Nutritionally-induced Differences in the Metabolic Profiles of *Chlamydomonas reinhardtii* and *Saccharomyces cerevisae* Determined by ^{1}H NMR and Cross Model Validation and Permutation Testing

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Introduction

- ^{1}H NMR-based metabolomics has been used successfully to distinguish between plant ecotypes (Ward *et al.*, 2003) and as a functional genomics tool to study metabolic pathways (Hagel *et al.*, 2008).
- The objectives of this research were
  - to establish the effect of nutrition on the metabolic profiles of the alga *Chlamydomonas reinhardtii* and the yeast *Saccharomyces cerevisae* using ^{1}H NMR.
  - to validate statistically the potential biomarkers identified on the basis of ^{1}H NMR spectra.
- The research was designed to form the basis for experiments that can be used to introduce ^{1}H NMR-based metabolomics into undergraduate biochemistry curricula.

Methods

- *Chlamydomonas reinhardtii* (wild type strain CC-125 m+) was grown in continuous culture systems at 30 °C.
- Twenty-four hours after start of heterotrophic growth (dark; 25 mM acetate) algae were harvested and processed according to the protocol below (*Figure 1*; modified from Ward *et al.*, 2003).
- *Saccharomyces cerevisae* (wild type strain) was grown in stirred flasks at 25 °C aerobically for 60 hours, followed by a 24-h anaerobic growth period.
- Yeast cells were harvested according to the protocol below (*Figure 1*; Villas-Bôas *et al.*, 2005).
- ^{1}H NMR spectra were processed unsupervised and reduced to integrated regions of equal width (0.005 ppm; Viant, 2003).
- Integrated ^{1}H NMR spectra were analyzed using PLSDA. Metabolic differences were statistically validated and potential biomarkers were identified by means of the cross validation, rank products (Breitling *et al.*, 2004), and permutation testing procedures (*Figure 2*) proposed by Westerhuis *et al.*(2008).
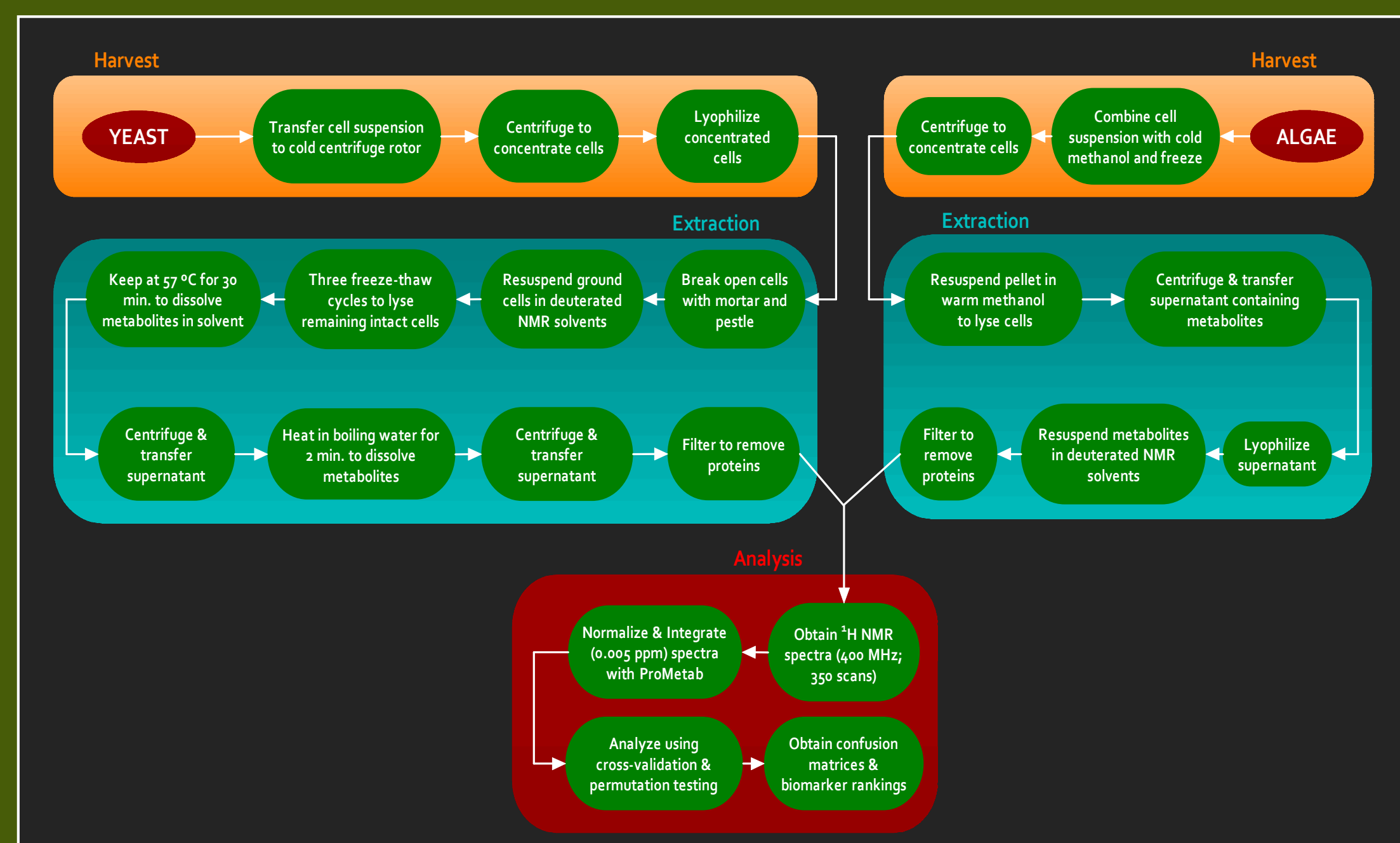

