PQI 5888
Fisiologia e Biotecnologia de Leveduras

Prof. Thiago Basso

29 de abril de 2020

[Aula 5 EaD]

Leveduras – Aplicações Industriais

(Engenharia Metabólica - Uso de CO_2)

Engenharia Metabólica (Exemplo #2)

Guadalupe-Medina *et al. Biotechnology for Biofuels* 2013, **6**:125
http://www.biotechnologyforbiofuels.com/content/6/1/125

Biotechnology for Biofuels

RESEARCH **Open Access**

Carbon dioxide fixation by Calvin-Cycle enzymes improves ethanol yield in yeast

Víctor Guadalupe-Medina[1,2], H Wouter Wisselink[1,2], Marijke AH Luttik[1,2], Erik de Hulster[1,2], Jean-Marc Daran[1,2], Jack T Pronk[1,2] and Antonius JA van Maris[1,2*]

Exemplo #2

Aumentar o rendimento em etanol (Y_{SE})

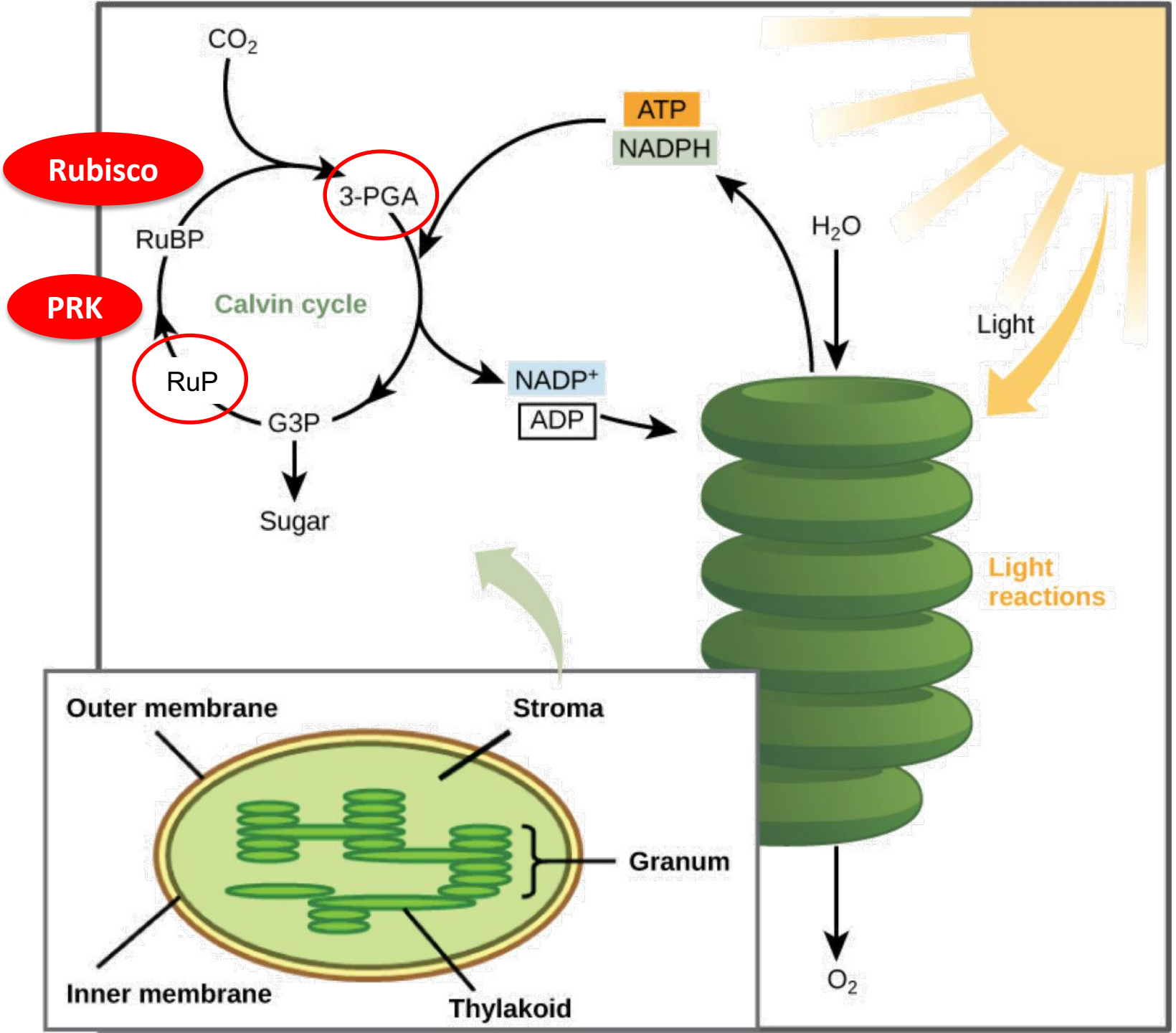
pela captura de CO_2 em leveduras

DSM
BRIGHT SCIENCE. BRIGHTER LIVING.

Can *S. cerevisiae* be engineered to reduce CO_2 to ethanol?

