Supplementary information

Physicochemical properties, molecular structure, antioxidant activity, and biological function of extracellular melanin from *Ascosphaera apis*

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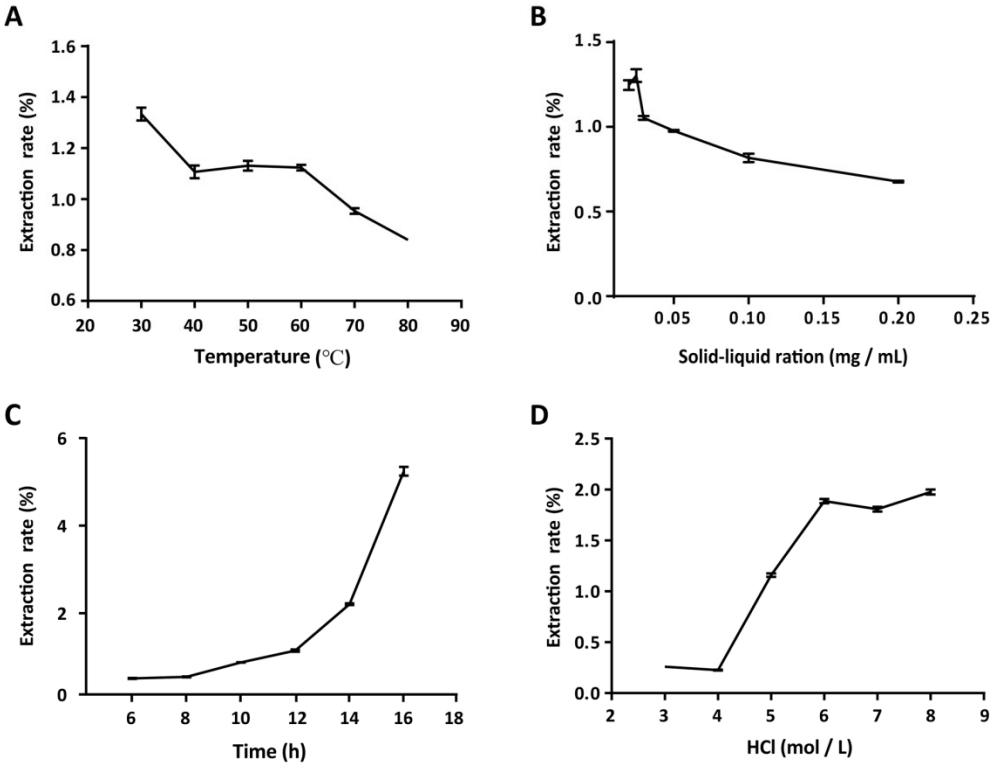

Fig. S1 The extraction rate of *Ascosphaera apis* melanin under different conditions. Optimization of melanin extraction conditions was performed at different hydrolysis temperatures (A) and times (C) of NaOH, the solid-liquid ratio (B), and the HCl concentration (D).

