Protocol - ***Cryosectioning/ cryo-embedding of symbiotic tissue for MALDI-MSI***

In order to retain as much metabolic and proteomic information as possible without adding chemicals (fixatives etc.) during sample preparation for MALDI-MSI, cryo-fixation and cryo-sectioning were optimized. Most commercial embedding media, such as Tissue-Tek® O.C.T.TM are unsuitable for MALDI imaging, since they produce too much noise in the mass spectrum. Therefore, different, potentially MALDI-suitable embedding media were tested:

- Carboxymethylcellulose (CMC, 2-4% in water) and gelatin (10% in water).
- Peel-A-Way embedding molds (E6032 SIGMA Square S-22) were applied, which are designed for paraffin embedding.
- The in liquid nitrogen shock-frozen pieces of tissue, intended for embedding, were then plunged into the media and frozen until completely hardened.
- After the block turned solid, it was frozen onto a flat disk of cork (1.5 mm) and the disk attached to specimen holders.
- Trimming of the block was done in the mount with a precooled razorblade.

2% Carboxymethylcellulose was selected for further experiments as it provided the best sectioning properties.