the entrance into S phase (P_S). These values are nutritionally modulated: higher growth rates and larger P_S are observed in rich media. The aim of this line of work is first to generate a coarse-grain scaffold model of growth and cycle (GCM) and then to utilize new experimental findings and the previously described network analysis to achieve a finer-grain modeling of specific modules of GCM. Iteratively improved hybrid models for yeast growth and cycle would thus be achievable.

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[P-S.37]
Equation for predicting shelf life of an apple
Ajit BHosale1,*, K.K. Sundaram2
1 Cummins College of Engineering for Women, Pune, India
2 Vishvakarma Institute of Technology, India
Keywords: Shelf Life; Firmness; Respiration rate

The shelf-life of a Fruit is critical in determining both its quality and profitability. Everyone benefits from healthy produce. Approximately Rs. 105 Cr (Rs200million) is lost each year due to waste caused by post harvest diseases, poor temperature management, bruising and other factors. This paper focuses in general on broadly defining a philosophy for predicting life of plant food and in particular attempt to predict the shelf life of apples. Earlier attempts are made to predict shelf life in terms of firmness or atmospheric conditions. Quality of apple can be evaluated against colour or sugar contents etc.

Presented in this paper is a mathematical relationship predicting the shelf life of an apple. An apple is not dead at the time of harvest. It remains a living, respiring organism and continues to exchange of gases through air circulation serve to slow those natural events as much as possible. Our study quantitatively takes in to account parameters such as firmness, colour of apple, atmospheric temperature, and respiration rate. This fundamental equation could be potentially used by farmers, food mals, and big food chains. This relationship can be extended to predict shelf life of perishable fruits.

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[P-S.38]
Effective synthesis of methyl (R)-mandelate by asymmetric reduction with a thermophilic NADH-dependent alcohol dehydrogenase
A. Pennacchio 1,*, A. Giordano 2, M. Rossi 1, C.A. Raia 1
1 Istituto di Biochimica delle Proteine, CNR, I-80131 Naples, Italy, Italy
2 Istituto di Chimica Biomolecolare, CNR, I-80078 Pozzuoli, Naples, Italy, Italy
Keywords: Asymmetric biocatalysis; Coenzyme regeneration; Dehydrogenases/reductases; Chiral alfa-hydroxy ester

Dehydrogenases/reductases (ADHs) are nowadays among the most requested enzymes used for the preparation of optically active compounds, for the inherent advantages over chemocatalysts in terms of their high chemo-, enantio-, and regioselectivity as well as to be environmentally friendly (Kroutil et al., 2004). An NAD(H)-dependent, highly enantioselective ADH (TtADH), identified in the eubacterium Thermus thermophilus HB27, was purified and characterized in our laboratory (Pennacchio et al., 2008). The thermophilic enzyme catalyses the following reactions at 1–3 mg scale with high yield conversion: the reduction of acetophenone, 2,2,2-trifluoroacetophenone, α-tetralone, and α-methyl benzoyl-formate (MBF) to (S)-1-phenylethanol (>99% enantiomeric excess, ee), (R)-α-(trifluoromethyl)benzyl alcohol (93% ee), (S)-1-tetralol (>99% ee), and methyl (R)-mandelate (R-MM) (91% ee), respectively. Noteworthy, the optically active alcohols produced are used as valuable chiral building blocks in organic synthesis (Kroutil et al., 2004; Pennacchio et al., 2008).

To demonstrate the preparative applicability of TtADH we focused on the chemoenzymatic synthesis of MBF to R-MM. Two in situ NADH-recycling systems based on the enzyme-coupled approach were developed: the first system included a thermophilic NAD(P)-dependent glucose dehydrogenase and glucose, and the second system involved a thermophilic NAD(H)-dependent ADH from Bacillus steathermophilus and an alcohol substrate as hydrogen donor. The effects of various parameters on the asymmetric synthesis such as pH, temperature and reaction time, agitation speed, solubility, enzymes and cofactor stability, substrate and product inhibition, and tolerance to organic solvents were evaluated. Both recycling systems proved to be highly efficient and enantioselective in producing R-MM with 95–93% ee, 98–99% conversion and 74–76% isolated yield when reduction of the keto ester was scaled up to 0.2–0.5 g under established optimal conditions. This work was funded by the ASI project MoMa 1/014/06/0 and by FIRB grant RBNE034XS.

References


[P-S.39]
Key Enzymes for the Optimization of CO2 Uptake and Nitrogen Consumption in the C3 Photosynthetic Carbon Metabolism
A. Papini 1,*, G. Nicosia 2, G. Stracquadanio 2, P. Liò 4, R. Umeton 3
1 Università di Firenze, Italy
2 University of Catania, Italy
3 Massachusetts Institute of Technology, United States
4 University of Cambridge, United Kingdom
Keywords: Photosynthesis; CO2 uptake vs. Nitrogen; Pareto Optimality