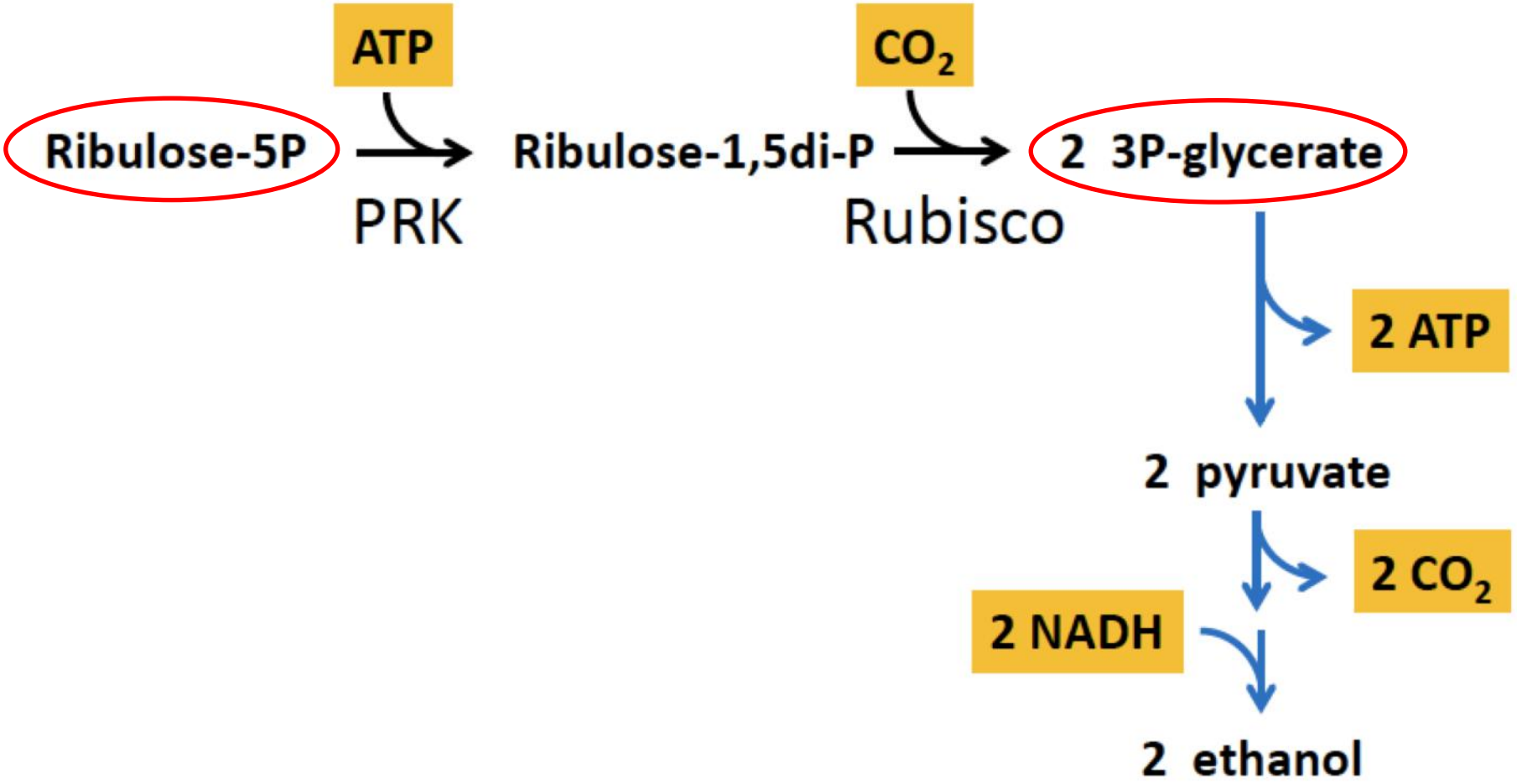
Ethanol production from CO_2 as (alternative) redox sink

Victor Guadalupe *et al.* (2013)
Biotechnology for Biofuels - accepted

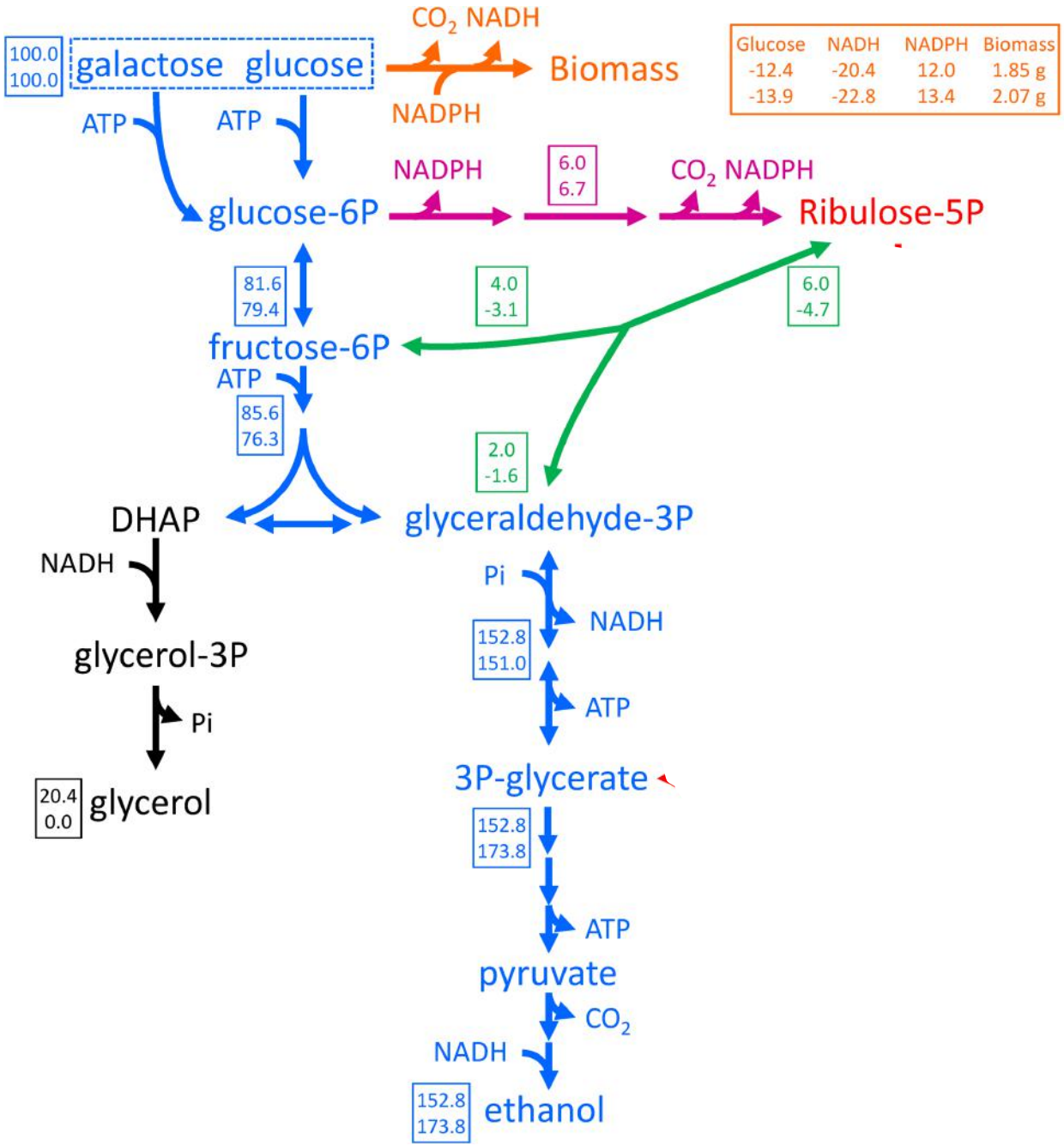

CO_2
Rubisco
3-PGA
RuBP
PRK
Calvin cycle
RuP
G3P
Sugar
ATP
NADPH
NADP+
ADP
H_2O
Light
Light reactions
O_2
Outer membrane
Stroma
Granum
Inner membrane
Thylakoid

