Evrimsel strateji...

Mutasyon hızını belirleyen araçlar:
- Replikasyon
- Tamir
- Transpozon aktivitesi

Esherichia coli'de mutasyon hızı

5.4 x 10^-10 mutasyon/bç/genom replikasyonu
Genom boyutu: 4.6 x 10^6 bç/genom
0.0025 mutasyon/genom/replikasyon
(Sinonim / non-sinonim SNP'ler dahil)

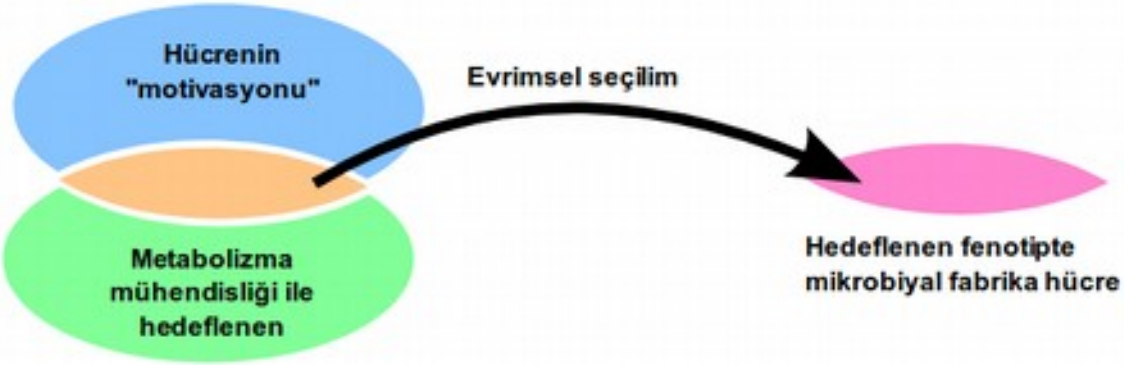
STRES

Hücreler mutasyon hızlarını ayarlayabilir
>>> Mutator phenotype <<<

Daha büyük boyutlu mutsayonlar:
- rekombinasyon
- insersiyon / delesyon
- genomik yeniden düzenlenme

Escherichia coli hücresinde bilinen en az 5 çeşit DNA'ya bağımlı DNA polimeraz bulunmakta
- pol II >> Hatasız DNA replikasyonu
- pol III / IV / V >> Artmış replikasyon hatası hızı
- Metilasyona bağımlı tamir >> hata hızında x100 artış

1. Kunkel TA. DNA Replication Fidelity. J Biol Chem. 2004 Apr 23;279(17):16895–8.
2. Fijalkowska IJ, Schaaper RM, Jonczyk P. DNA replication fidelity in Escherichia coli: a multi-DNA polymerase affair. FEMS Microbiol Rev. 2012 Nov;36(6):1105–21.

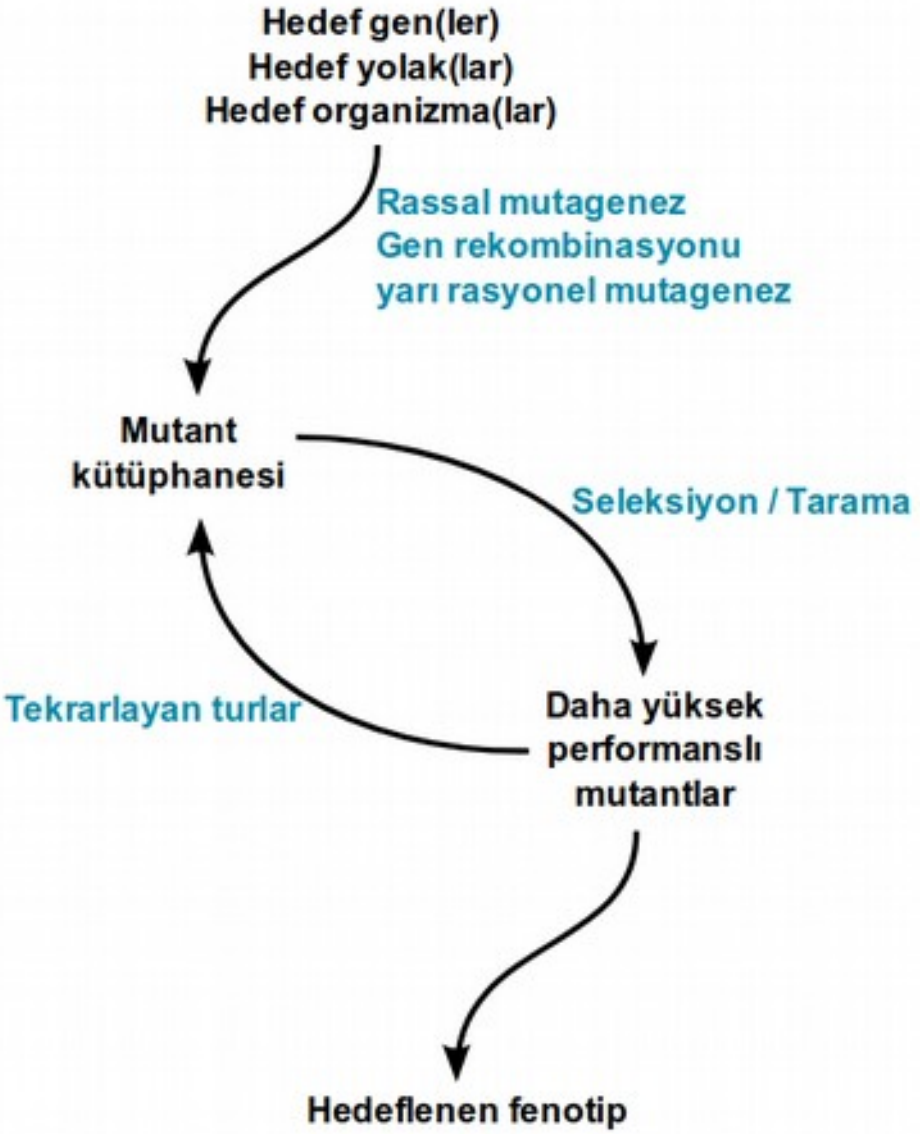


Sonuçta,
fenotipte gördüğümüz değişiklikler:
- **Artmış üreme hızı**
- **İyileşmiş biyokütle verimi**
- **"Generalist / Specialist" hücreler**

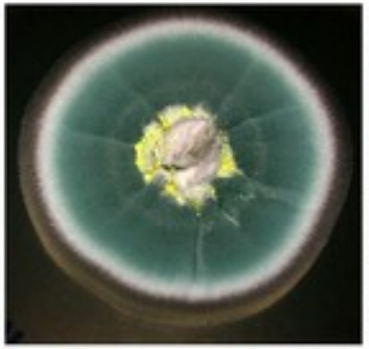
Her ortamın / koşulun mikrobu (Olmasını istediğimiz)

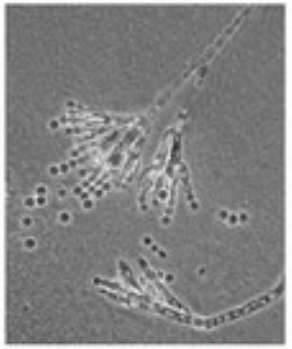
Belirli koşullarda yüksek performans (Gerçekte olan...)

Mueller's Ratchet:
Aseksüel çoğalan hücrelerin genomlarında zararlı mutasyonların geri dönüşümsüz olarak birikmesi...


Hedef gen(ler)
Hedef yolak(lar)
Hedef organizma(lar)
Rassal mutagenez
Gen rekombinasyonu
yarı rasyonel mutagenez
Mutant
kütüphanesi
Seleksiyon / Tarama
Tekrarlayan turlar
Daha yüksek
performanslı
mutantlar
Hedeflenen fenotip

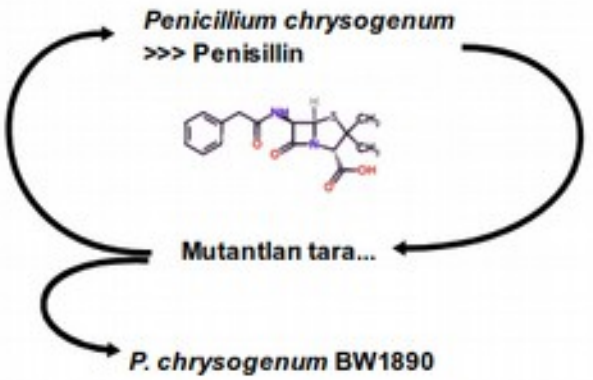

STRES TOLERANSI
antibiyotikler
toksik kimyasallar
ekstrem sıcaklıklar
besin kısıtlaması
SUBSTRAT KULLANIMI
organizmanın belirli bir substratı kullanmaya zorlanması
YENİ SEÇİLİM KRİTERLERİ
Kaynaklar
Metabolit sentezi
Metabolik olarak aktif, ancak bölünmeyen hücrelerin seçilimi
Üreme