Figure 1. Harvest, extraction, and analysis protocols for *Chlamydomonas reinhardtii* (algae) and *Saccharomyces cerevisae* (yeast).
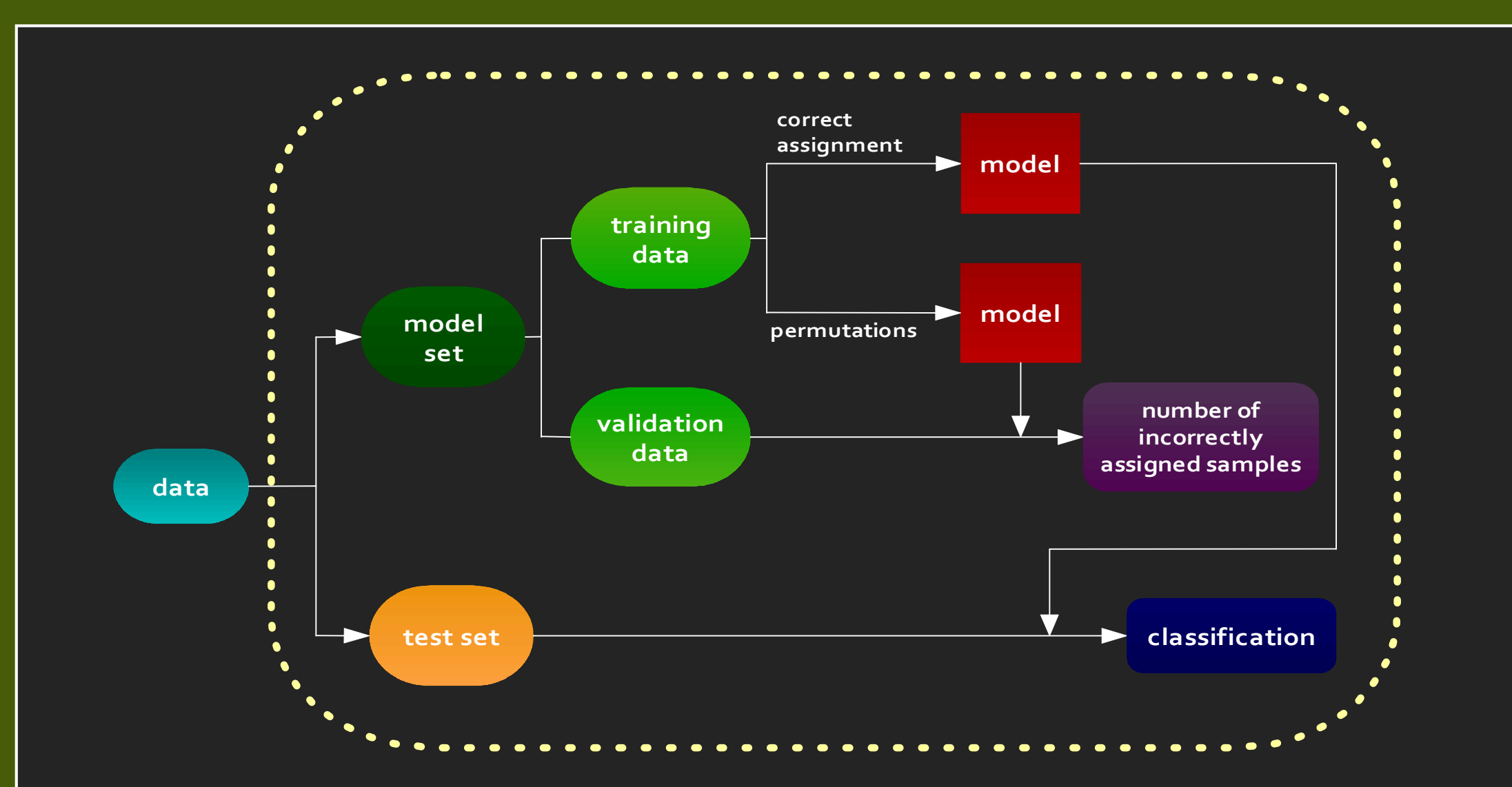