Phosphoribulokinase (PRK) and ribulose-1,5-bisphosphate carboxylase (Rubisco)

key enzymes in the Calvin cycle for autotrophic CO_2 fixation



Victor Guadalupe *et al.* (2013)
Biotechnology for Biofuels - accepted

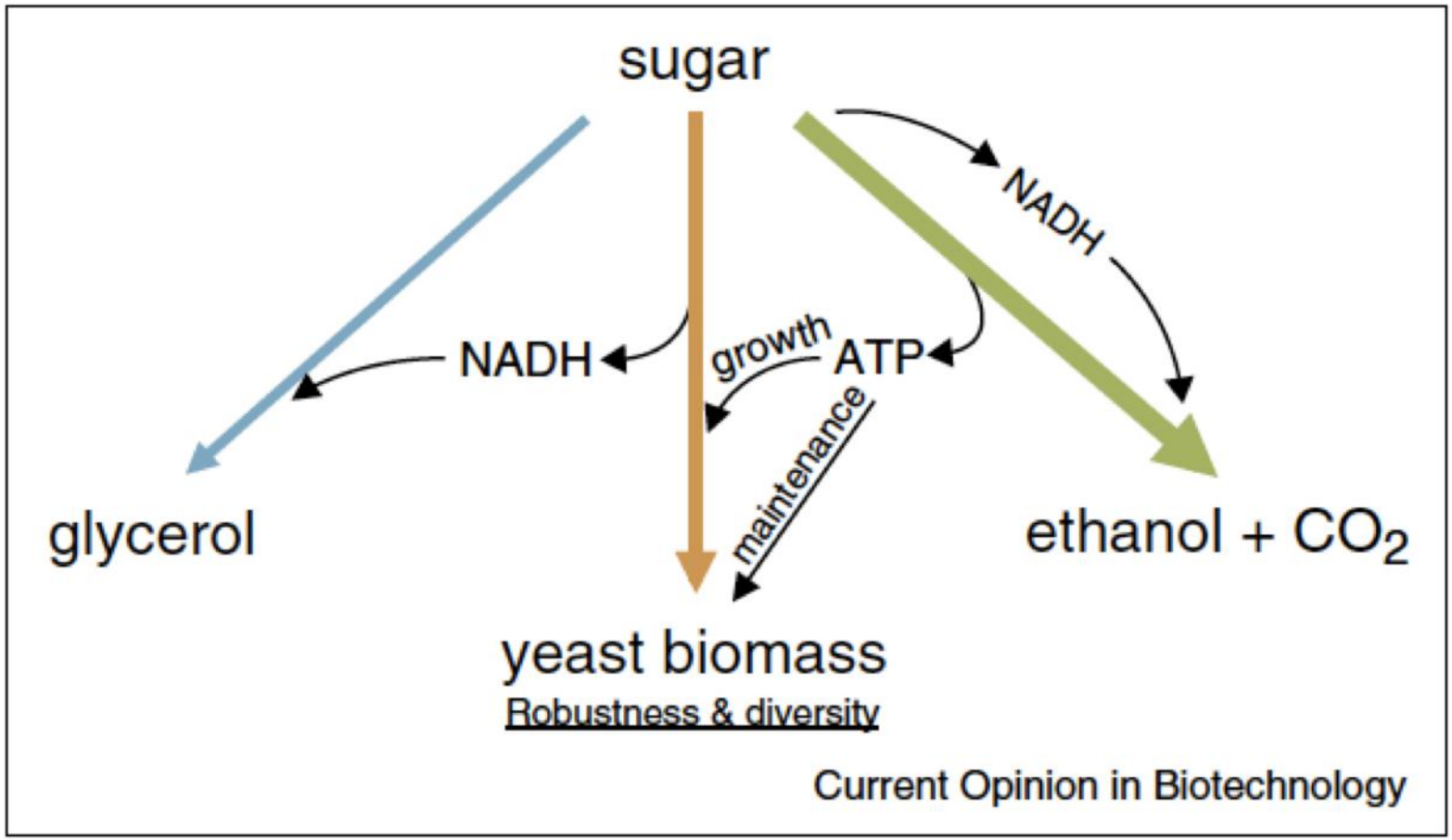

100.0
100.0
galactose glucose
CO2 NADH
Biomass
NADPH
ATP
ATP
NADPH
6.0
6.7
CO2 NADPH
Ribulose-5P
glucose-6P
81.6
79.4
4.0
-3.1
6.0
-4.7
fructose-6P
ATP
85.6
76.3
2.0
-1.6
DHAP
glyceraldehyde-3P
NADH
Pi
NADH
152.8
151.0
ATP
glycerol-3P
3P-glycerate
Pi
20.4
0.0
glycerol
152.8
173.8
ATP
pyruvate
CO2
NADH
152.8
173.8
ethanol
Glucose NADH NADPH Biomass
-12.4 -20.4 12.0 1.85 g
-13.9 -22.8 13.4 2.07 g

Qual deve ser a função do glicerol?

Tabela 1.1 — Proporção dos diversos produtos da fermentação alcoólica, em g/100g de glicose metabolizada, de acordo com várias fontes e para diferentes eficiências fermentativas

Produto da fermentação	Pasteur 95%	Jackman, 1987 90—95%	Basso et al. 1996 85—92 %
Etanol	48,5	45,0—49,0	43,0—47,0
Gás carbônico	46,4	43,0—47,0	41,0—45,0
Glicerol	3,3	2,0—5,0	3,0—6,0
Ácido succínico	0,6	0,5—1,5	0,3—1,2
Ácido acético	—	0,0—1,4	0,1—0,7
Óleo fúsel	—	0,2—0,6	—
Butilenoglicol	—	0,2—0,6	—
Biomassa (massa seca)	1,2	0,7—1,7	1,0—2,0

Figure 1



Schematic representation of the distribution of sugar for ethanol production, formation of yeast biomass, and formation of glycerol as a by-product. To achieve a high ethanol yield on sugar, the robustness of the process and yeast strains are essential.

