Exploring functional lipids in acetic acid bacteria using untargeted lipidomics

Nobuyuki Okahashi

Graduate School of Information Science and Technology, The University of Osaka

Research objective

Acetic acid bacteria, which are gram-negative microorganisms belonging to the class Alphaproteobacteria, have long been used in the fermentation of food products such as vinegar, Caspian Sea yogurt, cocoa, nata de coco, and kombucha, making them a microbial group of considerable importance in the food industry. In recent years, increasing attention has been paid to the cell membrane lipids and lipopolysaccharides (LPSs) of acetic acid bacteria. These molecules not only contribute to stress tolerance but also exert functional effects on the host through their modulation of immune responses and inflammation. The biological activity of LPS depends mainly on its lipid component, lipid A. The structural variations of lipid A including the number, length, and saturation of acyl chains critically influence its recognition by the host immune system and the magnitude of the inflammatory response. However, previous studies have been limited to analyses of the phospholipid composition and partial characterization of lipid A in LPSs from representative *Acetobacter* species, leaving much of its structural diversity and strain-specific distribution unexplored. In this study, we aimed to comprehensively elucidate the lipid profiles of diverse acetic acid bacterial species using untargeted lipidomics, which enables a broad and unbiased analysis of lipid diversity.

Methods

Eleven species across six genera belonging to the family Acetobacteriaceae—*Acetobacter* (*A. pasteurianus*, *A. orientalis*, and *A. aceti*), *Gluconobacter* (*G. frateurii* and *G. oxydans*), *Gluconoacetobacter* (*Ga. takamatsuzukensis* and *Ga. liquefaciens*), *Asaia* (*As. siamensis* and *As. bogorensis*), *Novacetobacter* (*N. hansenii*), and *Acidomonas* (*Ac. methanolica*)—were aerobically cultivated in GYC medium in shake flasks. The cells were harvested at the exponential growth phase and homogenized by bead beating. The total lipids were extracted using a single-phase extraction method with methanol/chloroform/water (10:5:1, v/v/v). The membrane lipids were analyzed using liquid chromatography coupled to quadrupole time-of-

flight mass spectrometry (LC-QTOF/MS) operated in data-dependent acquisition mode, and the data were processed using MS-DIAL software. For LPS analysis, crude LPS was extracted from cells using TRI-Reagent, and the polysaccharide moiety was cleaved by acid hydrolysis to isolate lipid A, the hydrophobic anchor portion.

Results

Untargeted lipidomics of the 11 acetic acid bacterial strains identified 195 lipid species, including glycerolipids, sphingolipids, and prenol lipids. The total signal intensities of representative phospholipid species within each class showed that phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylglycerol (PG) accounted for the majority in all strains (Figure 1). This result is consistent with those of a previous report,[1)] confirming the reliability of the present analysis.

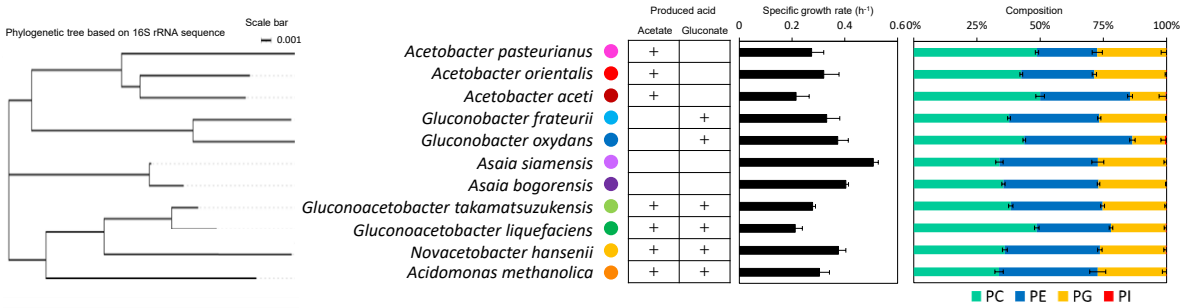

Figure 1. Phylogenetic tree of the bacterial species, specific growth rates, and relative proportions of phospholipids.