Figure 2. Partial Least Squares Discriminant Analysis using cross validation and permutation testing according to Westerhuis *et al.* (2008).

References

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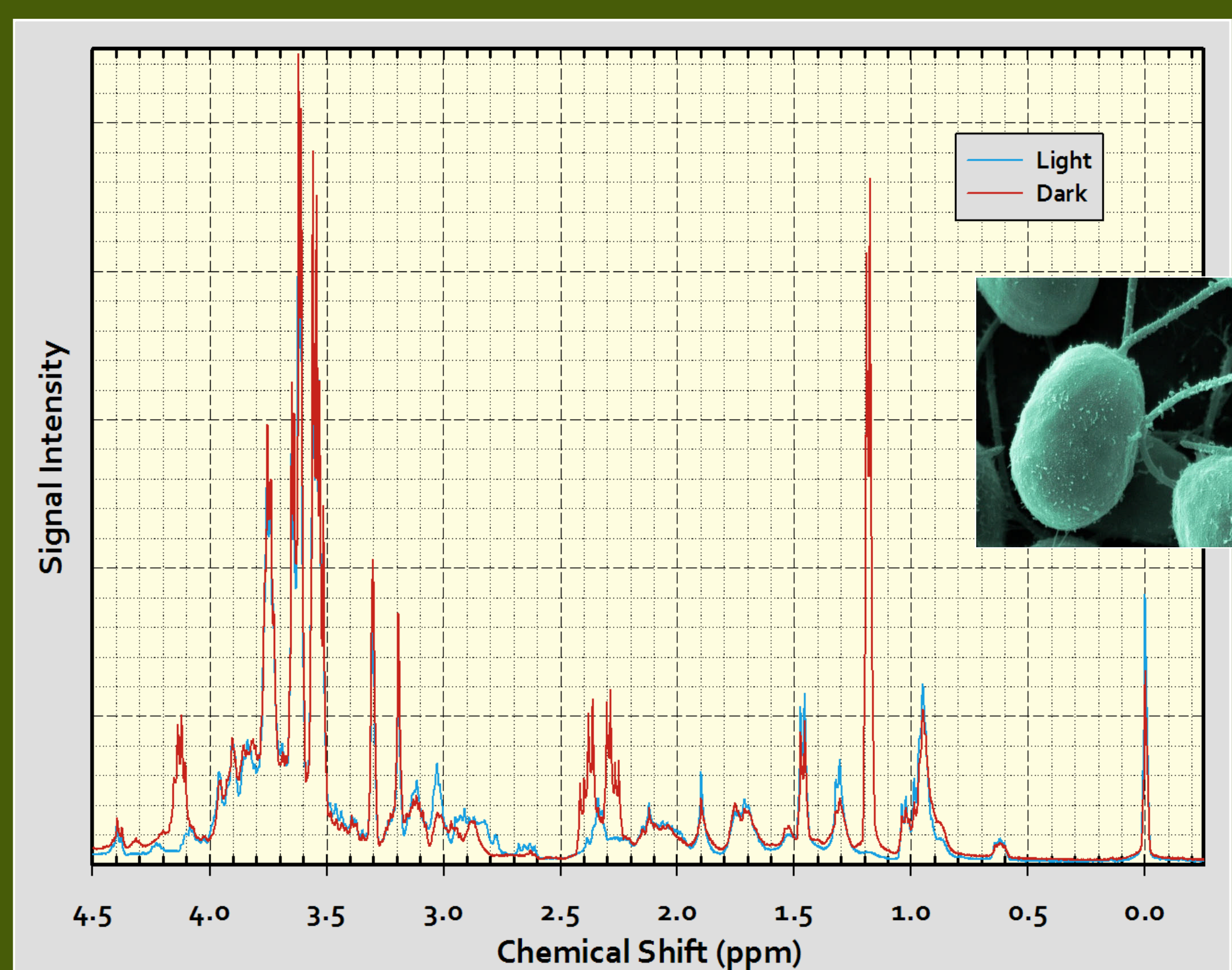
Results