Rubisco and PRK-expressing *S. cerevisiae*

Product yields in anaerobic, sugar-limited chemostat cultures (D = 0.05 h^{-1}, N_2-sparged, equimolar glucose/galactose feed)

Relevant genotype	reference	*cbbM, PRK, groEL/ES*
Biomass yield on sugar ($g\ g^{-1}$)	0.083 ± 0.000	0.093 ± 0.000
Glycerol yield on sugar ($mol\ mol^{-1}$)	**0.14 ± 0.00**	**0.04 ± 0.00**
Ethanol yield on sugar ($mol\ mol^{-1}$) *Corrected for evaporation*	1.56 ± 0.03	1.73 ± 0.01

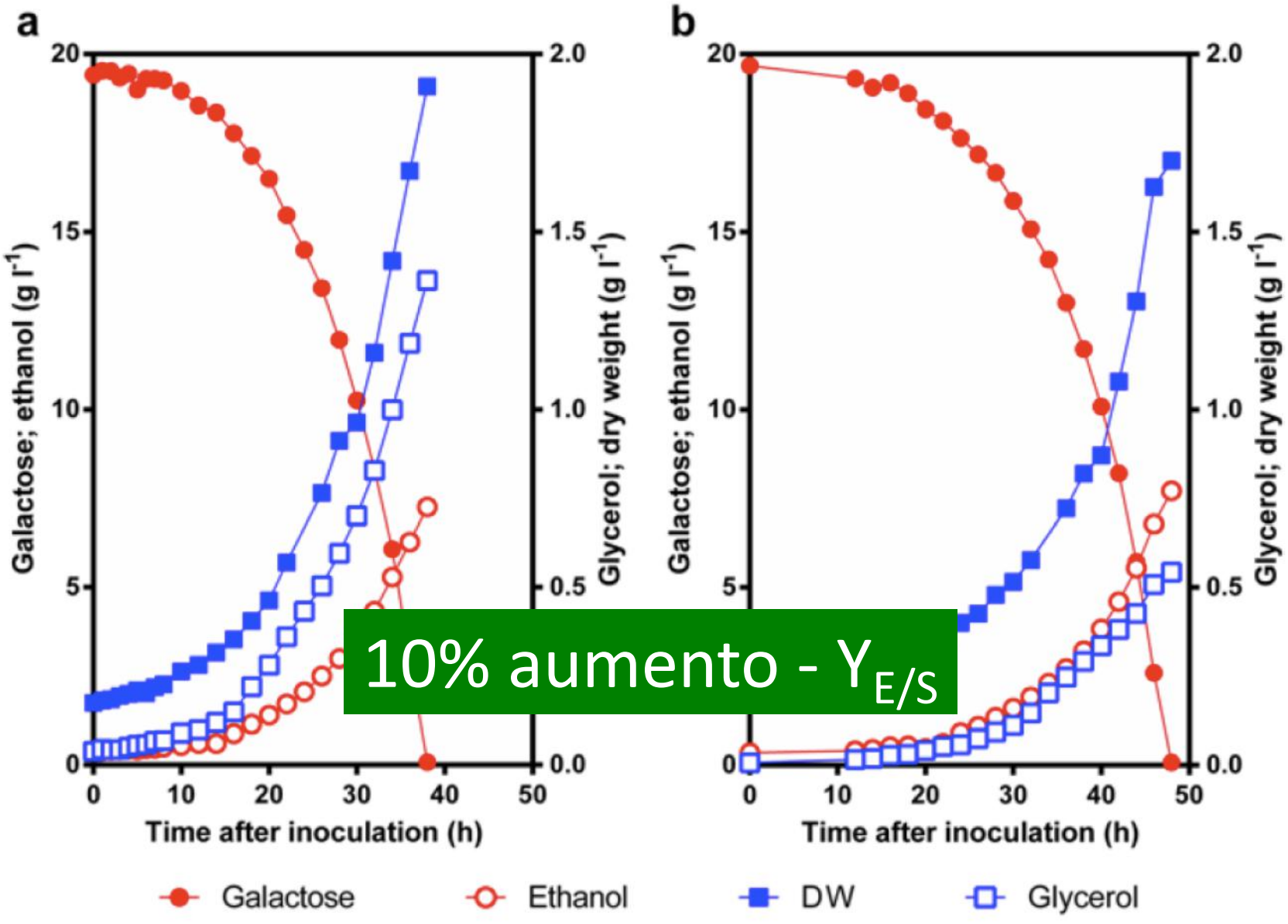
70 % reduction of glycerol production
Rubisco/PFK competes with native glycerol pathway

11% aumento - $Y_{E/S}$

Victor Guadalupe *et al.* (2013) Biotechnology for Biofuels - accepted

Levedura selvagem
(sem Rubisco)

Levedura modificada
(com Rubisco)



10% aumento - $Y_{E/S}$

Engenharia Metabólica (Exemplo #3)

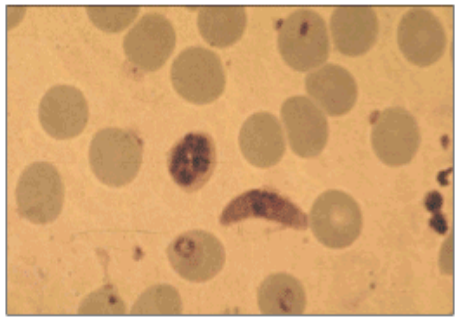
nature Vol 440|13 April 2006|doi:10.1038/nature04640

