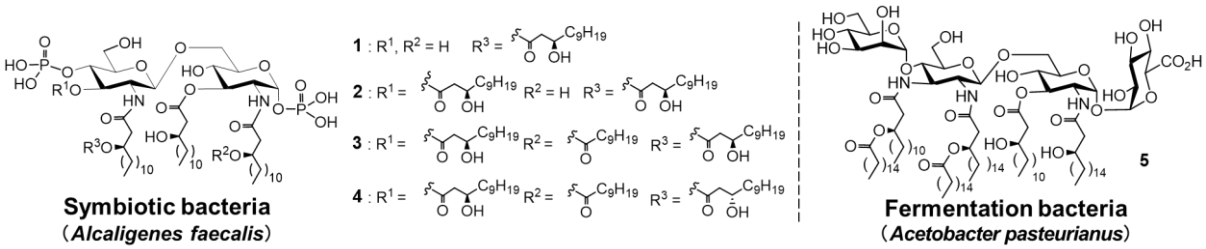
Lipopolysaccharide (LPS) is a major glycoconjugate in outer membrane of Gram-negative bacteria and canonical *Escherichia coli* LPS activate innate immunity to induce lethal strong inflammation. The terminal glycolipid lipid A is the active principle of LPS. Low inflammatory lipid A have been expected as vaccine adjuvants.

We hypothesized that co-evolved parasitic and symbiotic bacterial components should modulate host immunity moderately with low toxicity. We synthesized parasitic [1] and symbiotic [2] bacterial lipid A and elucidated the molecular basis of immunoregulation, and developed safe and useful adjuvants. In this presentation, we introduce chemical synthesis and functions of lipid A from *Alcaligenes faecalis* inhabiting gut-associated lymphoid-tissue (GALT) that is responsible for the mucosal immunity regulation.

We synthesized *A. faecalis* lipids A **1**-**3** with diverse acyl group patterns and identified the active center as hexa-acylated **3** [2]. Lipid A **3** was confirmed to exhibit non-toxic but useful adjuvant function (enhancing antigen-specific IgA and IgG production) [3-5], and the vaccine model using **3** was found to be significantly protective against bacterial infection [4]. Since IgA is responsible for mucosal immune homeostasis, we found a promising adjuvant that can safely regulate mucosal immunity by focusing on GALT symbiotic bacteria. Furthermore, lipid A **4**, which reversed the stereochemistry of the acyl side chain hydroxy group, was found to be more active than **3**, and the molecular basis of the adjuvant function is also becoming clear.

Acetobacter pasteurianus is a Gram-negative bacteria used for the fermentation process of traditional Japanese black rice vinegar (kurozu). *A. pasteurianus* LPS, which is a candidate of immunostimulatory component of kurozu, contains lipid A with a distinctive structure [6]. Here, we considered *A. pasteurianus* lipid A as a pool of acid resistant and safe immunostimulants. We achieved the systematic synthesis of three kinds of *A. pasteurianus* lipid A and identified the active center as lipid A **5** [7]. Structure-activity relationship studies revealed that the glucuronic acid residue, a characteristic structure of *A. pasteurianus* lipid A, is important for both immune function and acid resistance ability.



References:
1. A. Shimoyama, Y. Fujimoto, K. Fukase et al, *Chem. Eur. J.* **2011**, *17*, 14464-14474.
2. A. Shimoyama, F. D. Lorenzo, A. Molinaro, K. Fukase et al, *Angew. Chem. Int. Ed.* **2021**, *60*, 10023-10031.
3. Y. Wang, K. Hosomi, A. Shimoyama, K. Fukase, J. Kunisawa et al, *Vaccines* **2020**, *8*, E395.
4. K. Yoshii, K. Hosomi, A. Shimoyama, K. Fukase, J. Kunisawa et al, *Microorganisms* **2020**, *8*, 1102.
5. X. Sun, K. Hosomi, A. Shimoyama, K. Fukase, J. Kunisawa et al, *Int. Immunol.* **2024**, *36*, 33–43.
6. M. Hashimoto, K. Fukase, Y. Fujimoto, et al., *J. Bio. Chem.*, **2016**, *291*, 21184–21194.
7. H. Yamaura, A. Shimoyama, J. Kunisawa, K. Fukase et al, *Angew. Chem. Int. Ed.* **2024**, e202402922.