Alternative menaquinone biosynthetic pathway

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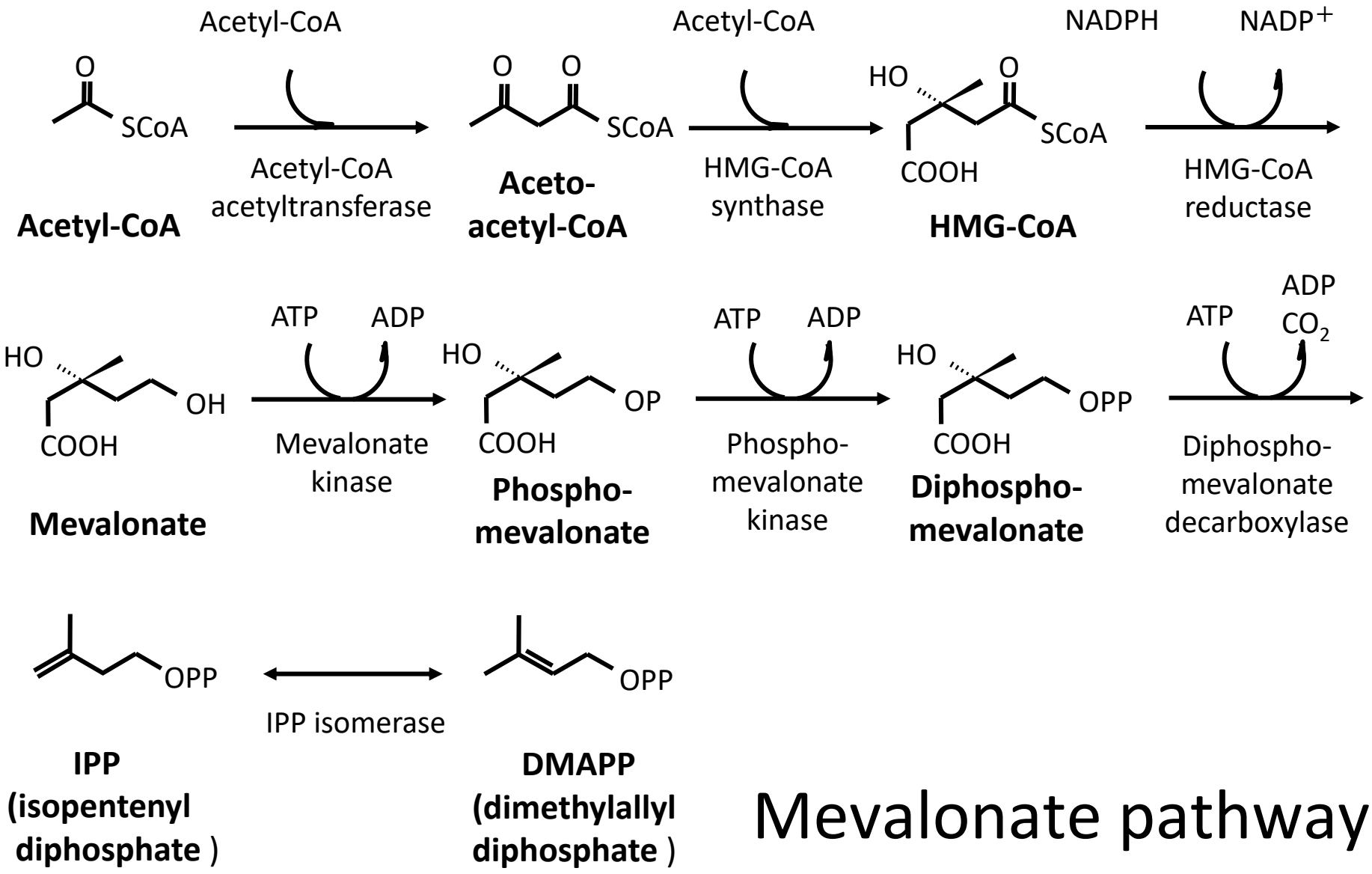
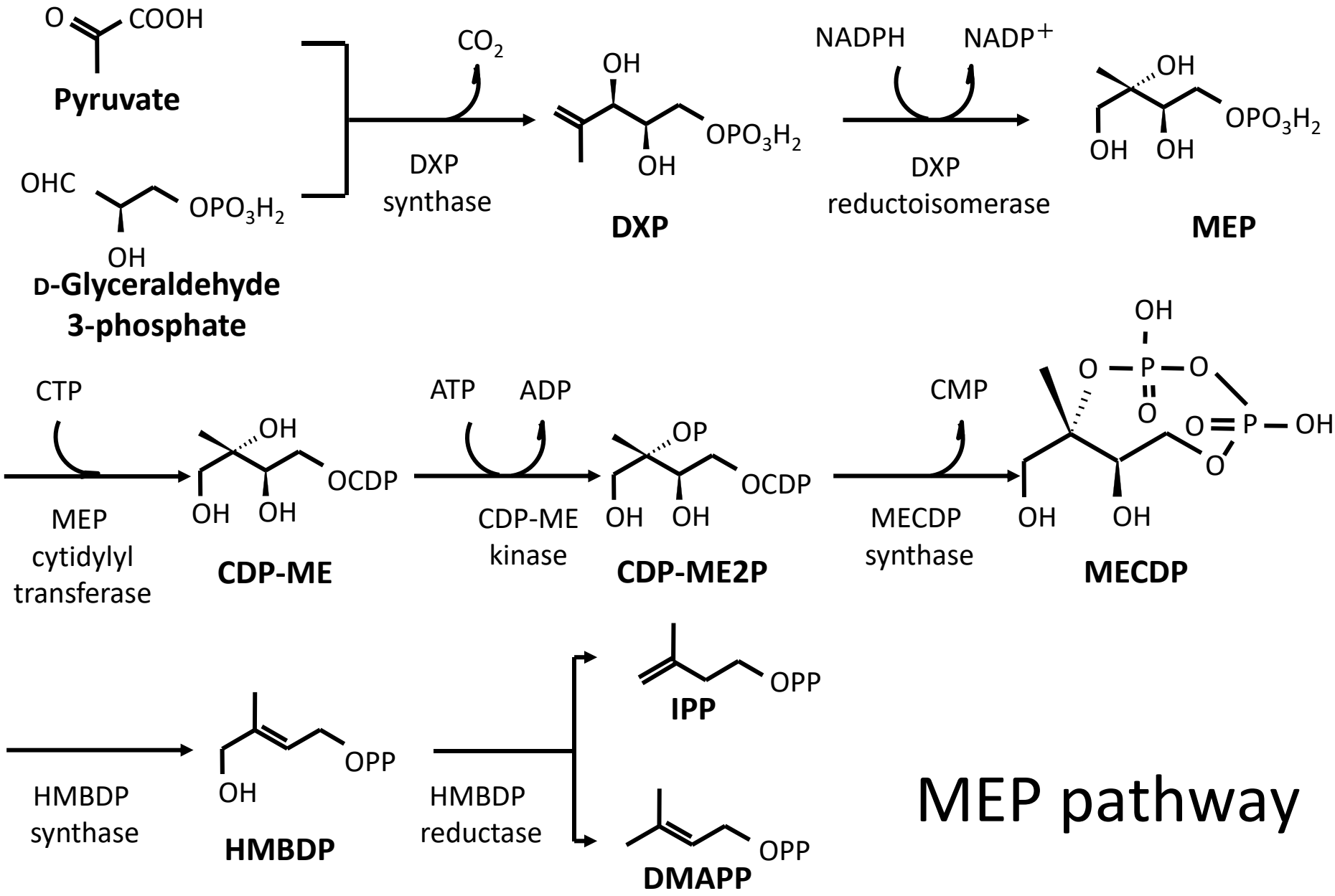
Alternative Biosynthetic Pathway