Penisillin üretimini artırmak:

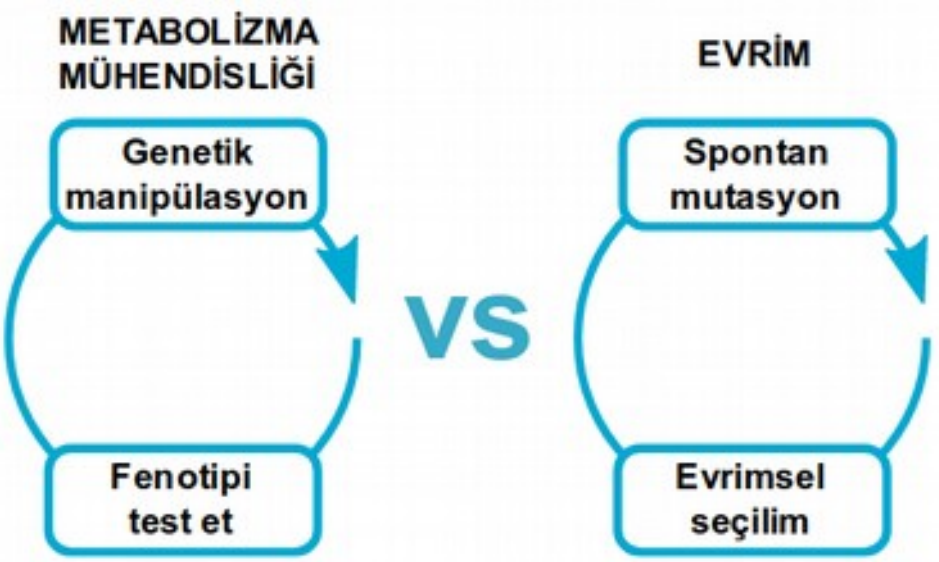
http://botit.botany.wisc.edu/toms_fungi/nov2003.html



penisillin biyosentetik genleri 8 - 16 ardışık kopya halinde bulunuyor!...

- Penisillin biyosentezinde yer alan genlerin artmış ifadelenmesi
- Penisillin yapısal genlerinin promotorlarının kuvvetinin artması
- "Genome-wide" analiz... Penisillin metabolizmasında neler değişiyor??
- Biyosentetik öncüllerin artırılması Metabolik darboğazların aşılması için gerekli enzimlerin rDNA teknikleri ile sentezlenmesi
- Tüm penisillin sentez yolağının heterolog / sentetik olarak oluşturulması

1. Wang F-Q, Zhong J, Zhao Y, Xiao J, Liu J, Dai M, et al. Genome sequencing of high-penicillin producing industrial strain of Penicillium chrysogenum. BMC Genomics. 2014 Jan 24;15(Suppl 1):S11.
2. Jami M-S, Barreiro C, García-Estrada C, Martín J-F. Proteome Analysis of the Penicillin Producer Penicillium chrysogenum. Mol Cell Proteomics. 2010 Jun;9(6):1182–98.



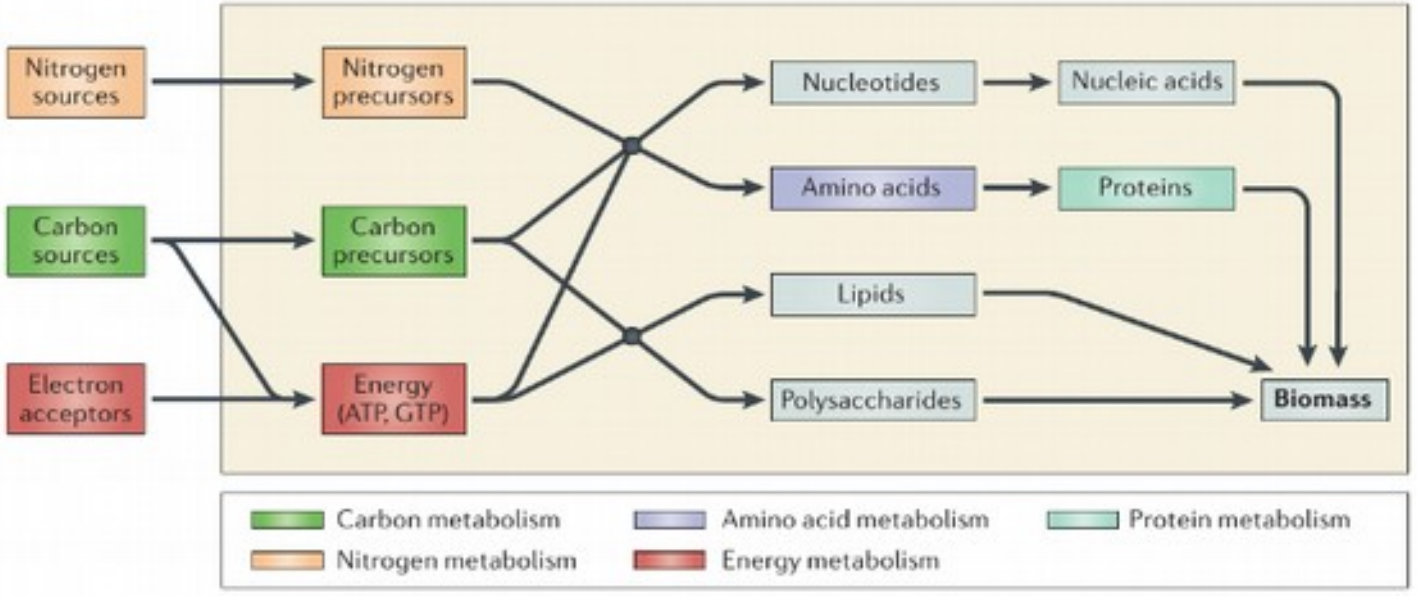
Hücresel metabolizmayı şekillendirmek için
DAHA RASYONEL YAKLAŞIMLAR...

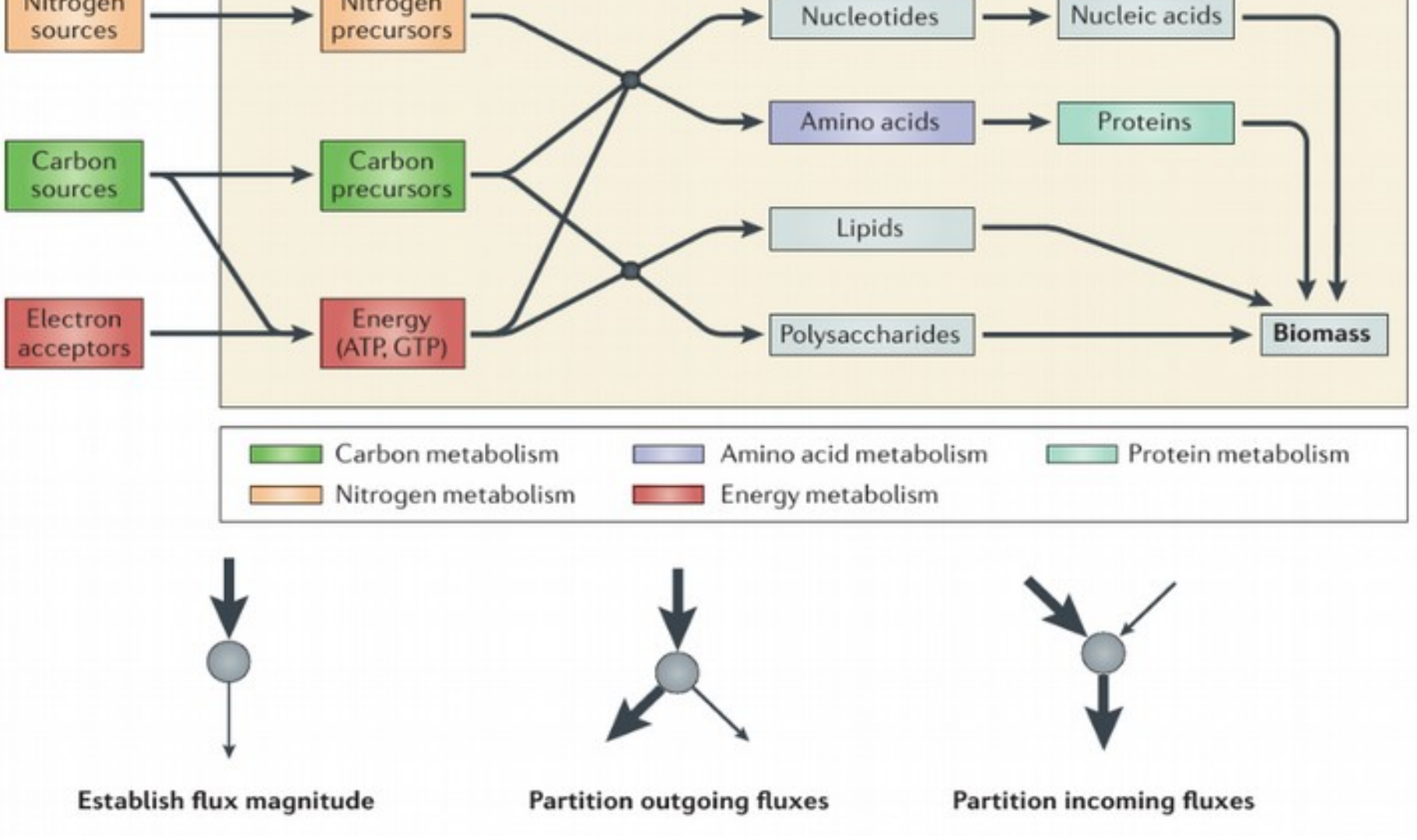
promotor mutagenezi... -10, -35
kromozomal integrasyon
rekombinasyon
yapay "construct"lar
RNA düzeyinde regülasyon

