Preparation and GC-MS analysis of pollen coat lipids in Arabidopsis

A. Isolation of Arabidopsis pollen grains

1. Harvest flowers that have fully opened in the past 24 hours and put them into a microcentrifuge tube (100-150 flowers per tube). Keep the tube on ice.
2. Add ice-cold 1 ml 50 mM NaPi buffer (pH 7.0).
3. Shake vigorously for 2 min at 4 C with a mixer (mixing speed 8; MT-360, TOMY).
4. Transfer the buffer containing pollen grains into a new microcentrifuge tube. Keep the tube on ice.
5. Once again add ice-cold 1 ml 50 mM NaPi buffer (pH 7.0) to the first tube.
6. Repeat Steps 3 and 4.
7. Centrifuge the two tubes (from Step 3 and 6) at 10,000 rpm for 1 min at 4 C.
8. Remove sup, but retain a small volume (ca. 20ul).
9. Combine the pollen grains in the two tubes as follows. Suspend the pollen grains of the second tube (with the smaller amount of pollen grains) in the remaining buffer and transfer them into the first tube. Then add 300 ul 50 mM NaPi buffer (pH7.0).
10. Centrifuge the tube at 10,000 rpm for 1 min at 4 C. Remove sup but retain a small volume (ca. 10 ul) . This remaining buffer will make it easy to extract pollen coat lipids in the next step.

B. Preparation of pollen coat lipids

11. Add 150 ul chloroform into the tube containing pollen grains and shake vigorously for 1 min at 4 C with a mixer (mixing speed 8; MT-360, TOMY).
12. Centrifuge the tube at 10,000 rpm for 1 min at 4 C.
13. Lay the tube gently so that the upper phase (buffer) and the boundary (pollen grains) form a small droplet. Transfer the lower (solvent) phase into a new microcentrifuge tube.
14. Repeat the steps 11 to 13 twice. The solvent phase is combined into the same tube.
15. Store the solvent phase containing dissolved pollen coat lipids at –80C.

C. Preparation of GC-MS samples and analysis

16. Evaporate the chloroform and dry the pollen coat lipids completely by N_2 gas flow.
17. Dissolve the lipids in 10-30 ul CH_2Cl_2 or hexane. Use 1 ul for GC-MS analysis.
18. For trimethylsilylation, transfer the pollen coat lipids (chloroform solution) into a small glass tube and dry them up completely by N_2 gas flow. Dissolve them in 30 ul hexane, add 200 ul BSTFA (N, O-bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane, Sigma), close the lid, and incubate it at 80 C for 30 min. After chilling to room temperature, dry up the sample by N_2 flow and dissolve it in 30 ul hexane. Use 1 ul for GC-MS analysis.

19. Condition for GC-MS analysis: Non-polar (ex. DB-1, Agilent Technologies) and polar (ex. Ultra ALLOY Capillary Column 65% Diphenyldimethylpolysiloxane, Tokyo Kasei) GC columns are used. Pulse Splitless, injection temperature 275 C, column temperature program (initial temperature 50C for 1 min, raised to 200 C at a rate of 50 C min^{-1}, then raised to 300 C at a rate of 5 C min^{-1}), ionization EI (70 eV), carrier gas He, flow rate 1.5 ml/min.

References

Saito, H., Tsunekawa, S., Kitamura, S., Ojika, H., Nakamura, K., and Ishiguro, S.
GC-MS analysis of pollen coat lipids in *Arabidopsis thaliana*. (in preparation).