LETTERS

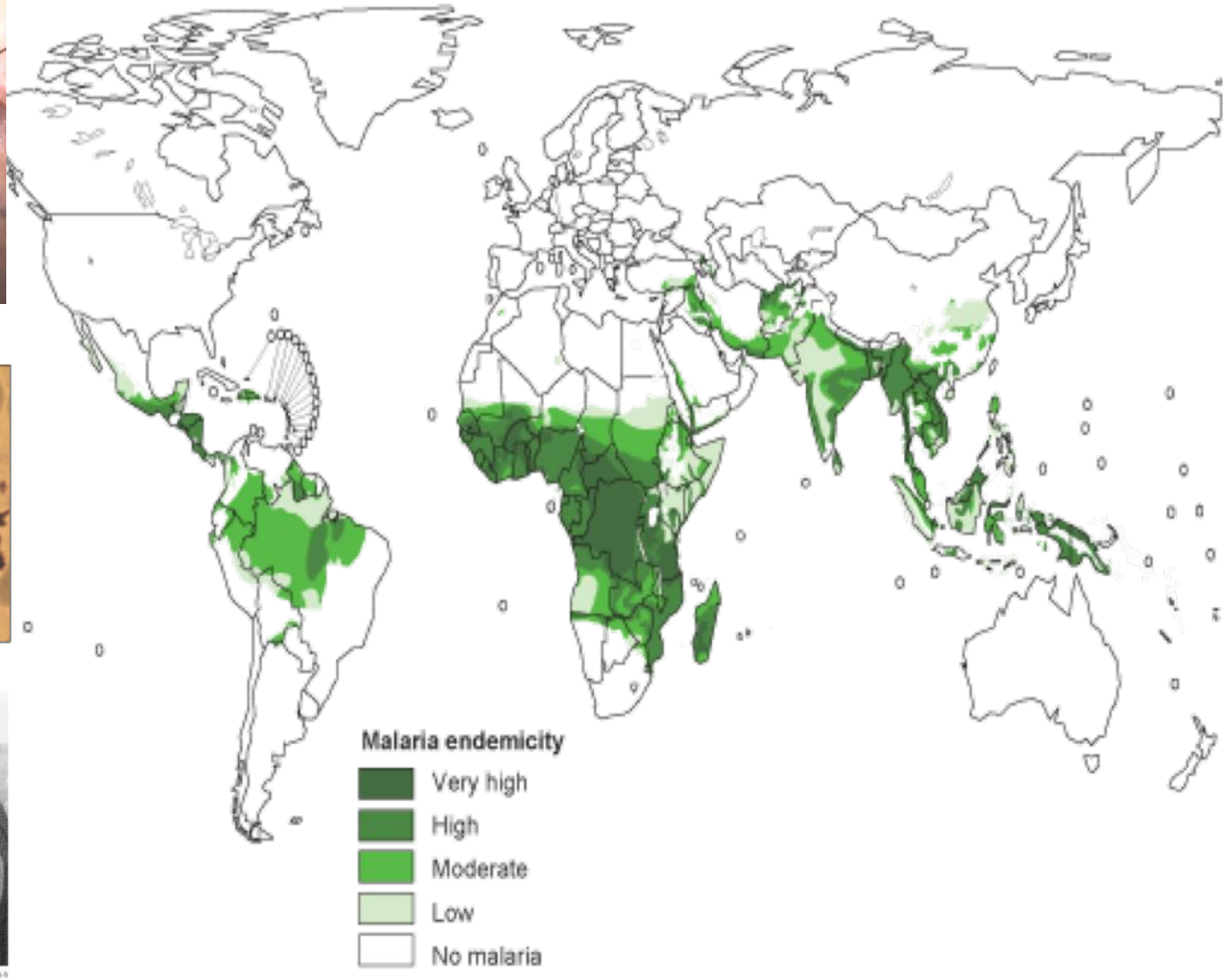
Production of the antimalarial drug precursor artemisinic acid in engineered yeast

Dae-Kyun Ro[1]*, Eric M. Paradise[2]*, Mario Ouellet[1], Karl J. Fisher[6], Karyn L. Newman[1], John M. Ndungu[3], Kimberly A. Ho[1], Rachel A. Eachus[1], Timothy S. Ham[4], James Kirby[2], Michelle C. Y. Chang[1], Sydnor T. Withers[2], Yoichiro Shiba[2], Richmond Sarpong[3] & Jay D. Keasling[1,2,4,5]

Malaria

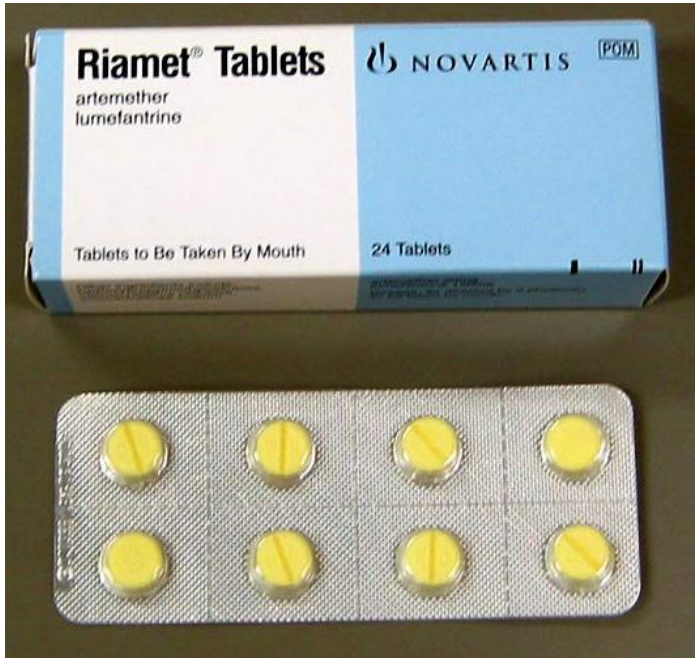


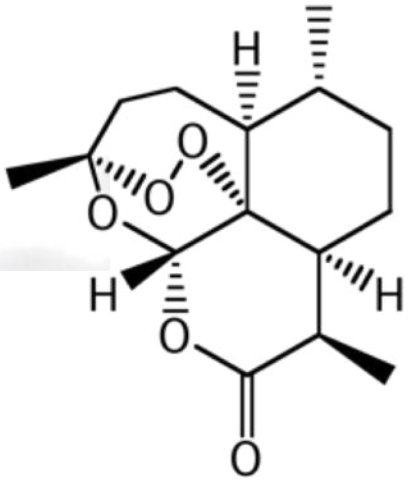
In Africa, where children are the main victims of malaria, delivery of antimalarial drugs through routine vaccination programmes is a promising new approach.