Figure 3. ^{1}H NMR spectrum of polar metabolites from *Chlamydomonas reinhardtii* grown in the light with CO_2 or in the dark with 25 mM acetate for 24 hours.
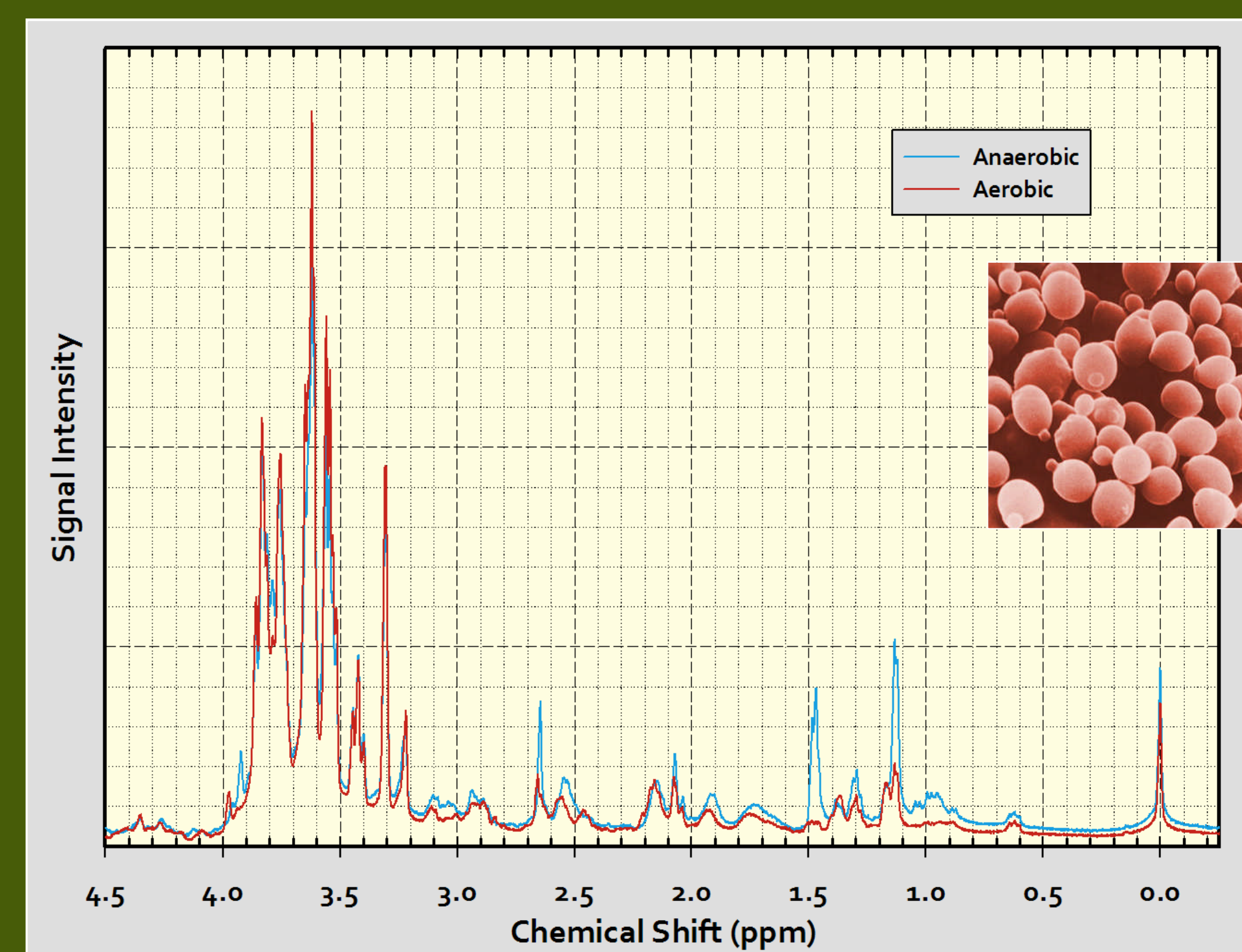

Figure 4. ^{1}H NMR spectrum of polar metabolites from *Saccharomyces cerevisae* grown aerobically or anaerobically for 24 hours.