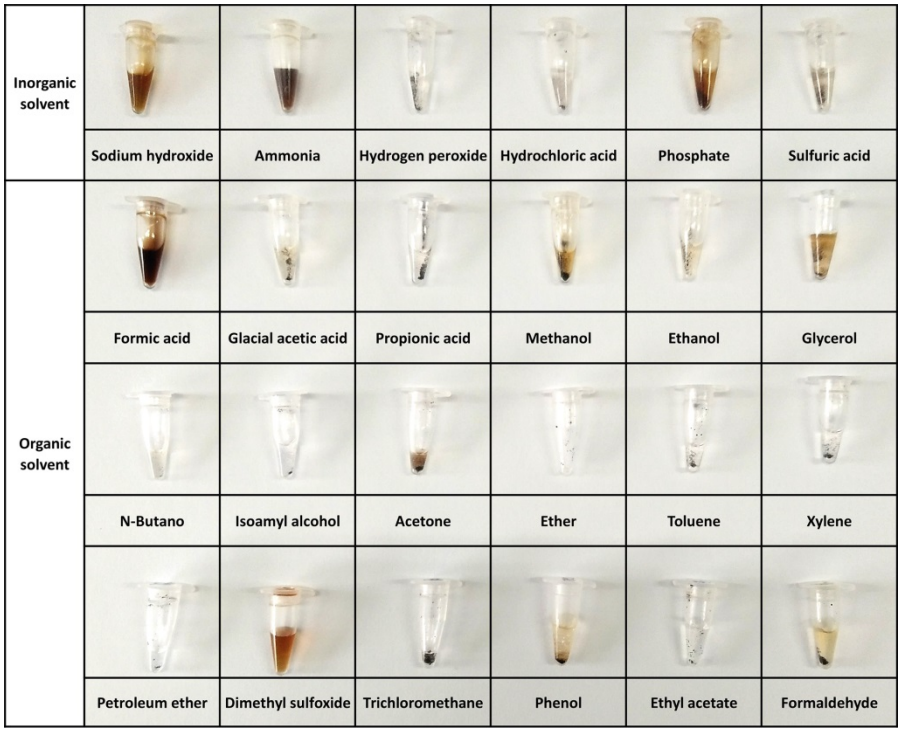

Fig. S2 The solubility of *Ascosphaera apis* melanin in different solvents. Purified *A. apis* melanin was dissolved and vortexed in different organic and inorganic solvents. After being left to stand for three hours, the solubility of melanin was observed at room temperature.

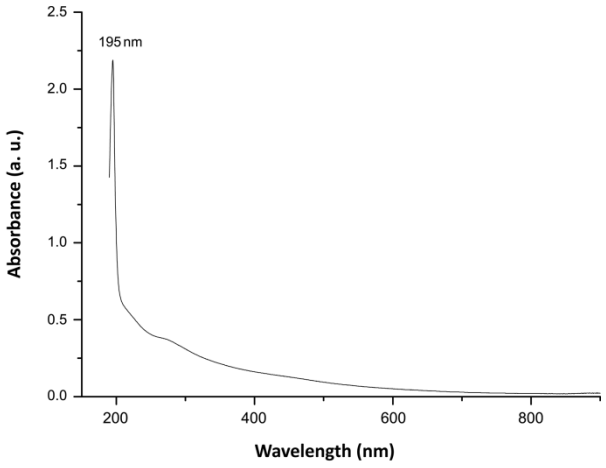

Fig. S3 UV-visible light absorption spectrum of melanin from *Ascosphaera apis*. The peak at 195 nm indicated the maximum absorption peak of *A. apis* melanin.

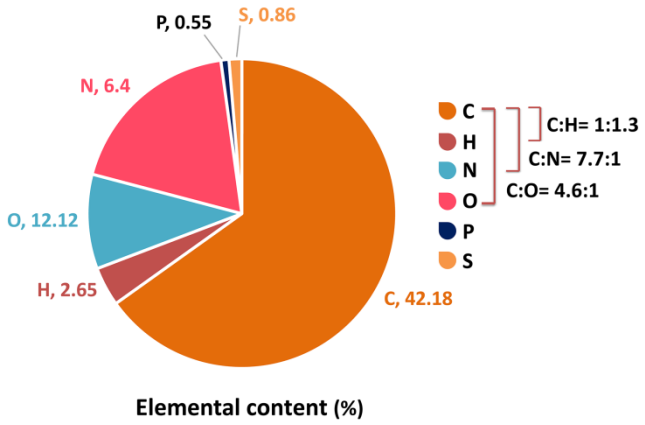

Fig. S4 Elemental composition of melanin from *Ascosphaera apis*.

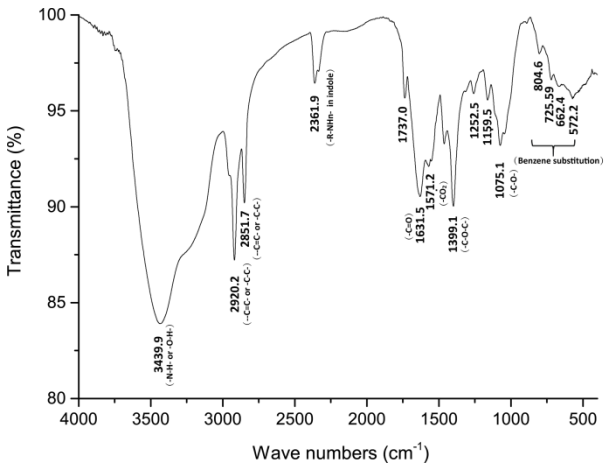

Fig. S5 Infrared spectra of melanin from *Ascosphaera apis*.

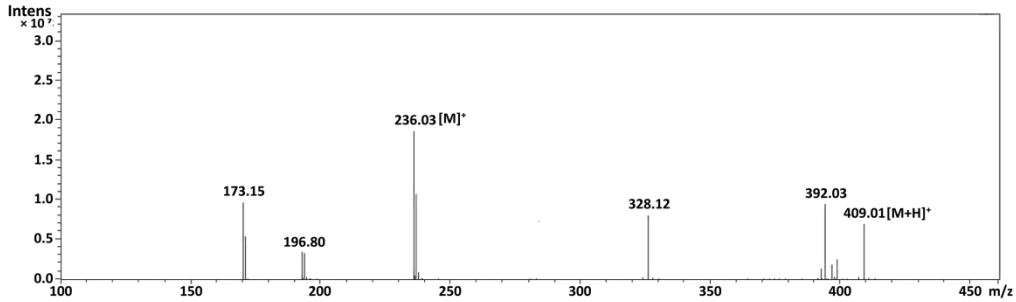

Fig. S6 GC-MS – $[M]^+$ and $[M+H]^+$ spectra of melanin from *Ascosphaera apis*.