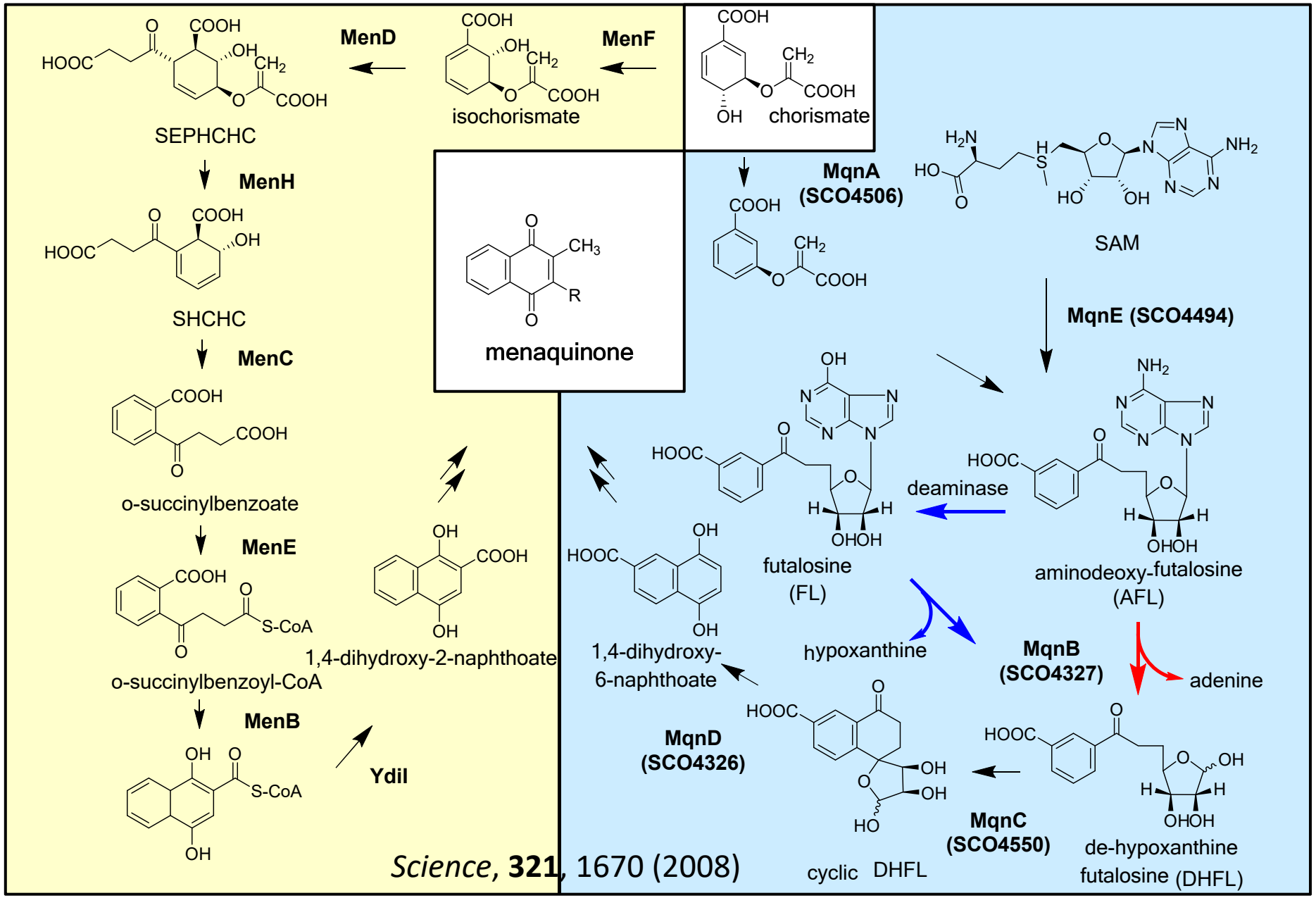
Mevalonate pathway

Alternative Biosynthetic Pathway

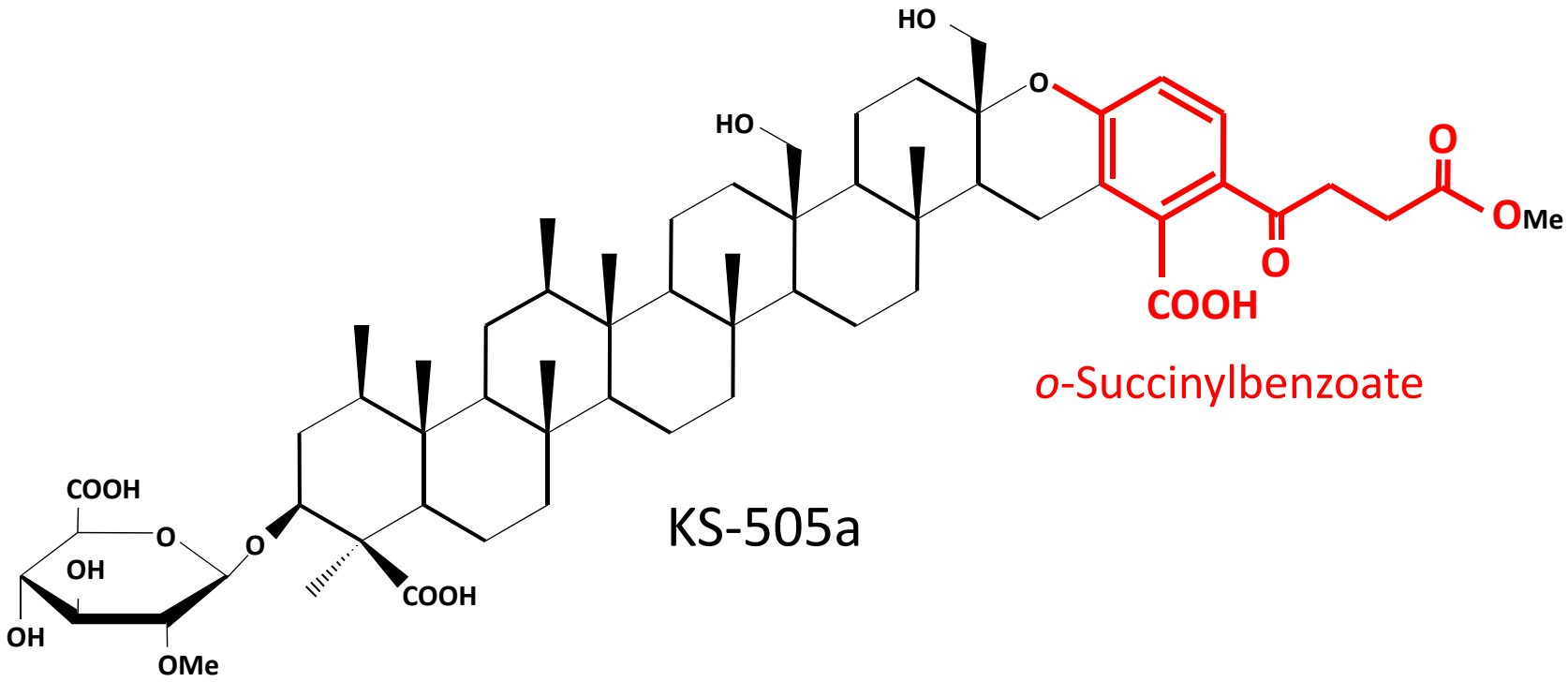


MEP pathway

Menaquinone Biosynthetic Pathways



KS-505a has a *o*-succinylbenzoate as a partial structure



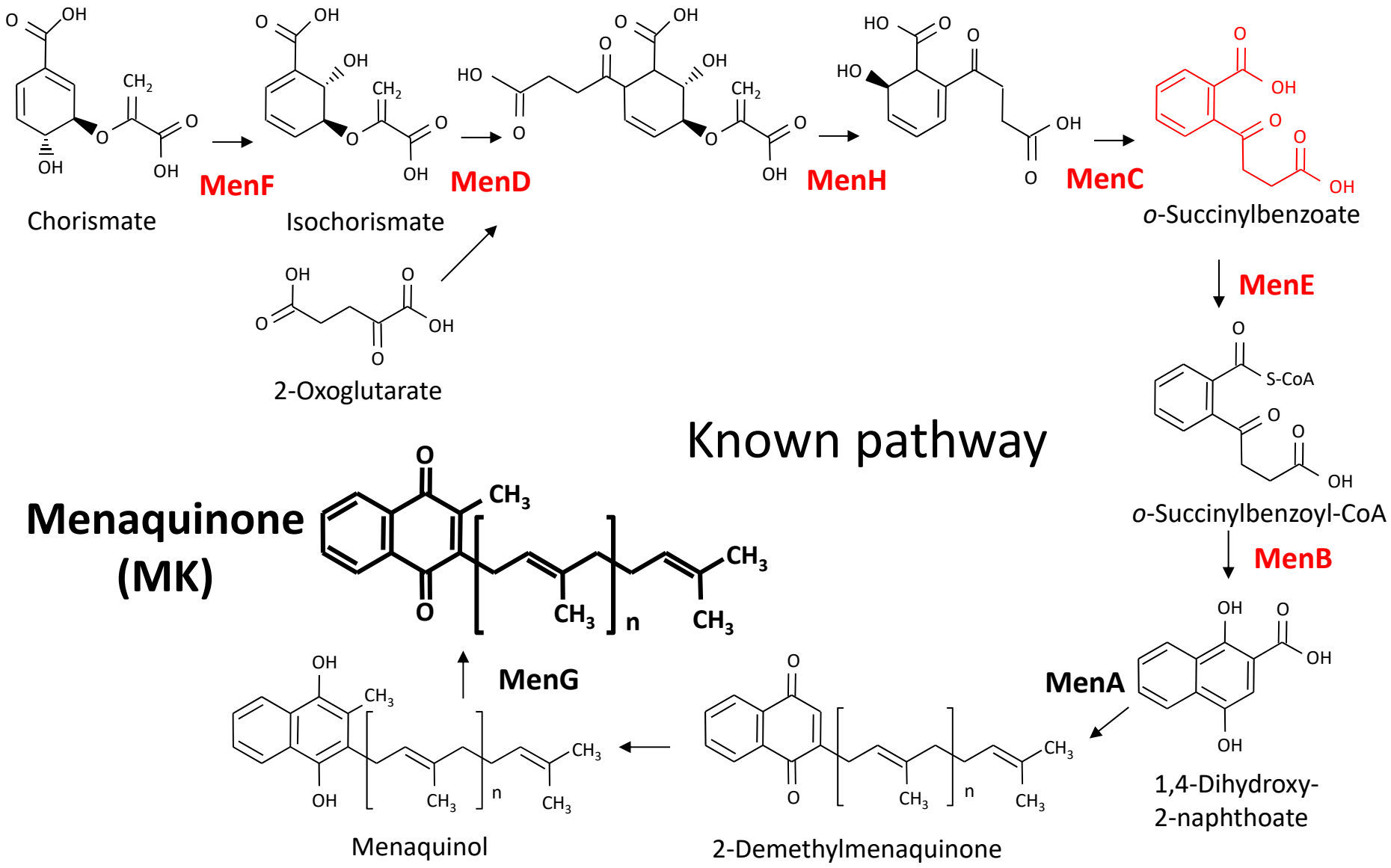
o-Succinylbenzoate

KS-505a

Producer; *Streptomyces argenteolus* strain A-2

Biosci. Biotech. Biochem., **71,** 3072-3081 (2007).

S. coelicolor and *S. avermitilis* have no *men*F, D, H, C, E and B genes



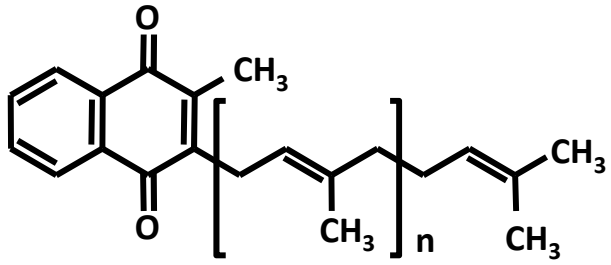
The function of MK in organisms

For human

Essential vitamin for

- blood coagulation,
- bone metabolism,
- cell-cycle regulation

Supplied *via* diet.



