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Leveraging 13C-Labeling to Assign Molecular Formulas to Unknown Yeast Metabolites

Background/Objective

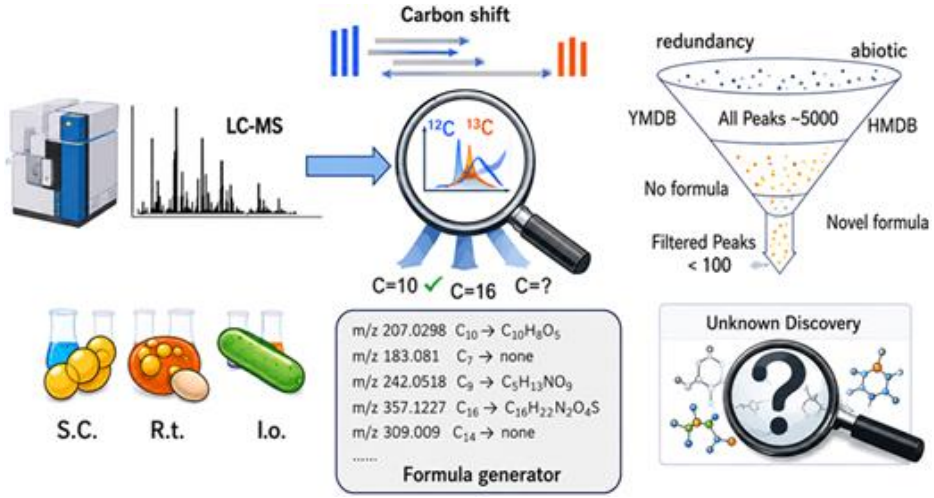
Novel metabolite discovery in non-model yeasts is essential for uncovering previously uncharacterized metabolic pathways and enabling new strategies for fuel/chemical production and metabolic engineering. A major bottleneck lies in distinguishing genuine unknown metabolites from thousands of LC–MS features, which are often dominated by redundancies and artifacts. This work leverages ^{13}C-isotopic labeling in yeast to achieve high-confidence formula assignments, integrating AI-driven tools to prioritize novel metabolite candidates for further evaluation.

Approach

We developed a computational pipeline to reduce LC–MS features into a prioritized set of high-confidence unknown metabolites for downstream structural elucidation. It integrates ^{13}C-labeling data to distinguish biologically derived peaks and infers carbon numbers for each feature. These carbon constraints are incorporated into a molecular formula generator with a classification model that evaluates its chemical plausibility. This approach was evaluated in three yeast species.

Results

The pipeline reduced thousands of LC-MS features to ~100 high-confidence unknown metabolite candidates for each yeast species. Notably, most features were species-specific rather than conserved, and non-model yeasts contained a greater number of candidate novel metabolites compared to *S. cerevisiae*, highlighting their enriched and underexplored metabolic diversity.



General strategy for yeast metabolite discovery in non-model yeasts *Saccharomyces cerevisiae* (S.C.), *Rhodotorula toruloides* (R.t.), and *Issatchenkia orientalis* (I.o.).

Significance/Impacts

This work establishes an AI-integrated, generalizable strategy for discovery of unknown metabolites in untargeted LC–MS datasets, underscoring the value of enriched novel metabolites in non-model yeasts as powerful systems for expanding yeast metabolism and bioengineering applications.

Xing et al. 2026. "Leveraging 13C-Labeling to Assign Molecular Formulas to Unknown Yeast Metabolites." *Journal of the American Society for Mass Spectrometry*. DOI: 10.1021/jasms.6c00012.