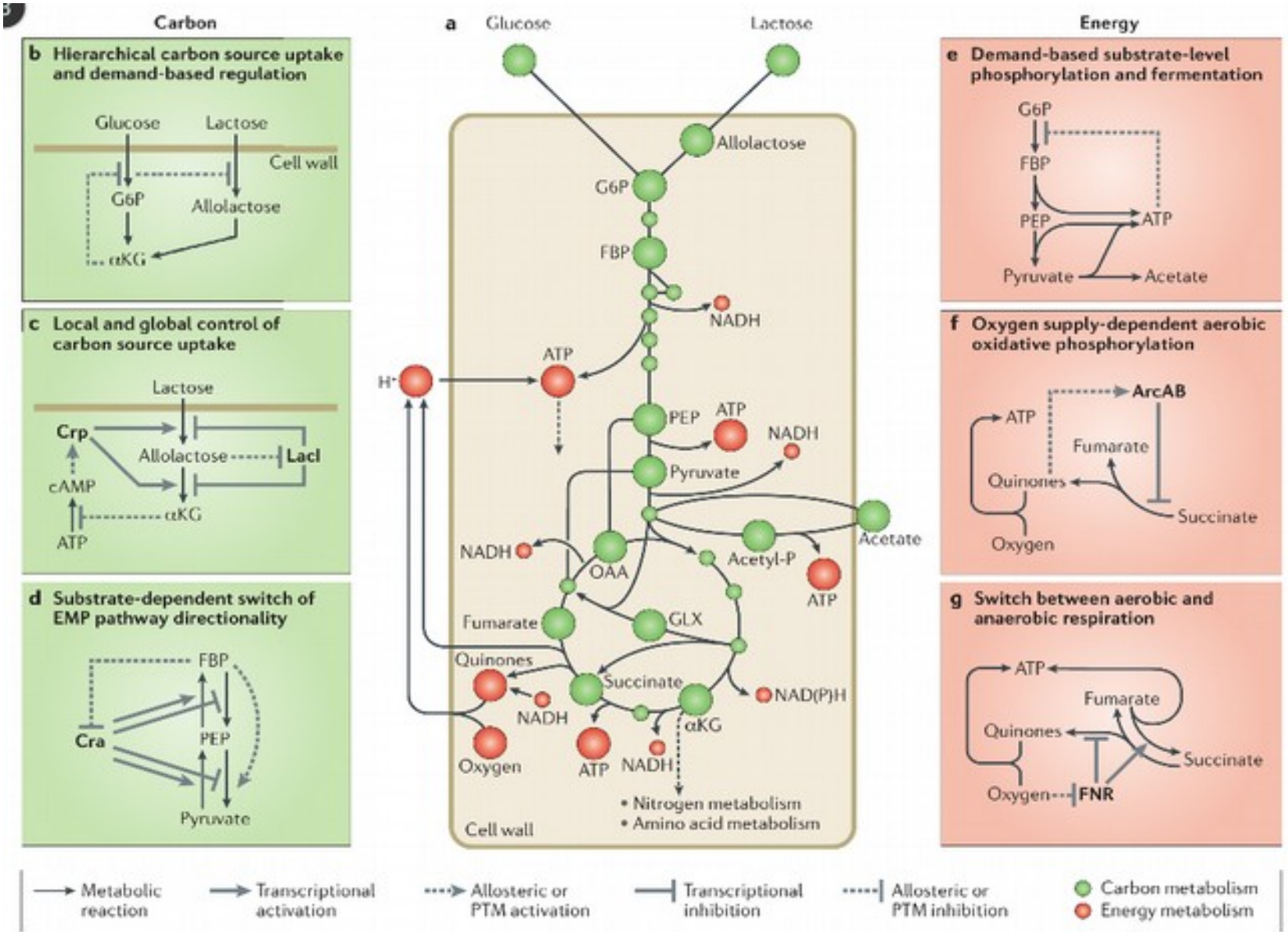
HEDEFE YÖNELİK

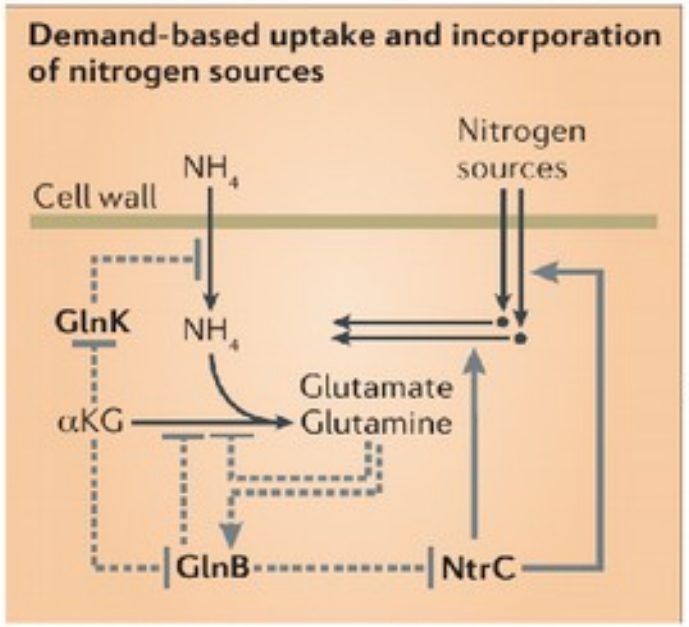
SENTETİK BİYOLOJİ

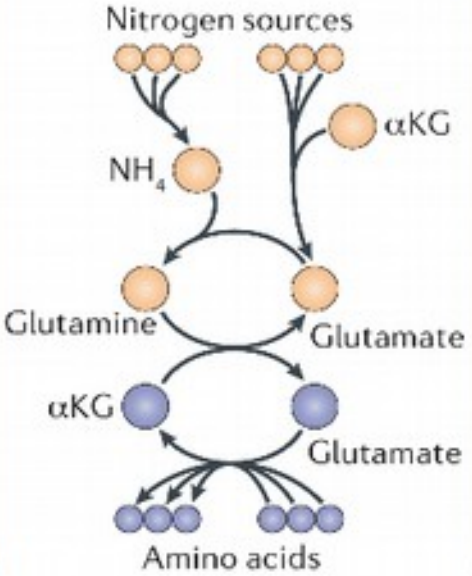
Metabolizmanın kontrolu

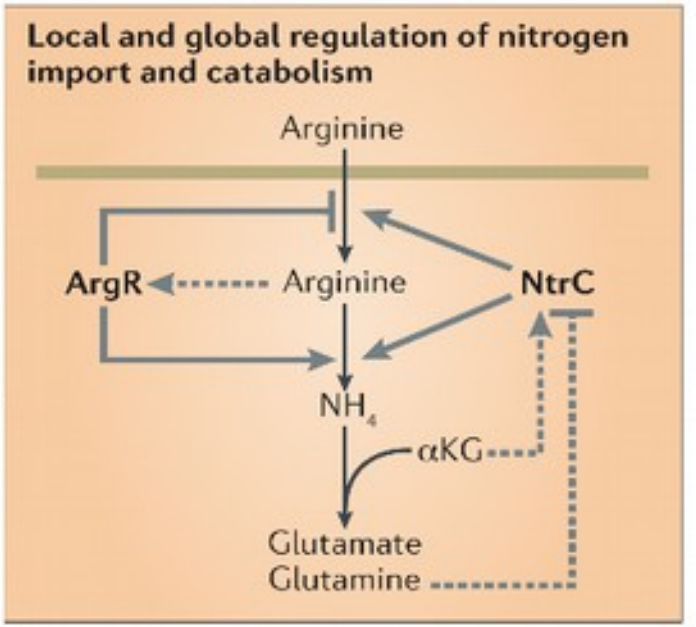



Nitrogen sources
Carbon sources
Electron acceptors
Nitrogen precursors
Carbon precursors
Energy (ATP, GTP)
Nucleotides
Nucleic acids
Amino acids
Proteins
Lipids
Polysaccharides
Biomass
Carbon metabolism
Amino acid metabolism
Protein metabolism
Nitrogen metabolism
Energy metabolism
Establish flux magnitude
Partition outgoing fluxes
Partition incoming fluxes