Artemisinin

the best anti-malarial drug

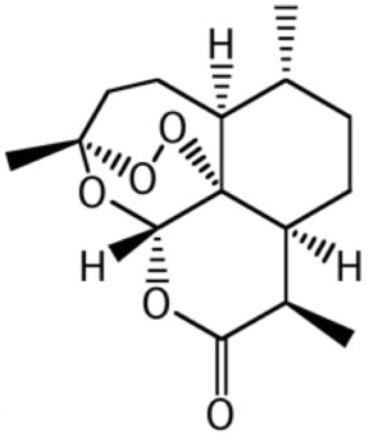




Current process

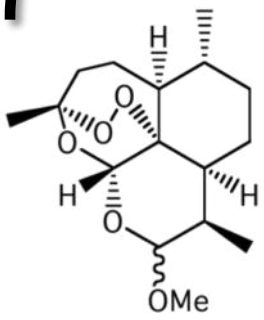
Plant synthesis

Purification

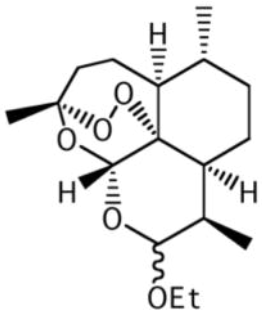


Artemisinin

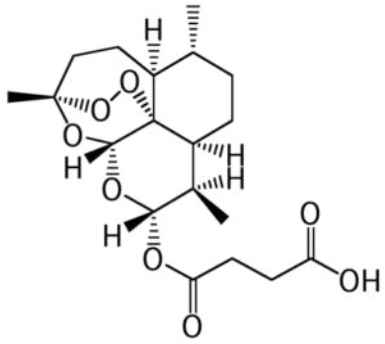
Chemical Conversions



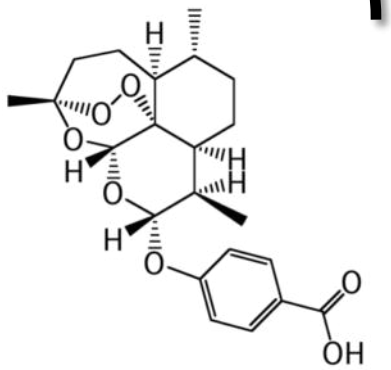
Artemether



Arteether

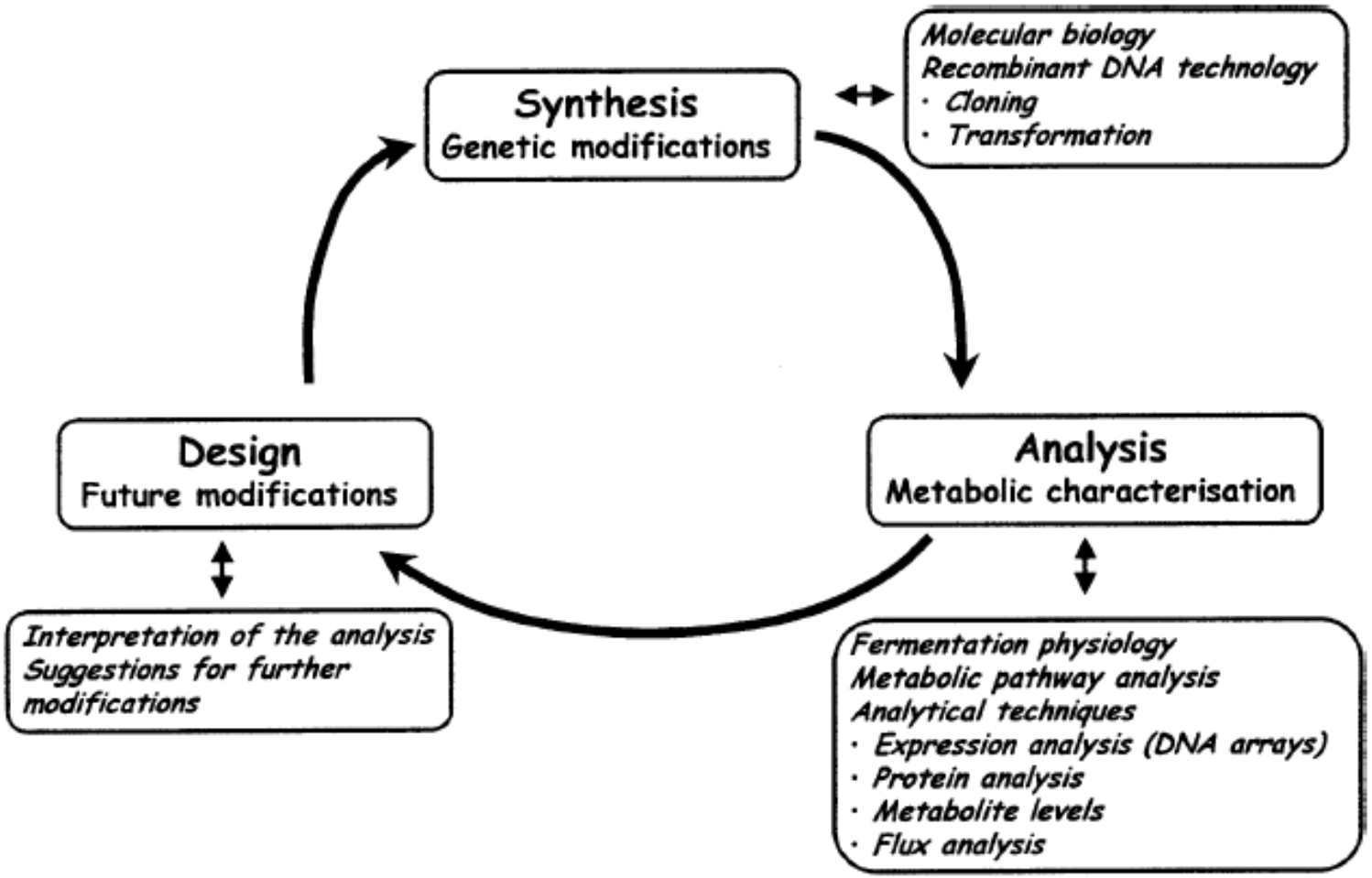