Menaquinone （MK)

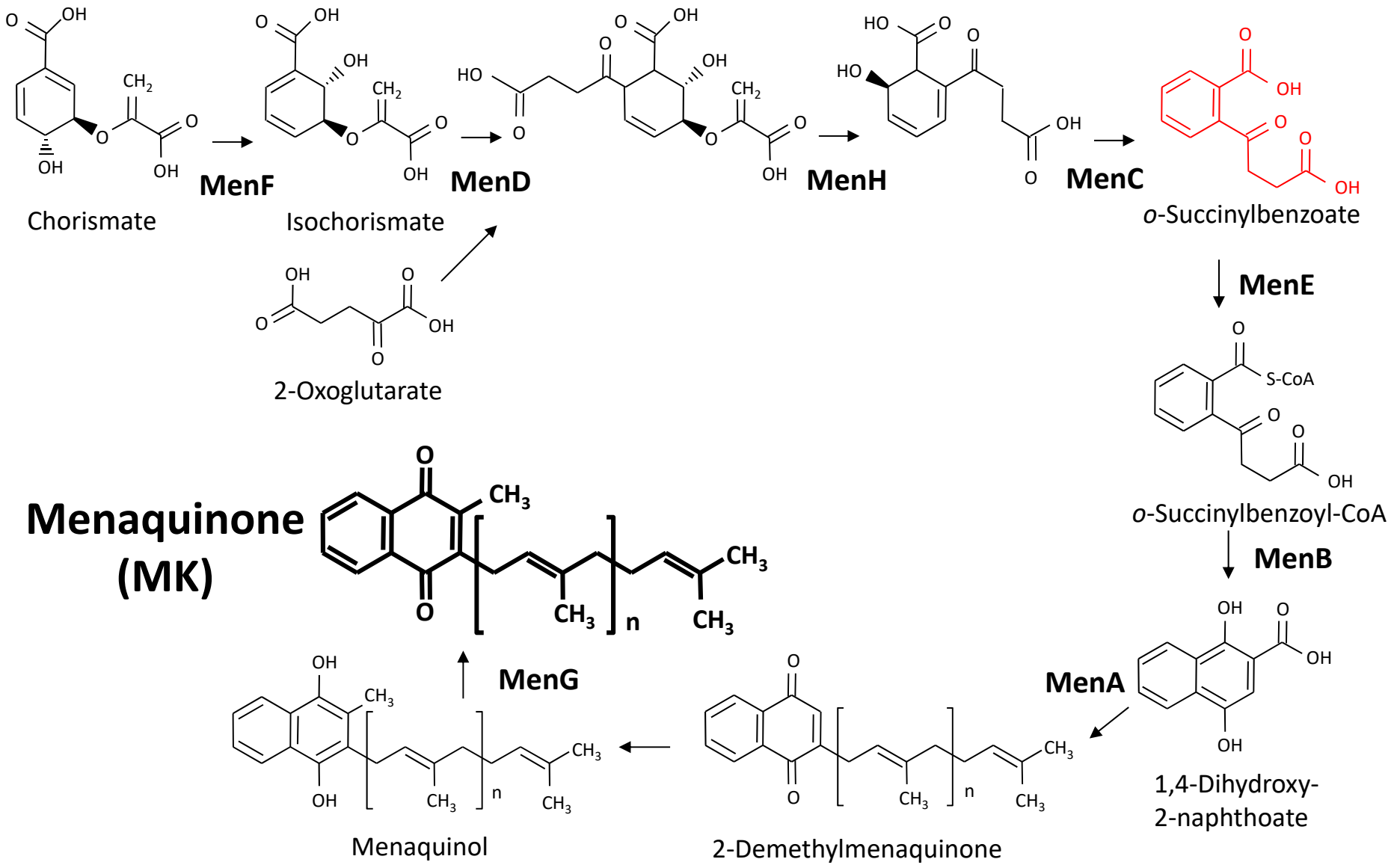
For bacteria

Essential for

- respiration {mainly in Gram (+) bacteria}.