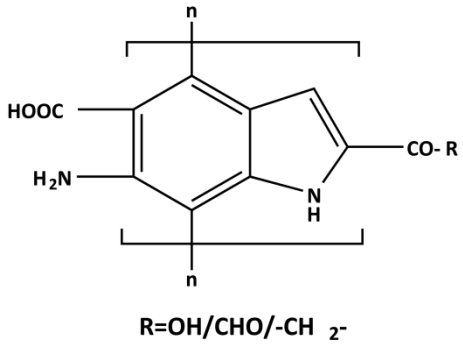

Fig. S7 The proposed structural formula of melanin from *Ascosphaera apis*.

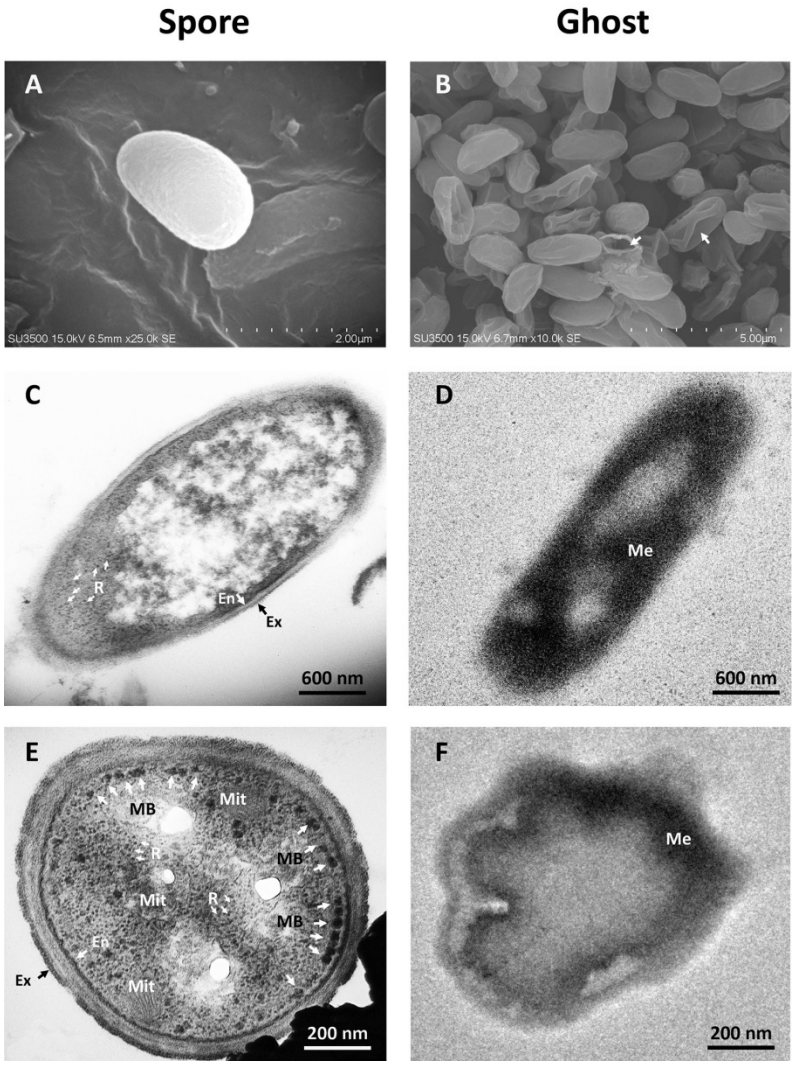

Fig. S8 Electron microscopic analysis of the subcellular localization of melanin from *Ascosphaera apis*. Analysis of the spores of *Ascosphaera apis* by scanning electron microscopy. (A) Normal spore. (B) Melanin ghost; analysis of the spores of *A. apis* by transmission electron microscopy. (C, E) Cross-section and longitudinal sections of spores, respectively. (D, F): Cross-section and longitudinal section of melanin ghost, respectively. Me: melanin; En: endospore of the spore; Ex: exospore of the spore; Mit and R: mitochondrion and ribosome, respectively; MB: microbody.