Artesunate



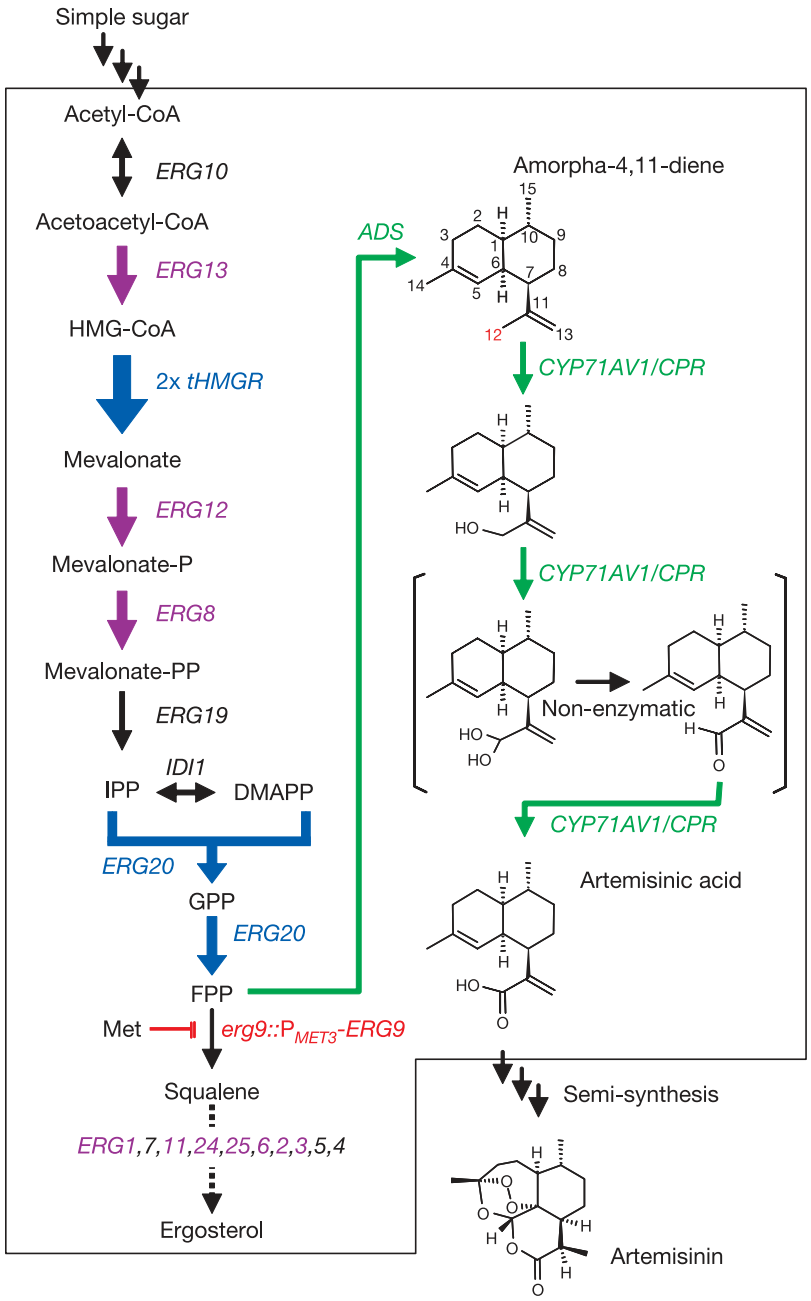
Artelinate

O Ciclo da Engenharia Metabólica

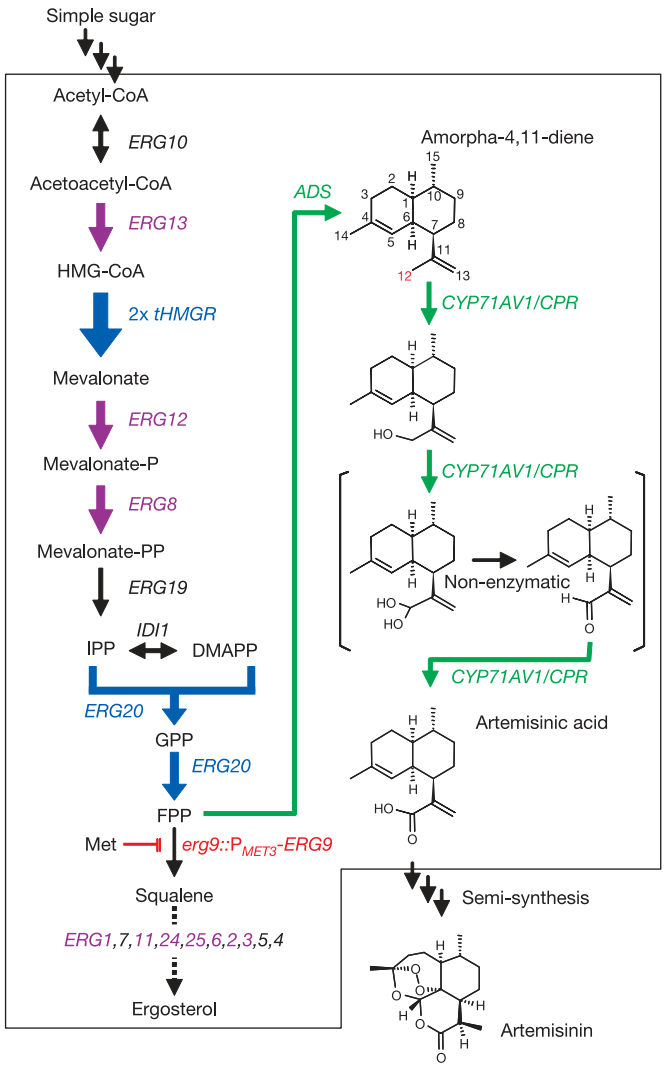


Jens Nielsen, 2001. Appl Microbiol Biotechnol (2001) 55:263–283

Projeto (design)



Projeto, Síntese, e Análise



Linhagem	Modificação	[amorphadiene]
EPY201	ADS	5
EPY208	ERG13, 12, 8 (up)	25
EPY225	ERG9 down	50
EPY213	UPC2 (transcrição)	100
EPY224	tHMG, ERG20 (up)	150