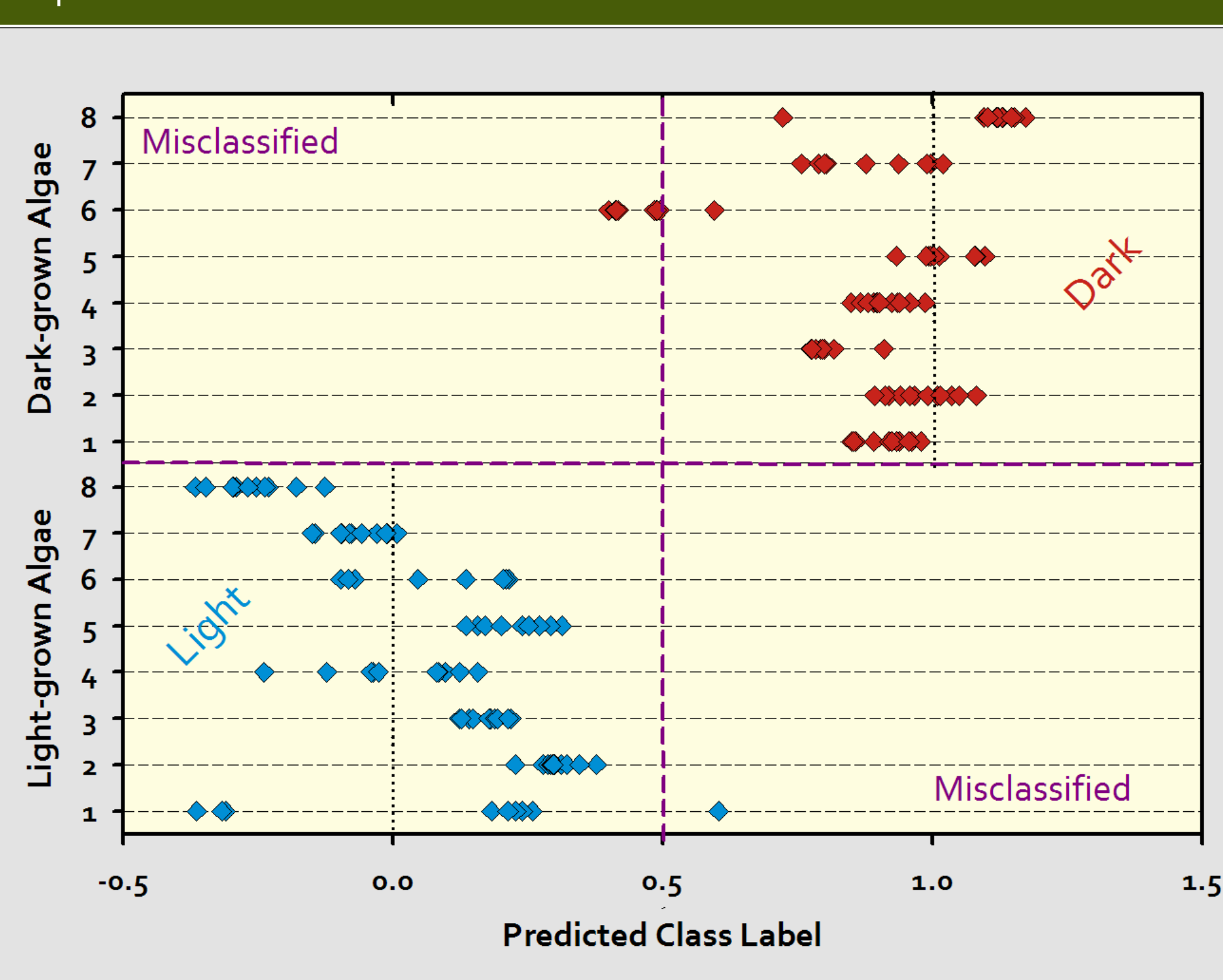*Figure 5.* Confusion matrix of predicted *y*-values for *Chlamydomonas* cultured under different growing conditions (light + CO_2 and