In this research work have been identified the key enzymes to target in order to maximize CO2 uptake rate and minimize the protein-nitrogen consumption. The aim is to re-arrange resource allocation enzymes-wise in order to obtain a robust trade-off between CO2 uptake and the total amount of protein-nitrogen. The designed methodology, including multi-objective optimization, unravelled that Rubisco, Sedoheptulosebisphosphatase (SBPase), ADP-Glc pyrophosphorylase (ADPGPP) and Fru-1,6-bisphosphate (FBP) aldolase are the most influential enzymes in carbon metabolism model (Fig. 1) where CO2 uptake maximization is concerned (our methodology raises this rate up to 36.149 µmol/m2s; natural leaf value is 15.486 µmol/m2s). Interesting insights include the fact that the Rubisco enzyme participate with a very high concentration; additionally, some of the photosynthetic enzymes that should be almost switched off to reach the best configurations known (Zhu’07) cannot be effectively switched off because they are involved in other processes carried by C3 plants. The pathway
enzymes that lead to sucrose and starch synthesis were shown not to affect CO₂ uptake rate if maintained at their natural concentration levels. The importance of SBPase has been already pointed out by antisense transgenic plants studies (Raines’03). We have modeled the C₃ photosynthetic carbon metabolism in terms of concurrent optimization of two conflicting biological strengths: maximization of CO₂ uptake and contextual minimization of the total protein-nitrogen employed to gain that CO₂ uptake rate. We have inspected the problem at three CO₂ concentration (Ci) in the atmosphere (25 M years ago environment, nowadays one, and the one predicted for the end of the century) and two triose-P (PGA, GAP, and DHAP); low and high export rates (Fig. 2 right plot). In this context, our analysis has detected Pareto-optimal leaves in the six Ci/triose-P conditions studied. Among the others, two promising candidates for leaf re-engineering are further inspected and compared with the natural leaf enzyme configuration (Fig. 2 left plot). For the first time, has been individuated a reasonably small set of key enzymes whose targeted tune gives rise to a maximization of the photosynthetic rate, contextually with an efficient protein-nitrogen employment.

Fig. 1. The ratio of the enzyme concentrations optimized (CO₂ uptake = 36.149 μmol m⁻² s⁻¹) compared to the initial concentrations of the natural leaf (CO₂ uptake = 15.486 μmol m⁻² s⁻¹).

Fig. 2. (right plot) Leaf CO₂ uptake rate vs. protein-nitrogen consumption. Moving beyond the natural operative area (green area), we found leaf configurations that expose a Pareto-optimality in the six conditions considered. Point B represents a leaf with a naturally CO₂ uptake ability but employs 47% of the naturally needed protein-nitrogen. Point A2 needs exactly 50% of the naturally employed protein-nitrogen to gain up to 10% CO₂ uptake capacity when compared to the natural leaf. Fig. 2 (left plot) shows the comparison among “leaf B” (total conc. of Nitrogen equal to 99027 mg l⁻¹) and the natural leaf (Nitrogen 208333 mg l⁻¹).

Keywords:
Recombinant protein; CPOT; Secretory pathway

Transcriptional profiles of Saccharomyces cerevisiae during chemostat cultivation at different temperatures and oxygenation levels

L. Dato¹,∗, M. Dragosits², A. Graf², G. Frascotti¹, P. Branduardi¹, D. Mattanovich²

¹ Department of Biotechnology and Bioscience, University of Milano-Bicocca, Milan, Italy
² Institute of Applied Microbiology, University of Natural Resources and Applied Life Sciences, Vienna, Austria

Saccharomyces cerevisiae is among the most frequently used eukaryotic cell hosts for recombinant protein production. Process optimization has been so far mainly performed case by case, and therefore it is not easy to generalise the outcomes of such studies.

In this study, which was part of the European Genophys project, the impact of environmental parameters on the yeast physiology and on the productivity of a secreted heterologous protein was investigated.

The anti-idiotypic 3H6 Fab antibody fragment, directed against the HIV-1 broadly neutralising antibody 2F5, chosen by the Genophys consortium as a model protein, was expressed under the control of the constitutive triose phosphate isomerase promoter and secreted via the S. cerevisiae alpha mating factor leader sequence.

Two-color based microarrays were applied to chemostat cultures to study the effect of different temperatures (30 °C, 26 °C and 23 °C) and of different aeration conditions (full aeration, low oxygenation and hypoxia) on gene expression of the producing and control strains.

Data analysis evidenced a different regulation of cellular processes and metabolic pathways under the different conditions examined, that will be discussed.

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Insulin production in S. cerevisiae

Z. Liu∗, K. Tyo, D. Petranovic, J. Nielsen

Chalmers University of Technology, Sweden

Keywords: Recombinant protein; CPOT; Secretory pathway

With the rapid increase in diabetes there is an increasing demand for insulin, and improving insulin production has therefore drawn more and more attention. Nowadays, recombinant human insulin is produced in either yeast or Escherichia coli. Through detailed knowledge of the secretion pathway in yeast it has become possible to improve the secretion yield and efficiency through a combination of the different molecular techniques. Here we expressed insulin precursor in the yeast Saccharomyces cerevisiae using both the α factor leader and a synthetic leader. The synthetic genes of insulin were cloned into three different plasmids, a POT plasmid with TEF1 promoter and POT1 gene from Schizosaccharomyces pombe as complement for tpi1 deletion of the host strain, a CPOT plasmid with TPI1 promoter and POT1 gene marker, and a p426GPD plasmid with CDPH promoter and URA3 marker. Titters and other physiological parameters for the six engineered strains were evaluated and discussed in order to gain a deeper insight into the secretory pathway.

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