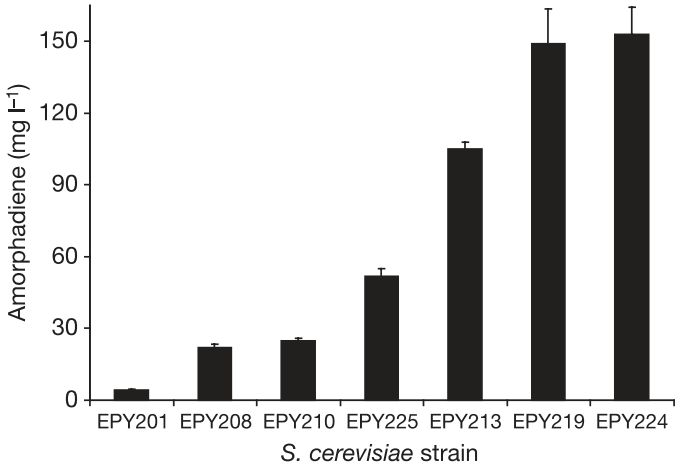
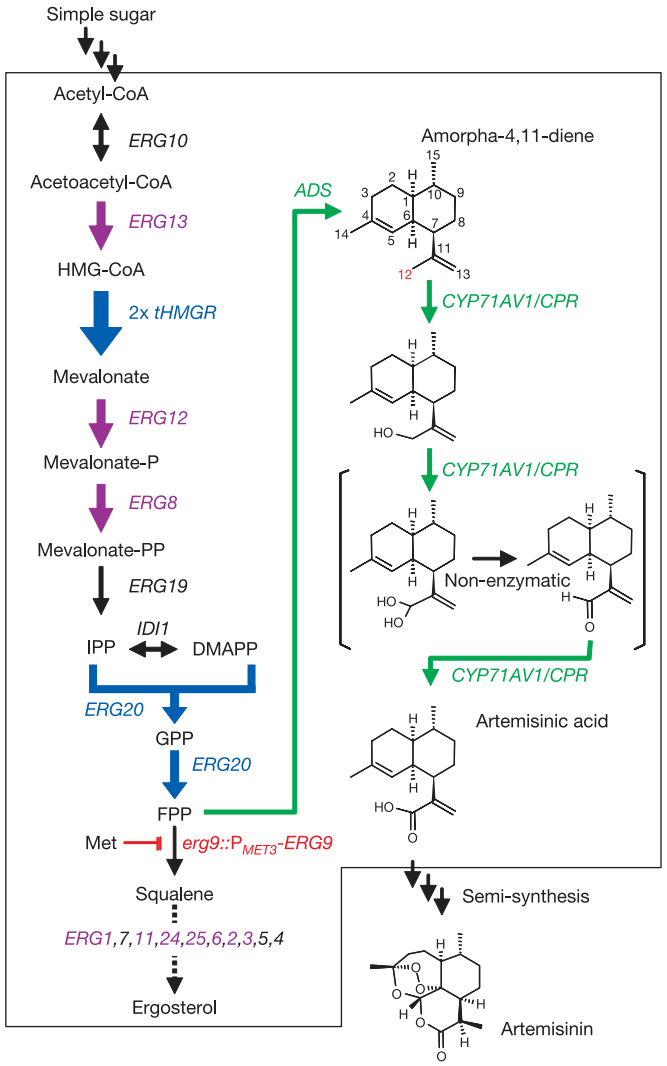
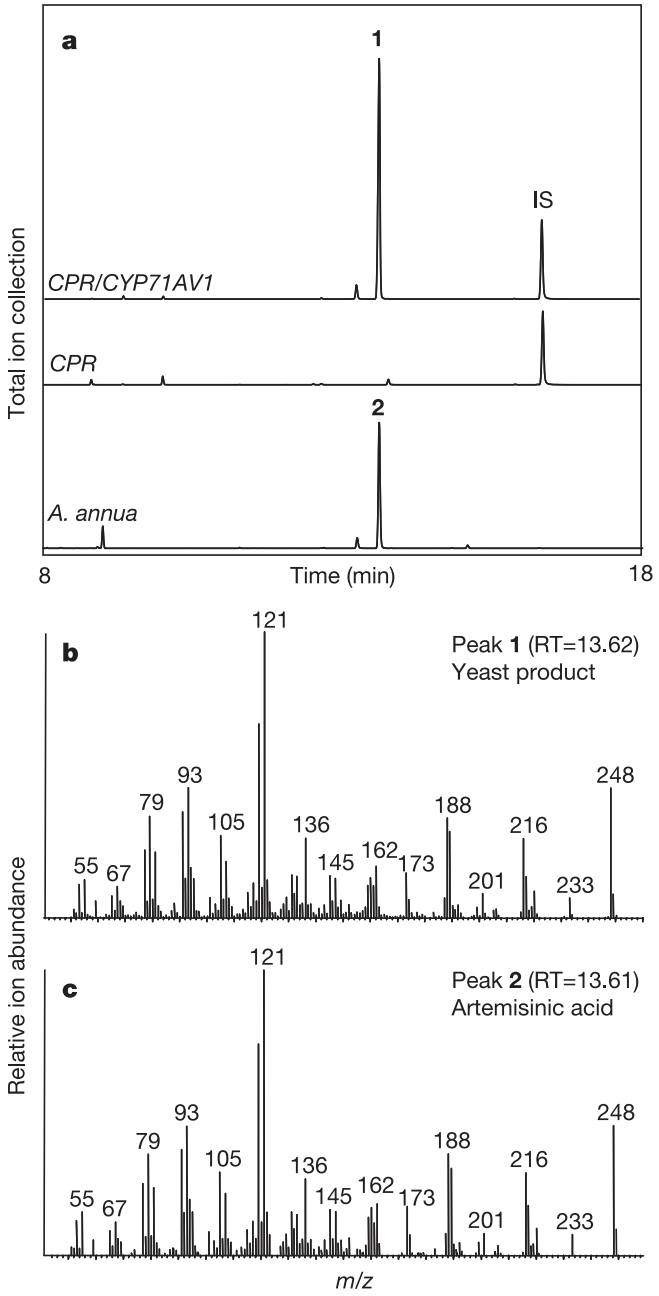


Figure 2 | Production of amorphadiene by *S. cerevisiae* strains. The various *S. cerevisiae* strains are described in the text. Cultures were sampled after 144 h of growth, and amorphadiene levels were quantified. Data, shown as total production, are mean ± s.d. ($n = 3$).

Análise


Simple sugar
Acetyl-CoA
ERG10
Acetoacetyl-CoA
ERG13
HMG-CoA
2x tHMGR
Mevalonate
ERG12
Mevalonate-P
ERG8
Mevalonate-PP
ERG19
IDI1
IPP
DMAPP
ERG20
GPP
ERG20
FPP
Met
erg9::P$_{MET3}$-ERG9
Squalene
ERG1,7,11,24,25,6,2,3,5,4
Ergosterol
ADS
Amorpha-4,11-diene
CYP71AV1/CPR
CYP71AV1/CPR
Non-enzymatic
CYP71AV1/CPR
Artemisinic acid
Semi-synthesis
Artemisinin


a
1
IS
CPR/CYP71AV1
CPR
2
A. annua
Total ion collection
8
Time (min)
18
b
Peak 1 (RT=13.62)
Yeast product
c
Peak 2 (RT=13.61)
Artemisinic acid
Relative ion abundance
m/z
121
93
79
105
136
188
216
248
55
67
145
162
173
201
233

Conclusões

- Prova-de-conceito para produção de artemisinina através de um bioprocesso através do uso de engenharia metabólica
- Produção de artemisinina (ácido artemisinico) com uma produtividade muito maior que da planta (*Artemisia annua*)
- Independência de fatores climáticos e políticos
- Sem risco de outros terpenos contaminantes