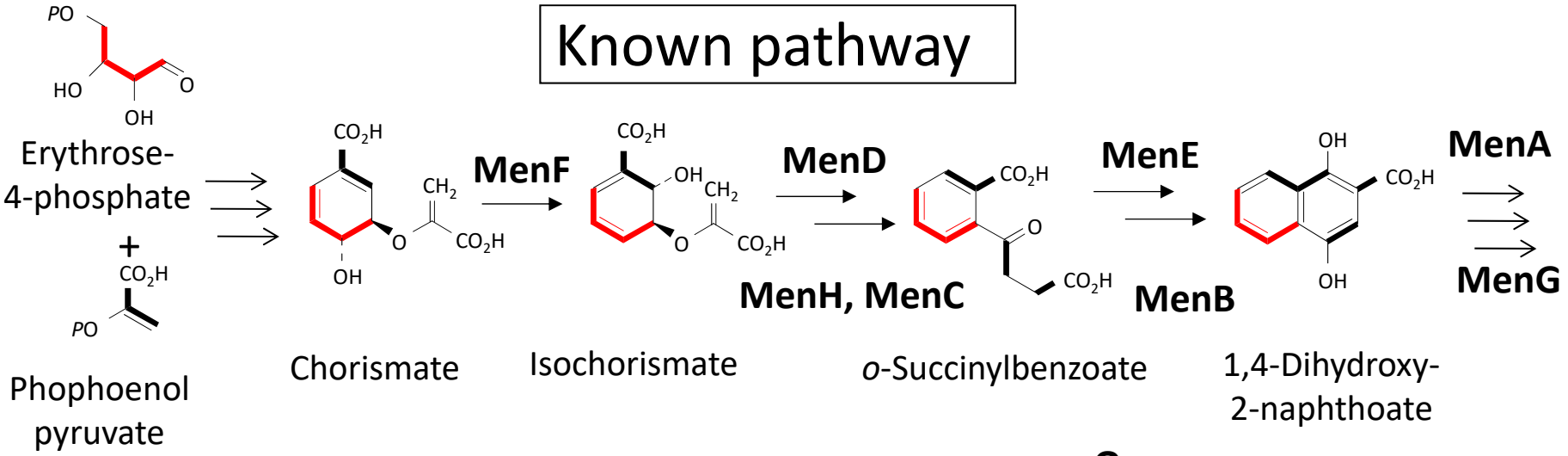
de novo synthesis

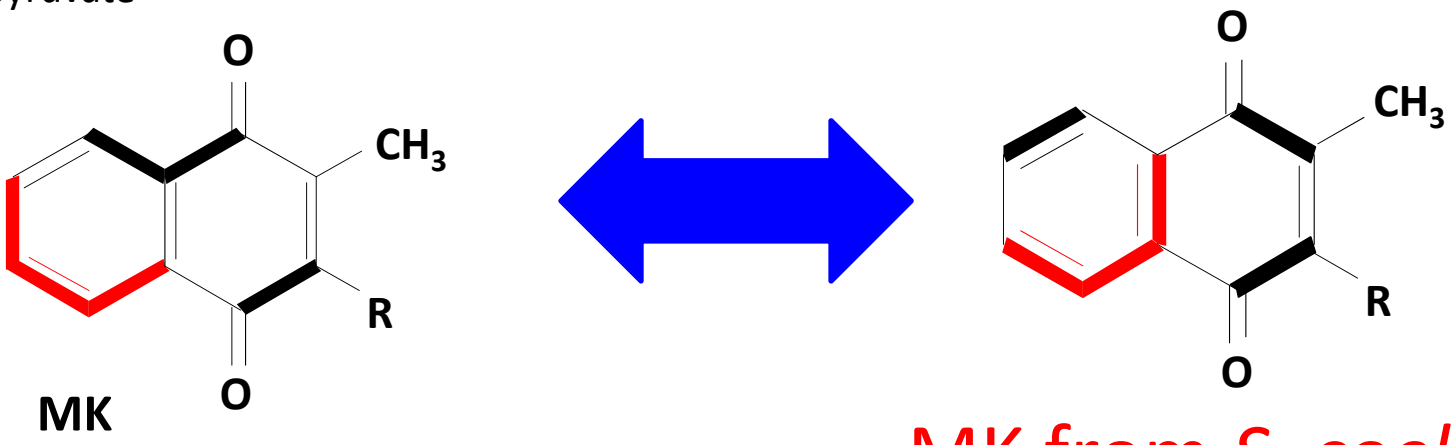
MK biosynthetic pathway in *E. coli*