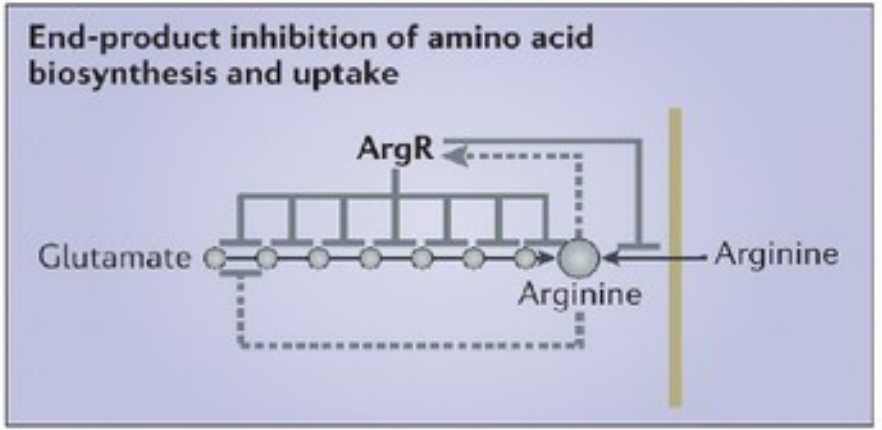

Carbon
Energy
a
Glucose
Lactose
b Hierarchical carbon source uptake and demand-based regulation
Glucose
Lactose
Cell wall
G6P
Allolactose
αKG
c Local and global control of carbon source uptake
Lactose
Crp
Allolactose
LacI
cAMP
αKG
ATP
d Substrate-dependent switch of EMP pathway directionality
FBP
Cra
PEP
Pyruvate
Allolactose
G6P
FBP
NADH
ATP
H+
PEP
ATP
NADH
Pyruvate
Acetate
NADH
OAA
Acetyl-P
ATP
Fumarate
GLX
Quinones
Succinate
NAD(P)H
NADH
αKG
Oxygen
ATP
NADH
• Nitrogen metabolism
• Amino acid metabolism
Cell wall
e Demand-based substrate-level phosphorylation and fermentation
G6P
FBP
PEP
ATP
Pyruvate
Acetate
f Oxygen supply-dependent aerobic oxidative phosphorylation
ArcAB
ATP
Fumarate
Quinones
Succinate
Oxygen
g Switch between aerobic and anaerobic respiration
ATP
Fumarate
Quinones
Succinate
Oxygen
FNR
Metabolic reaction
Transcriptional activation
Allosteric or PTM activation
Transcriptional inhibition
Allosteric or PTM inhibition
Carbon metabolism
Energy metabolism