dark + 25 mM acetate) using double cross validation. Replicate cultures 1-8 were coded 0 and replicate cultures 9-16 were coded 1.
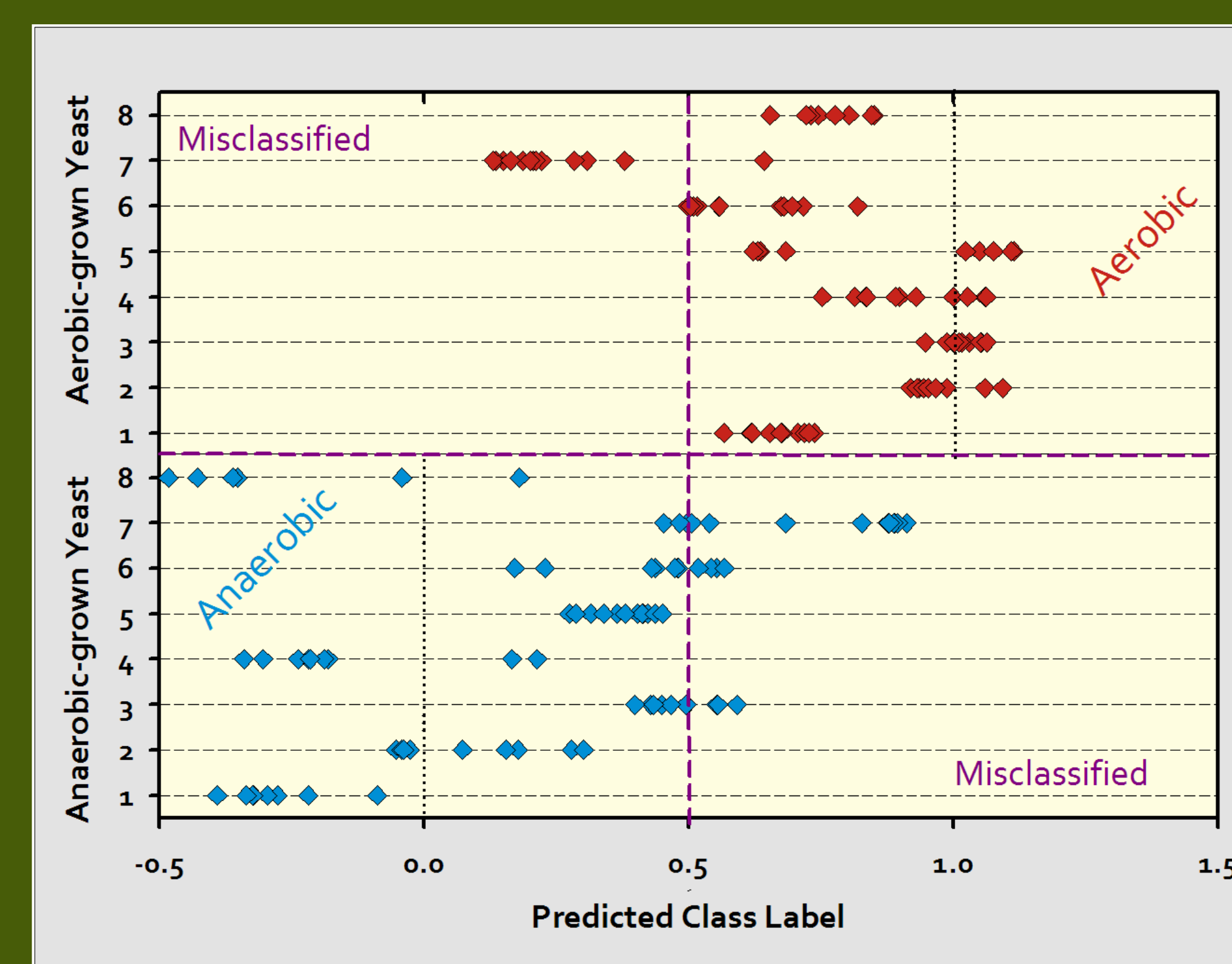