Tracer experiments with *S. coelicolor* by Prof. Seto *et al.*

Known pathway

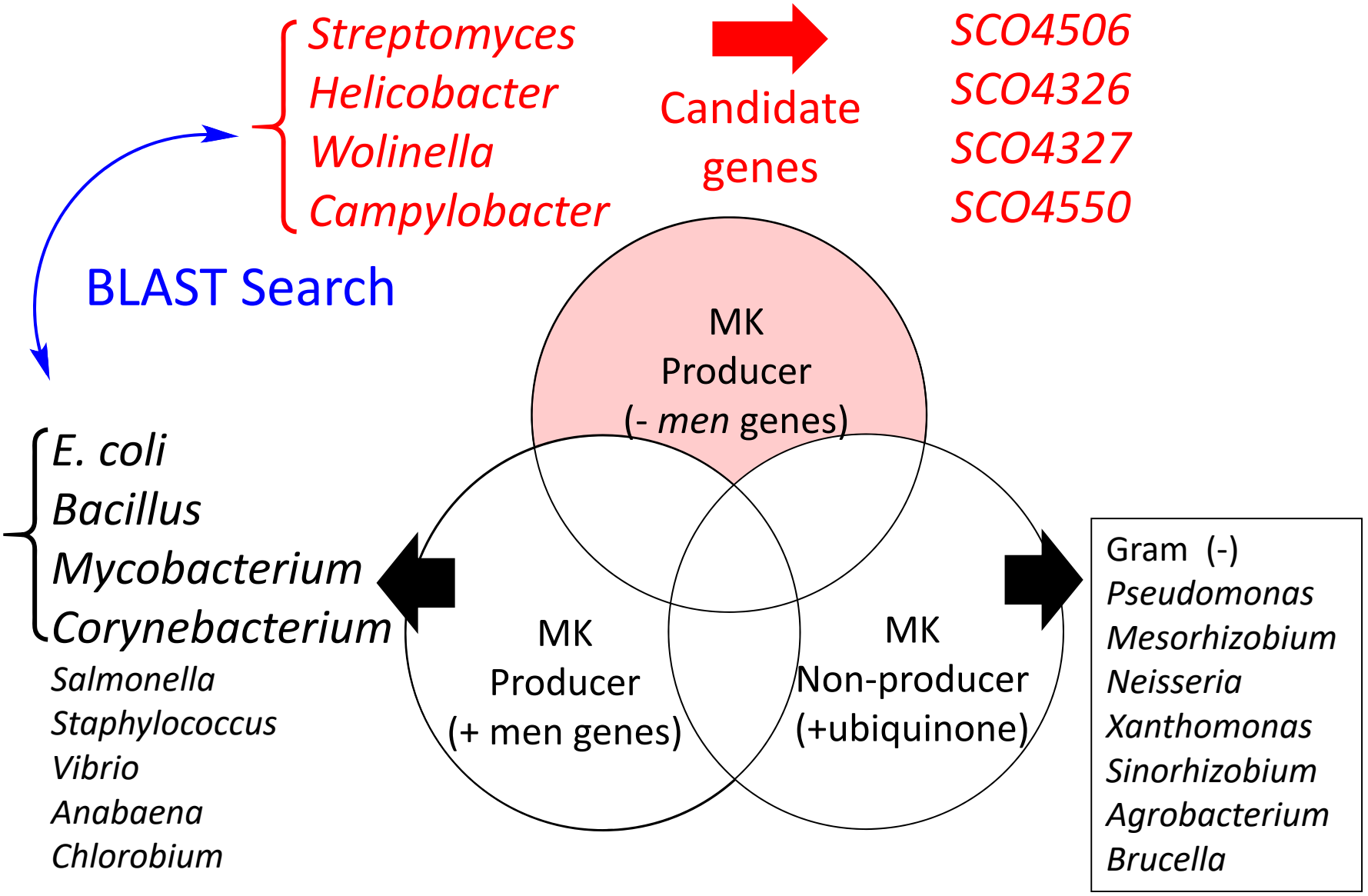




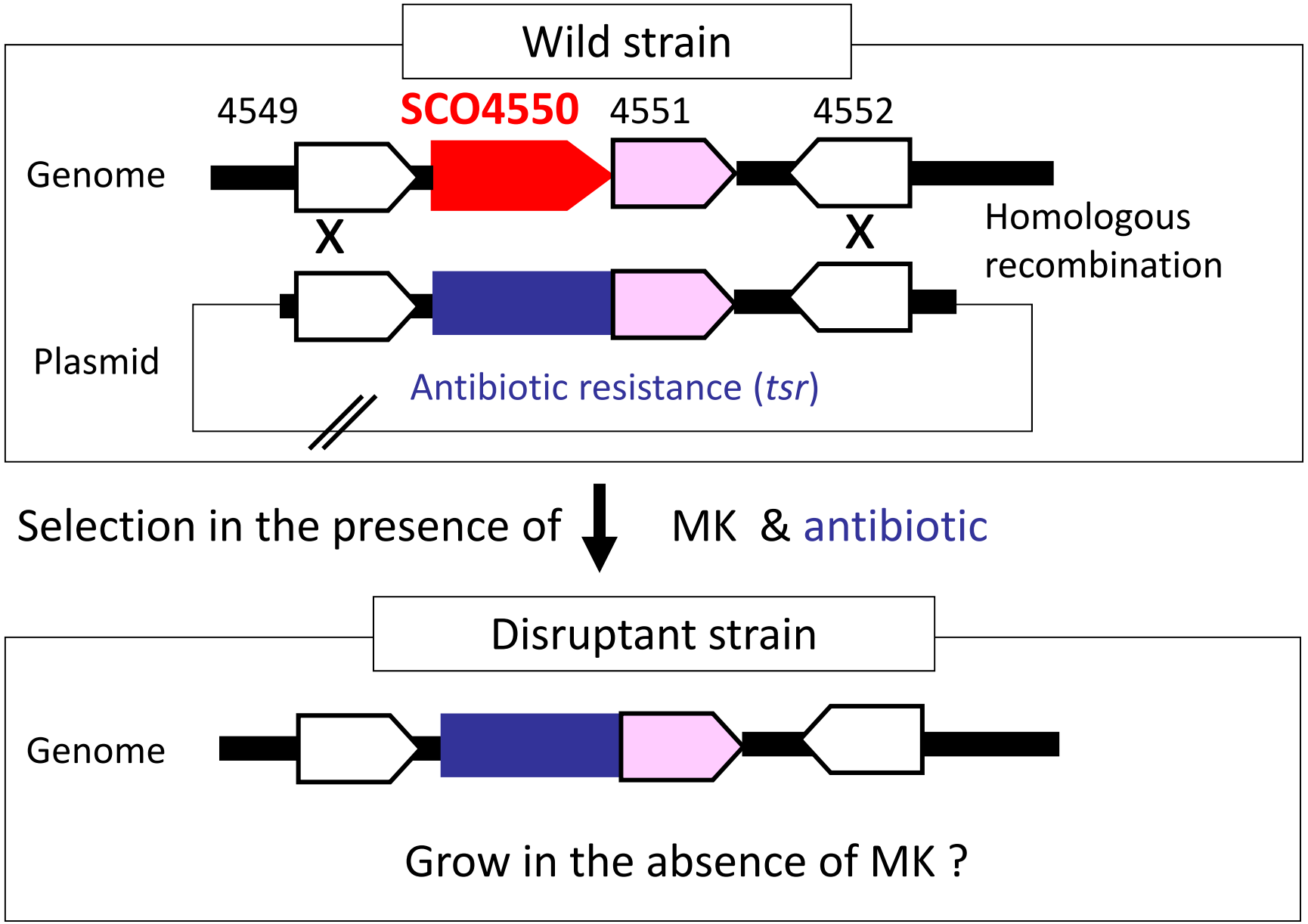