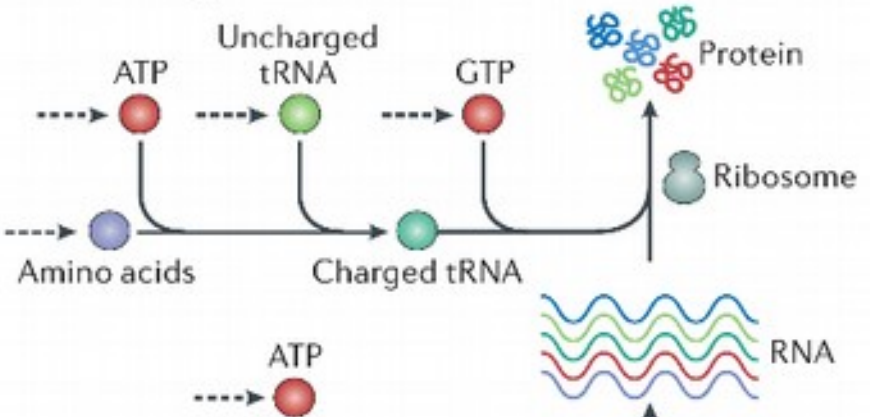


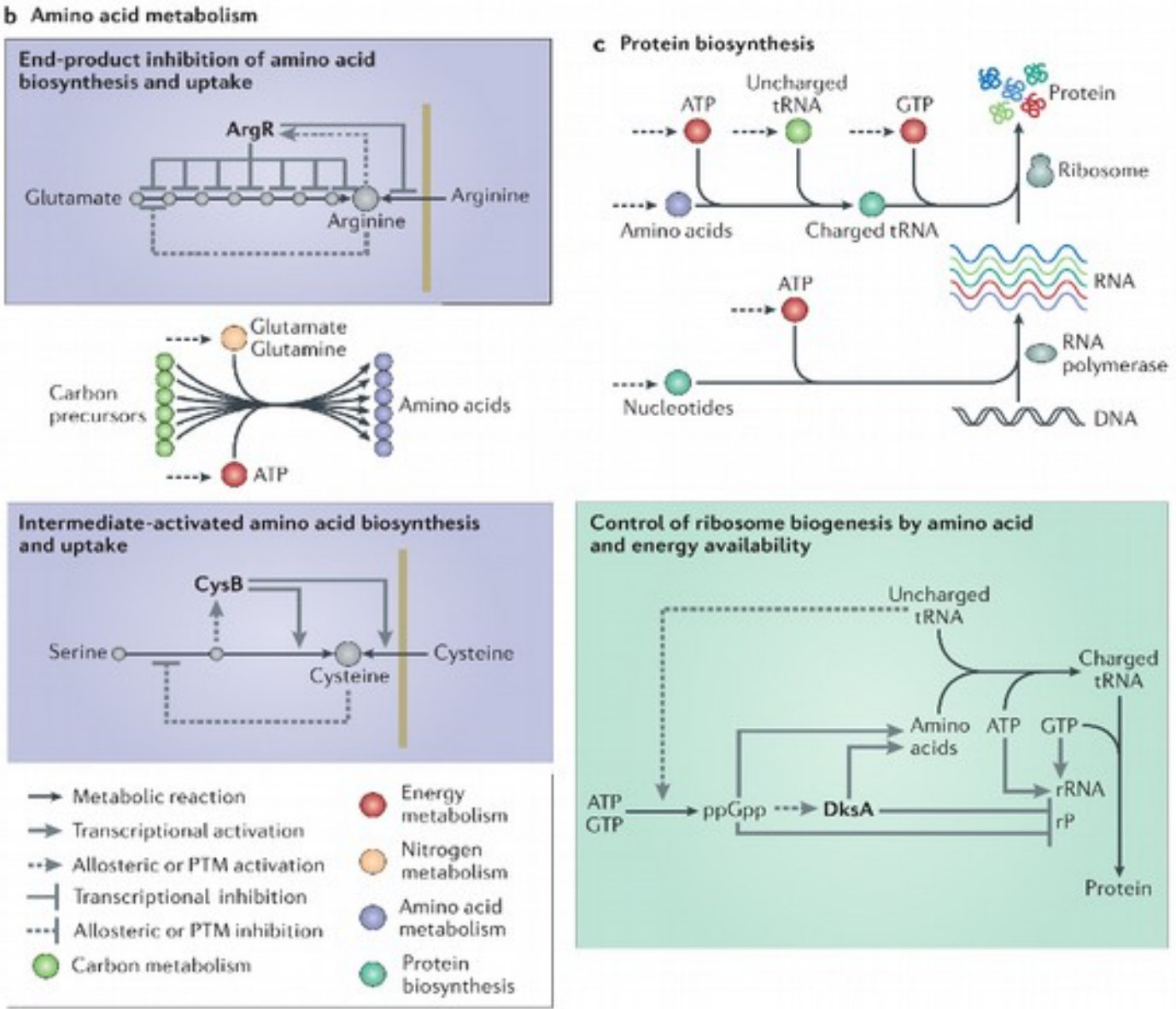


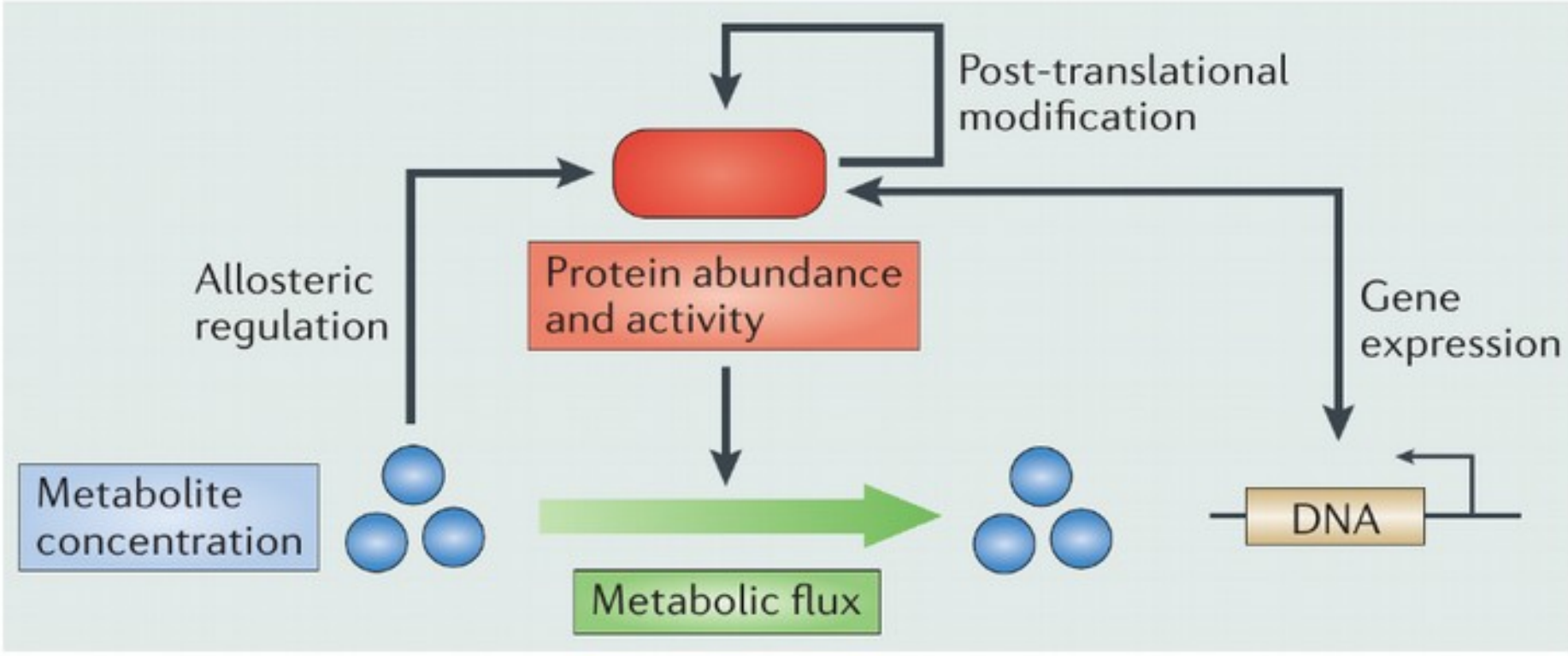
b Amino acid metabolism



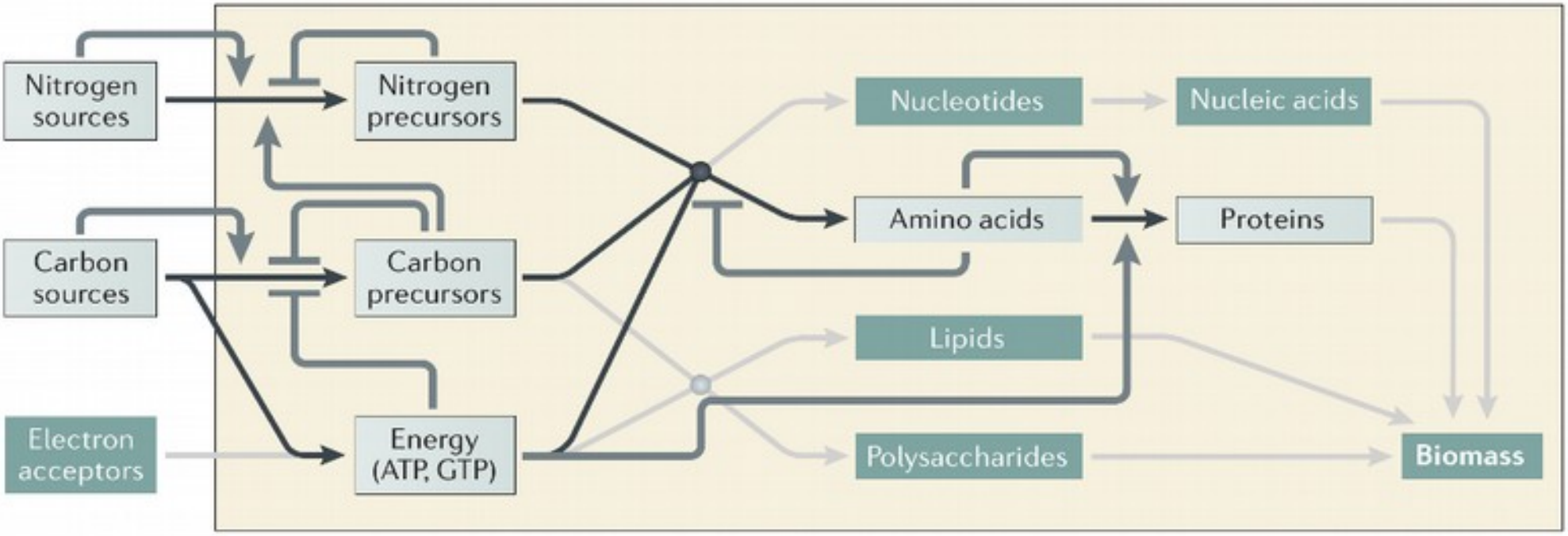
c Protein biosynthesis




b Amino acid metabolism
End-product inhibition of amino acid biosynthesis and uptake
ArgR
Glutamate
Arginine
Arginine
Glutamate
Glutamine
Carbon precursors
Amino acids
ATP
Intermediate-activated amino acid biosynthesis and uptake
CysB
Serine
Cysteine
Cysteine
Metabolic reaction
Transcriptional activation
Allosteric or PTM activation
Transcriptional inhibition
Allosteric or PTM inhibition
Carbon metabolism
Energy metabolism
Nitrogen metabolism
Amino acid metabolism
Protein biosynthesis
c Protein biosynthesis
ATP
Uncharged tRNA
GTP
Protein
Ribosome
Amino acids
Charged tRNA
ATP
RNA
RNA polymerase
Nucleotides
DNA
Control of ribosome biogenesis by amino acid and energy availability
Uncharged tRNA
Charged tRNA
Amino acids
ATP
GTP
rRNA
ATP
GTP
ppGpp
DksA
rP
Protein


Post-translational modification
Allosteric regulation
Protein abundance and activity
Gene expression
Metabolite concentration
DNA
Metabolic flux

Metabolite	Information transfer	Key regulatory interaction
FBP	Glycolytic flux[37,39,155]	Enzyme: pyruvate kinase Enzyme: PEP carboxylase Transcription factor: Cra
cAMP	α-ketoacid concentration[20]. Phosphorylation state of PTS system[16]	Transcription factor: Crp
L-glutamine	Nitrogen availability[60,89]	Signalling: GlnBK Transcription factor: NtrC (via GlnBK)
α-ketoglutarate	Ratio of carbon to nitrogen availability for amino acid biosynthesis[20]	Enzyme: EI (PTS component) Enzyme: adenylate cyclase (cAMP forming) Signalling: GlnBK
Other α-ketoacids (for example, pyruvate and oxaloacetate)	Ratio of carbon to nitrogen availability for amino acid biosynthesis[20]	Enzyme: several enzymes in TCA cycle (oxaloacetate) Enzyme: adenylate cyclase (cAMP forming) Transcription factor: PdhR (pyruvate)
L-leucine	Balance of L-leucine production, uptake and protein biosynthesis[110]. General nutrient abundance[110,111]	Enzyme: several steps in branched-chain amino acid biosynthesis Transcription factor: Lrp
ppGpp	Amino acid starvation[118]	Transcription: RNA polymerase Transcription factor: DksA
ATP	Energy starvation[117]	Enzyme: numerous enzymes in metabolism Transcription factor: RpoS via ClpXP Transcription: RNA polymerase (transcription of ribosomal promoters)
Quinones	Balance of respiratory capacity and oxygen supply[77,78]	Signalling: ArcAB



Flux push

A metabolite enhancing its own consumption (outcoming flux) when abundant

Flux pull

A metabolite enhancing its own production (incoming flux) when scarce

AMİNO ASİT ÜRETİMİ

1. Bott M. Offering surprises: TCA cycle regulation in Corynebacterium glutamicum. Trends in Microbiology. 2007 Sep;15(9):417–25.

2. Wendisch VF. Microbial production of amino acids and derived chemicals: Synthetic biology approaches to strain development. Current Opinion in Biotechnology. 2014 Dec;30:51–8.

3. Mitsuhashi S. Current topics in the biotechnological production of essential amino acids, functional amino acids, and dipeptides. Current Opinion in Biotechnology. 2014 Apr;26:38–44.