Figure 6. Confusion matrix of predicted *y*-values for *Saccharomyces* cultured under aerobic or anaerobic growing conditions using double cross validation. Replicate cultures 1-8 were coded 0 and replicate cultures 9-16 were coded 1.
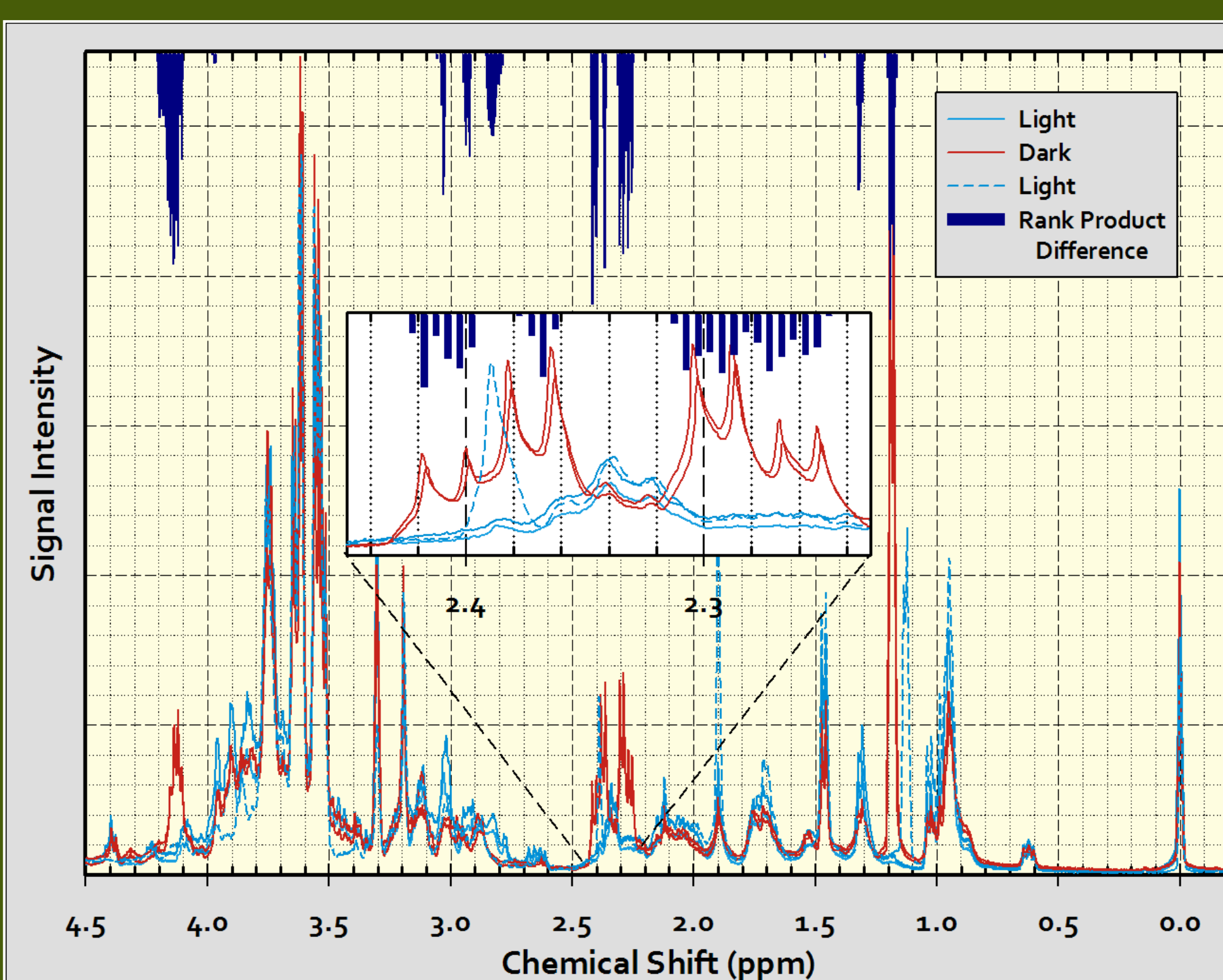

