**Pathway Databases**
- KEGG
- MetaCyc

**S. stipitis Genome**

**Draft Reconstruction**
- Obtain genome annotation
- Identify candidate metabolic genes
- Obtain candidate metabolic reactions
- Assemble draft metabolic reaction list

**Resources and Tools**
- The Comprehensive enzyme information system – BRENDA
- PASUB- Protein Sub-cellular Localization prediction Algorithm
- Mitoprot - Mitochondrial targeting sequence predictor
- Web GCM – Prediction of reaction directionality

**Completed Metabolic Reconstructions**
- BIGG Database
- BioMet Toolbox – Models
- Publications

**Refinement of Reconstruction**
- Curation of candidate metabolic reactions
- Substrate and cofactor specificity
- Reaction stoichiometry and directionality
- Gene-protein reaction association
- Reaction localization
- Assembly into pathways/sub-systems

**Refinement of the metabolic model**
- Identify and add new pathways/reactions
- Modifying existing information
- Add additional constraints

**Biomass Macromolecular Composition**
- Cell Wall
- Chitin
- Mannan
- Glucan
- Proteins
- Amino acids
- Fatty acid Composition
- Lipids
- Mono, Di and Tri-Acyl Glycerol
- Sterols
- Phospholipids
- Intracellular compounds
- Carbohydrates
- Glycogen
- Trehalose
- Nucleic acids
- DNA
- RNA

**Maintenance Coefficients**

**Creation of metabolic model**
- Load reconstruction into Matlab
- Define objective function
- Define minimal medium conditions

**Network Evaluation/In-Silico Analysis**
- Identify and fill gaps in metabolic pathways
- Identify dead-ends reactions
- Add missing exchange reactions
- Test for production of biomass precursors and secretion products
- Test for growth under minimal media conditions
- Test for growth on other media sources
- Compute single gene deletion phenotypes

**Validation of metabolic model**
- Compare in silico predictions with experimental growth and product formation
- Compare in silico predictions with phenotype microarray data
- Verify gene and reaction essentiality information

**Computational Tools**
- MATLAB
- COBRA Toolbox

**Publications and Review Articles**

**Cobalamin**

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- Compare in silico predictions with experimental growth and product formation
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**Metabolic model - Predictions**
- Metabolic bottlenecks for growth under anaerobic conditions
- Target genes/reactions for Metabolic Engineering
- Microbial physiology under different growth conditions

**Phenotype microarray data**