4. Leuchtenberger W, Huthmacher K, Drauz K. Biotechnological production of amino acids and derivatives: current status and prospects. Appl Microbiol Biotechnol. 2005 Nov 1;69(1):1–8.

amino asit üretimi

- Oksotrofik mutant izole et

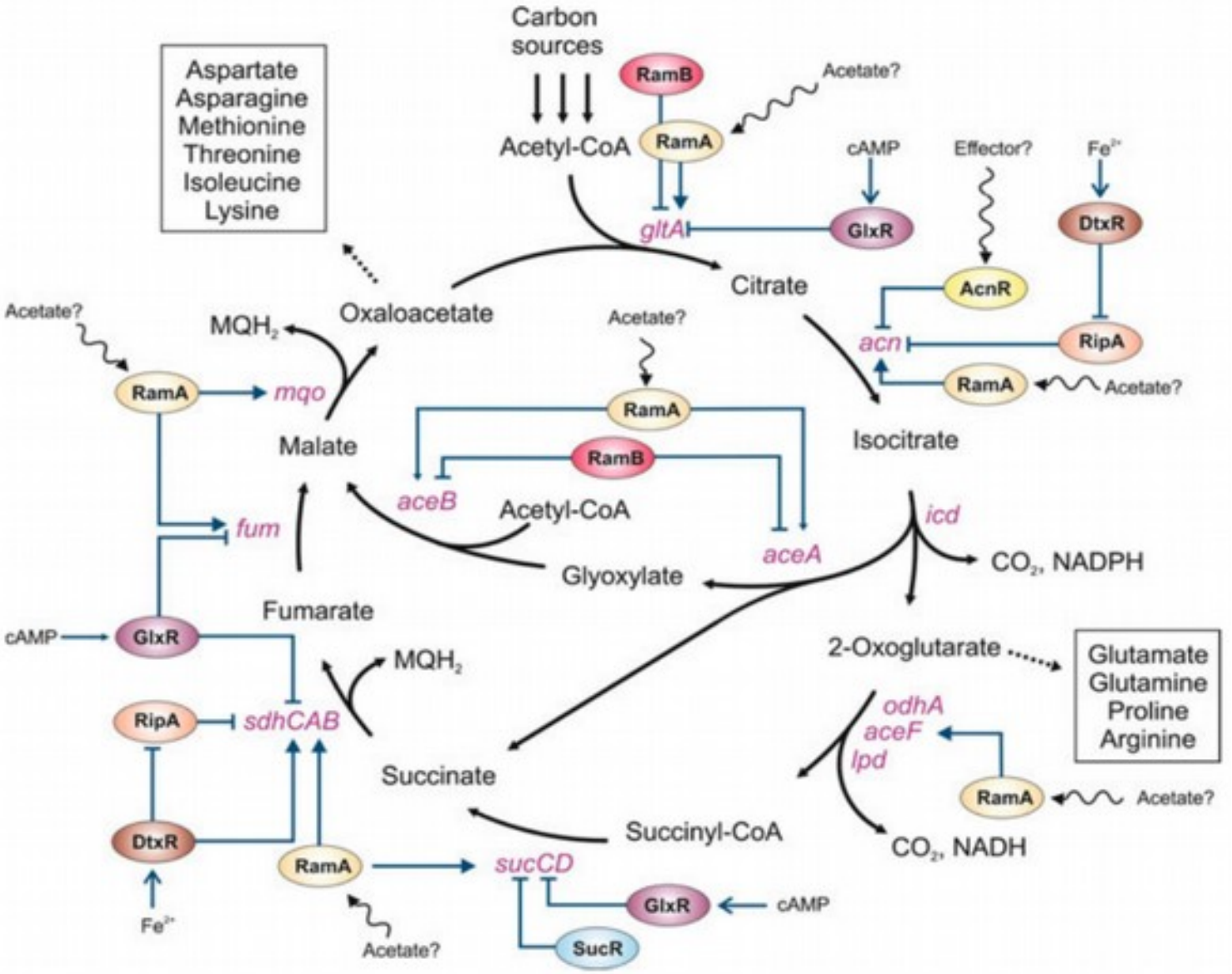
- Gereksinim duyulan (oksotrof olunan) substrat için parsiyel starvasyon koşullarında inkübe et

YA DA:

- Üretilecek metabolitin toksik analoğuna dirençli (antimetabolit dirençli) mutant seçimi yap...

YA DA:

- Üretilecek metabolitin hücre dışına permeabilitesini artır!


Carbon sources
Aspartate
Asparagine
Methionine
Threonine
Isoleucine
Lysine
RamB
Acetate?
RamA
Acetyl-CoA
cAMP
Effector?
Fe2+
gltA
GlxR
DtxR
Citrate
AcnR
Oxaloacetate
MQH2
Acetate?
acn
RipA
RamA
Acetate?
mqo
Malate
Isocitrate
aceB
Acetyl-CoA
icd
fum
aceA
CO2, NADPH
Glyoxylate
Fumarate
cAMP
GlxR
MQH2
2-Oxoglutarate
Glutamate
Glutamine
Proline
Arginine
RipA
sdhCAB
odhA
aceF
lpd
Succinate
RamA
Acetate?
DtxR
Succinyl-CoA
CO2, NADH
RamA
sucCD
Fe2+
GlxR
cAMP
Acetate?
SucR

