Appendix (Supplementary information)

Sleep-enhancing effects and mechanisms of *Bacillus coagulans* IDCC 1201: evidence from pentobarbital-induced sleep and electroencephalography analysis

1. High-performance liquid chromatography (HPLC) chromatographic conditions

The column temperature was maintained at 35°C, and acetonitrile and 25 mM acetate buffer (pH 4.8) were used as the mobile phase at a constant flow rate of 1 mL/min. GABA was separated by increasing the eluotropic strength as follows: 0–2 min, 20–25%; 2–32 min, 25–60%; 32–37 min, 60–20%; and 37–40 min, 20% acetonitrile. Finally, GABA was detected at 284 nm using a UV detector (G7115A; Agilent Technologies, Santa Clara, CA, USA), and its concentration was determined based on a calibration curve.

2. Gas chromatography-mass spectrometry (GC-MS) chromatographic conditions and data analysis

Derivatized samples were injected at 50 ℃ in split mode (split ratio 10:1), and metabolites were separated using the following oven program: held at 50 ℃ for 1 min, ramped to 280 ℃ at 15 ℃/min, and held at 280 ℃ for 10 min. Mass spectra were acquired over an m/z range of 50–550, with ion source and interface temperatures set at 250 ℃ and 280 ℃, respectively. Spectral data were processed using the Automated Mass Spectral Deconvolution and Identification System software to perform peak detection and deconvolution. The criterion for peak identification was mass spectral similarity.[1] Then, the processed data were uploaded to SpectConnect for peak alignment across all samples to generate a data matrix, including metabolite identifications, using the Golm Metabolome Database mass spectral reference library (http://gmd.mpimp-golm.mpg.de/).[2]

Notes and references

1. S. E. Stein, An integrated method for spectrum extraction and compound identification from gas chromatography/mass spectrometry data, *J. Am. Soc. Mass Spectrom.*, 1999, 10, 770–781.
2. M. P. Styczynski, J. N. Moxley, J. Cui, I. S. M. Liao and G. Stephanopoulos, Systematic identification of conserved metabolites in GC/MS data for metabolomics and biomarker discovery, *Anal. Chem.*, 2007, 79, 966–973.