Figure 7. Rank product identified biomarker regions in ^{1}H NMR spectra of polar metabolites of *Chlamydomonas*. The bars (log scale) at the top of the graph represent the rank product differences between the mean of the rank cross validations and the 10% quantile of 800 permutations.

Figure 8. Rank product identified biomarker regions in ^{1}H NMR spectra of polar metabolites of *Saccharomyces*. The bars (log scale) at the top of the graph represent the rank product differences between the mean of the rank cross validation and the 10% quantile of 800 permutations.
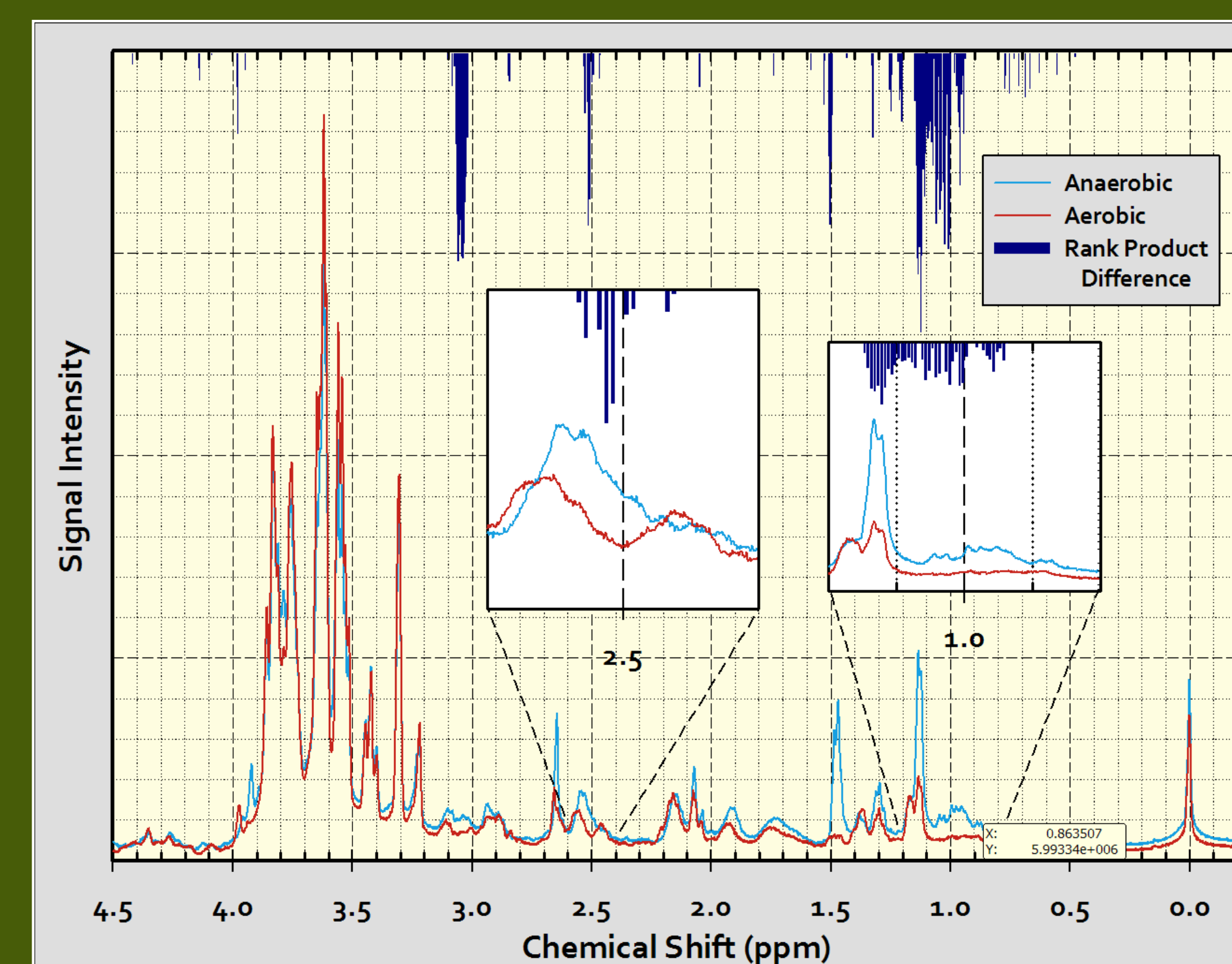
Summary

- Sample reproducibility was enhanced significantly by including a 3000 MW cut-off filtration step in the extraction procedures.
- The cross model validation and permutation testing procedures proposed by Westerhuis *et al.* (2008) validated the nutrition-based classification models in both *Chlamydomonas* and *Saccharomyces.*
- Based on the output from the cross validation and permutation testing, the rank product procedure (Breitling *et al.* 2004) identified integrated regions in the ^{1}H-NMR spectra of both *Chlamydomonas* and *Saccharomyces* that contributed significantly to the nutrition-based classification models for each species.

- The nutrition-induced biomarker regions in the ^{1}H NMR spectra of the polar metabolites of *Chlamydomonas* occurred at the chemical shifts indicated in Figure 7 (aliphatic region), and in addition at 8.2, 7.4, 7.3-7.1, 6.9 ppm (-NH region and aromatic regions).
- The nutrition-induced biomarker regions in the ^{1}H NMR spectra of the polar metabolites of *Saccharomyces* occurred at the chemical shifts indicated in Figure 8 (aliphatic region), and in addition at 8.6, 8.4, 7.6, 7.4, 7.1, 6.9 ppm (-NH region and aromatic regions).
- The chemical identities of the metabolites contributing to the significant biomarker regions are being determined by 2-D NMR.

Footnotes

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