L-Lizin Üretimi

Corynebacterium glutamicum

C-source + N-source + salts + trace elements

fermentation

cell separation → biomass

ion exchange → waste

evaporation → ammonia

cristallisation → mother liquor

drying

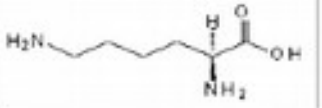
L-Lysine·HCl

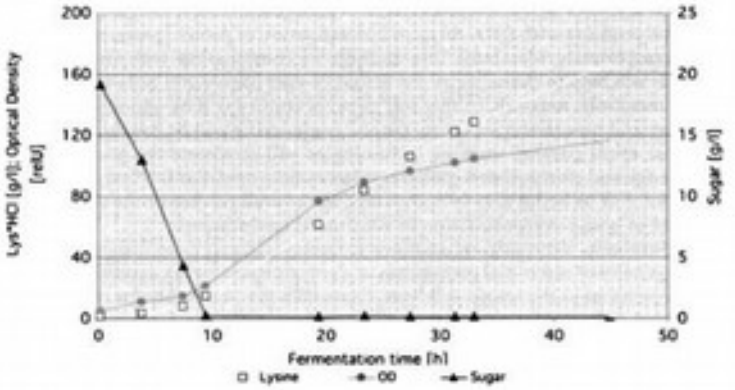
fermentation

evaporation

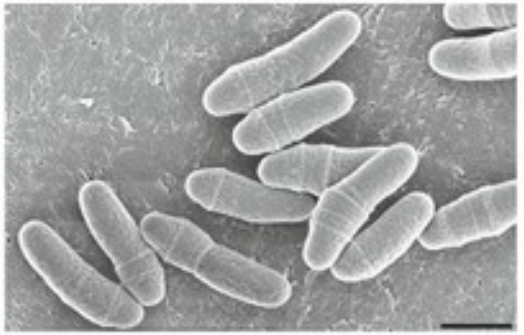
spray drying

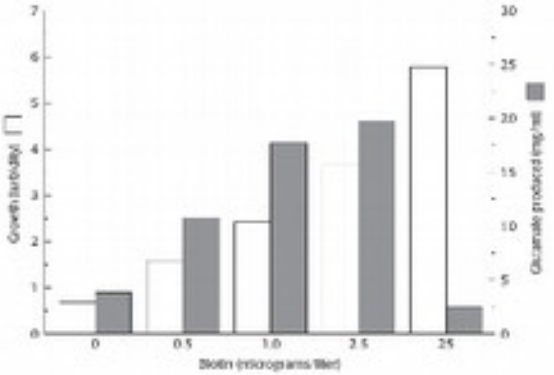
Biolys®

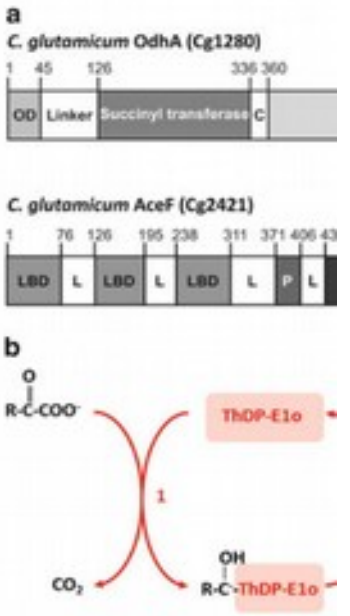


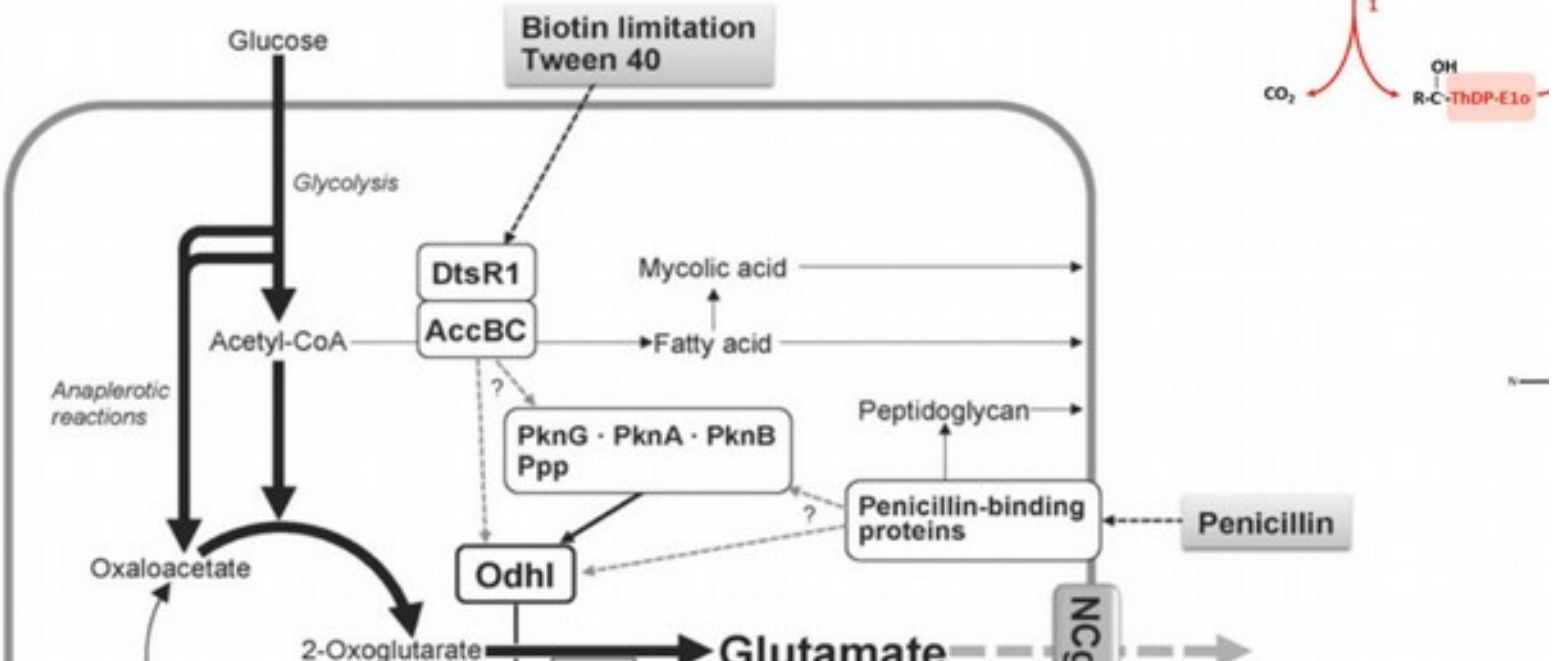


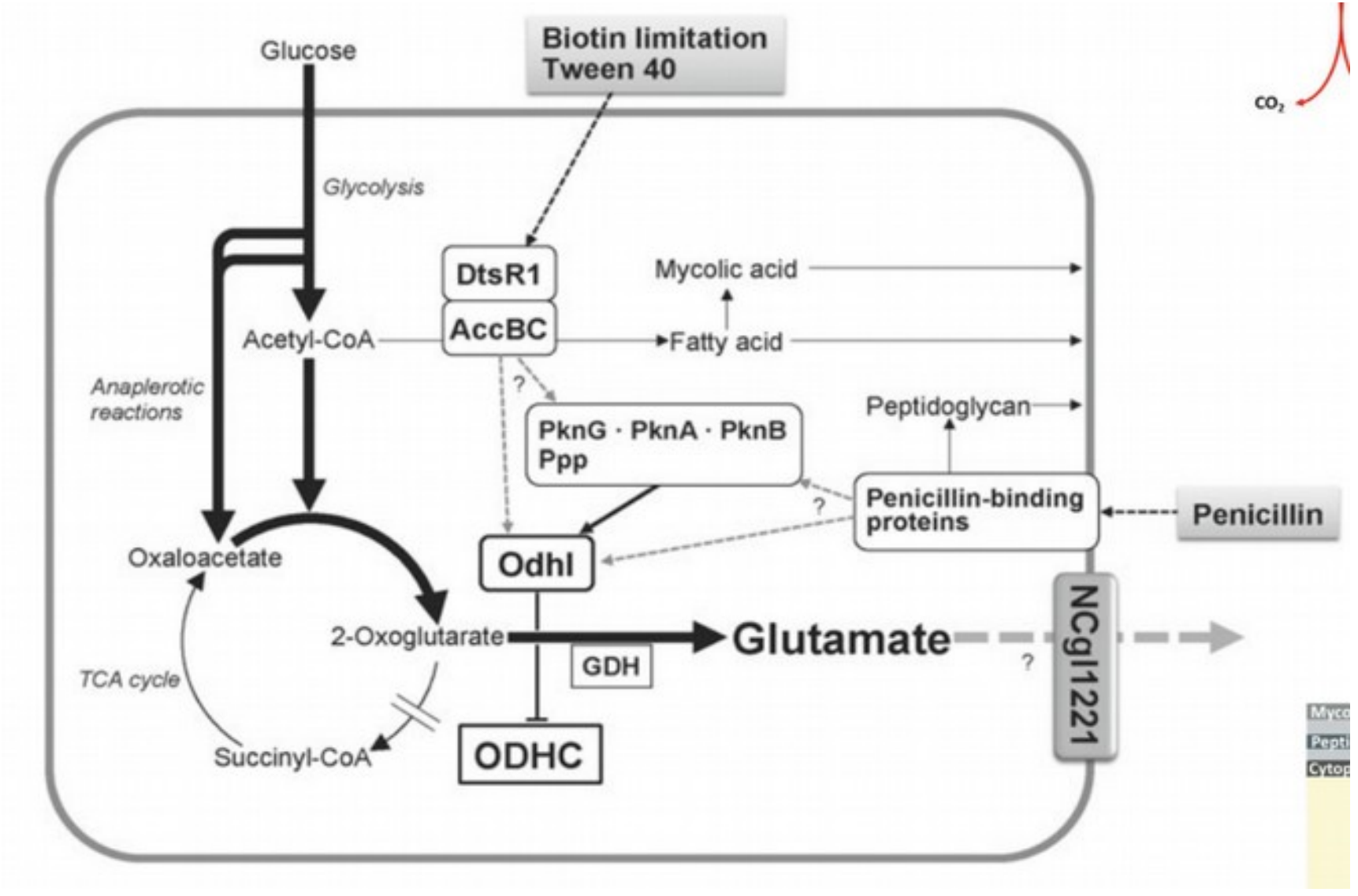
L-Glutamin Üretimi










Glucose
Biotin limitation
Tween 40
CO_2
Glycolysis
DtsR1
AccBC
Mycolic acid
Acetyl-CoA
Fatty acid
Anaplerotic reactions
?
PknG · PknA · PknB
Ppp
Peptidoglycan
Penicillin-binding proteins
Penicillin
?
Oxaloacetate
OdhI
NCgl1221
2-Oxoglutarate
Glutamate
GDH
?
TCA cycle
Succinyl-CoA
ODHC