The trends in the detected lipid profiles were visualized via principal component analysis. The score plot revealed clustering of the strains according to their genera, indicating that the lipid profiles corresponded to genus-level taxonomy (Figure 2). Examination of the individual profiles showed that the *Acetobacter* and *Gluconobacter* strains could be clearly distinguished by differences in the prenyl chain length of coenzyme Q. Additionally, the strains that produced higher levels of acetic acid possessed a greater abundance of lipids containing odd-chain fatty acids, suggesting a potential association with acid tolerance. Notably, among the

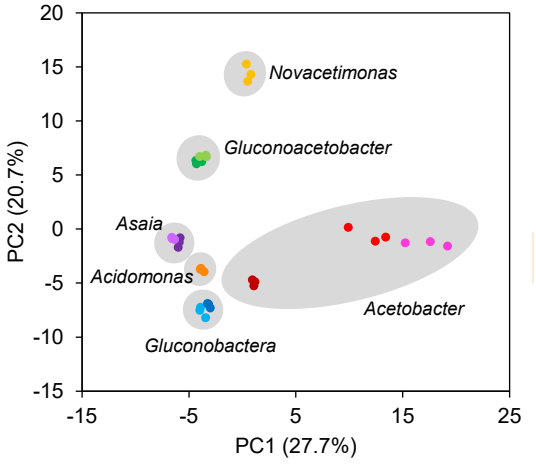

Figure 2. Principal component analysis score plot. The colors of the plot correspond to the strain colors shown next to the species names in Figure 1.

Acetobacter species, *A. aceti* was plotted apart from the other strains, indicating its taxonomic uniqueness.

Subsequently, lipid A, which exerts immunostimulatory activity, was extracted from the cells and analyzed using LC-QTOF/MS. In a previous report, lipid A of *A. pasteurianus* was described as being in a 6-acyl form with a total acyl chain length:total unsaturation composition of 96:0.[2)] By contrast, our analysis revealed coexistence of the 4-, 5-, and 6-acyl forms (Figure 3). Furthermore, the acyl chain properties of 6-acyl species ranged from 94:0 to 98:0, whereas that of the 5-acyl species ranged from 78:0 to 82:0, indicating that lipid A in acetic acid bacteria represents a highly heterogeneous mixture of molecular structures. Similar analyses of other acetic acid bacterial strains verified that the distribution of lipid A species corresponds with their taxonomic genera.

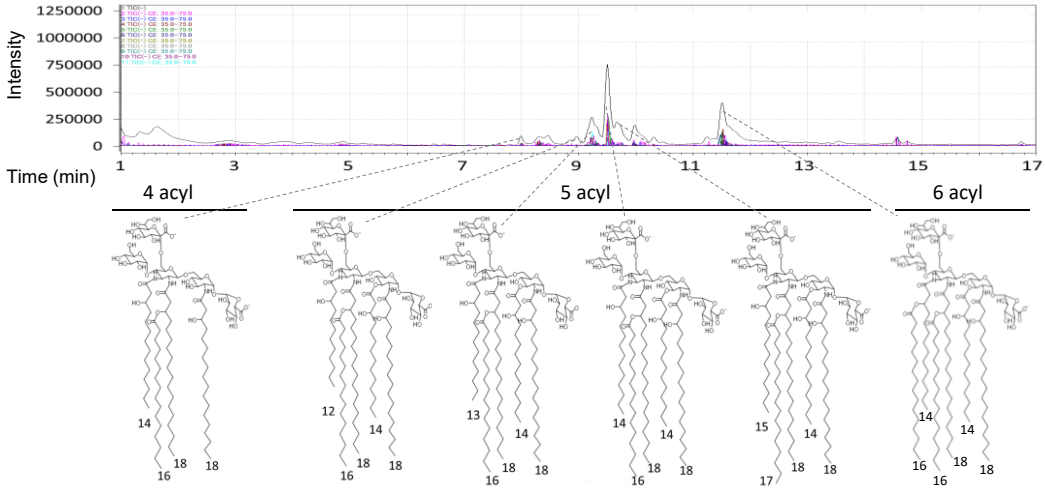

Figure 3. Structural assignments of lipid A extracts from *Acetobacter pasteurianus.* The numbers on the acyl chains represent the carbon chain lengths.

Conclusion

In this study, we analyzed the lipids of 11 acetic acid bacterial species using untargeted lipidomics and elucidated the structural diversity and species-specific heterogeneity of comprehensive lipid species, including the functional lipid.

References

1) Han, C., Xia, K., Yang, J., Zhang, H., DeLisa, M. P., and Liang, X. (2020) Investigation of lipid profile in *Acetobacter pasteurianus* Ab3 against acetic acid stress during vinegar production. *Extremophiles* **24:** 909–922.

2) Hashimoto, M. et al. (2016) Characterization of a novel D-glycero-D-talo-oct-2-ulosonic acid-substituted lipid A moiety in the lipopolysaccharide produced by the acetic acid bacterium *Acetobacter pasteurianus* NBRC 3283. *J. Biol. Chem.* **291:** 21184–21194.