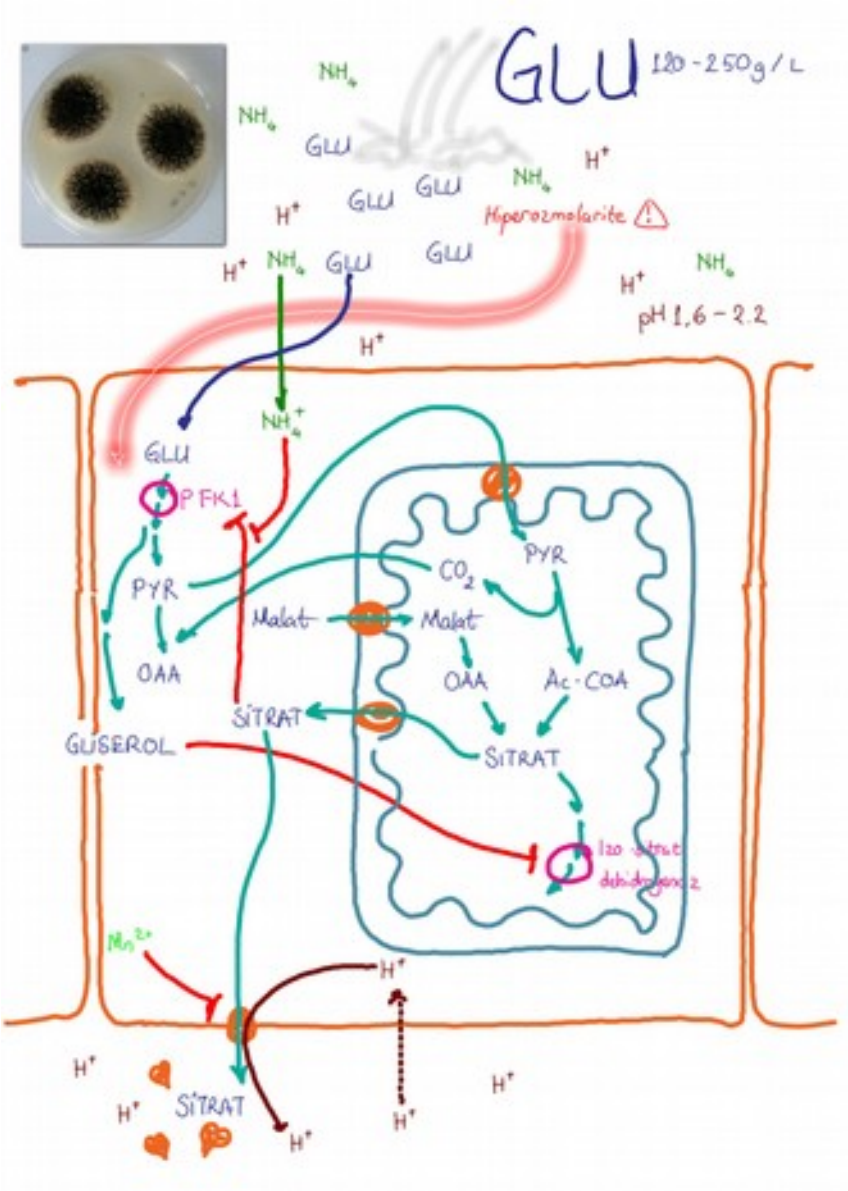
Sitrik Asit

Tatlandırıcı / Koruyucu

Etanolün aksine, sitrik asit enerji metabolizmasının tipik bir atık maddesi değil! ($\rightarrow CO_2$ ve H_2O)

Malzeme listesi:

- Aspergillus niger (durağan fazda kültür)
- Besiyeri pH 1.6 - 2.2 (kuvvetli asidik!)
- Yüksek şeker konsantrasyonu (120 - 250 g/L)
- Mn^{2+} iyonu bulunmamalı!
- Yüksek konsantrasyonda NH_4^+ iyonları


GLU 120 - 250g / L
Hiperozmolarite
pH 1,6 - 2,2
GLU
PFK1
PYR
OAA
Malat
CO2
PYR
Malat
OAA
Ac-COA
SITRAT
GLISEROL
SITRAT
Izo-sitrat dehidrogenaz
Mn2+
SITRAT

Geleneksel Yaklaşım

2° metabolit / antibiyotik üretim veriminin artırılması

ZBAHAM ROAR

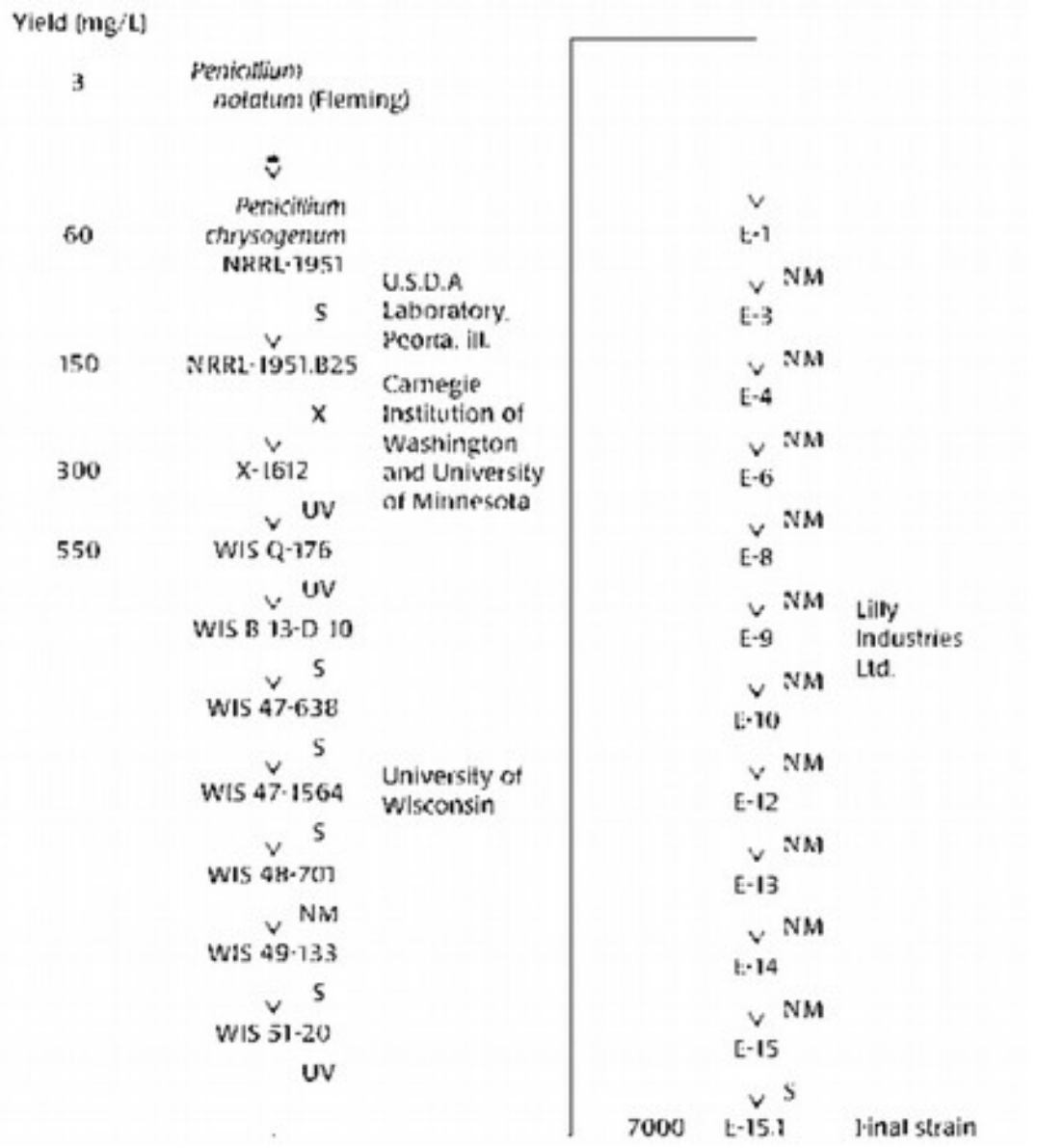
Rassal mutagenez

- Nitrogen mustard
- UV
- X-ray

BUF

→ Klonları üret

→ Kültür filtratları antimikrobiyal etki


Yield (mg/L)
3
Penicillium notatum (Fleming)
60
Penicillium chrysogenum NRRL-1951
U.S.D.A Laboratory, Peoria, Ill.
S
150
NRRL-1951.B25
Carnegie Institution of Washington and University of Minnesota
X
300
X-1612
UV
550
WIS Q-176
UV
WIS B 13-D 10
S
WIS 47-638
S
WIS 47-1564
University of Wisconsin
S
WIS 48-701
NM
WIS 49-133
S
WIS 51-20
UV
E-1
NM
E-3
NM
E-4
NM
E-6
NM
E-8
NM
E-9
Lilly Industries Ltd.
NM
E-10
NM
E-12
NM
E-13
NM
E-14
NM
E-15
S
7000
E-15.1
Final strain

OKSOTROFİK MUTANT OLUŞTURULMASI

Nasıl yapılır?

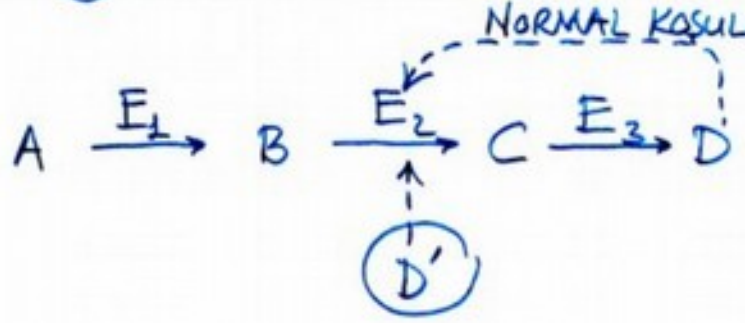
- mutajene maruz bırak, oksotrafu oluşturulmak istenen substrat bulunmayan besiyerinde inkübe et
- kültüre penisilin ekle → Enzim defektif hale gelip oksotrof olan hücreler üreyemeyecek/çoğalamayacak aktif bölünenler penisilin etkisi ile PARÇALANIR

- Penisilin(-) / Substract(+) besiyerinde oksotrofları seç!

Örnek:

L-ornitin üretimi için L-Arginin oksotrofu seçilmesi

Dallanmayan yolaklarda regülatuar mutantların seçimi



D': amino asit analoğu

TOKSİK!
E_2'yi inhibe eder
son ürün D
sentezlenemez

- Minimal besiyerinde D' kullanılarak seçim yapılır.
- Yalnızca E_2 regülasyon defekti dan mutantlar D üretebilir ve hayatta kalabilir!

Serratia marcescens'de prolin üretimi