MK from *S. coelicolor*

J. Am. Chem. Soc. **130,** 5614-5615 (2008).

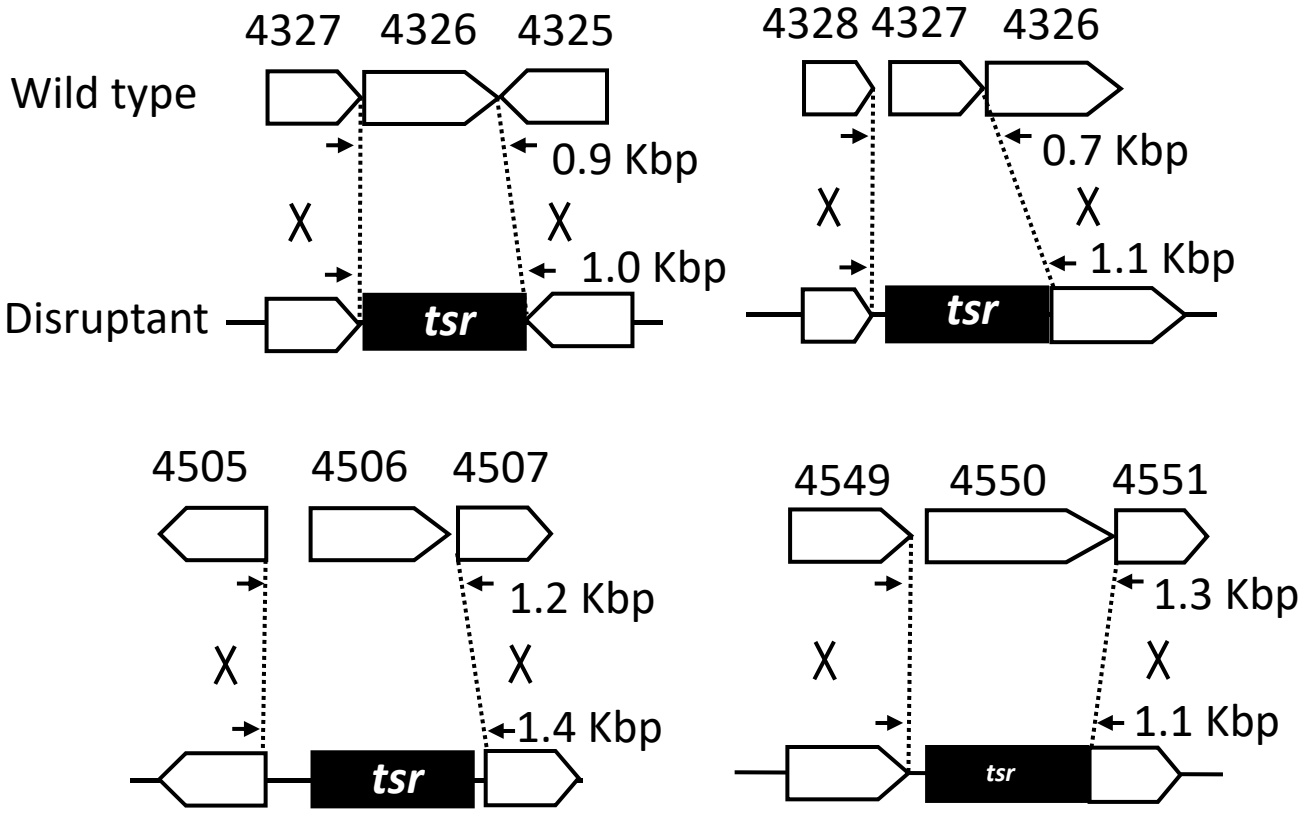
Candidate genes estimated by *in silico*

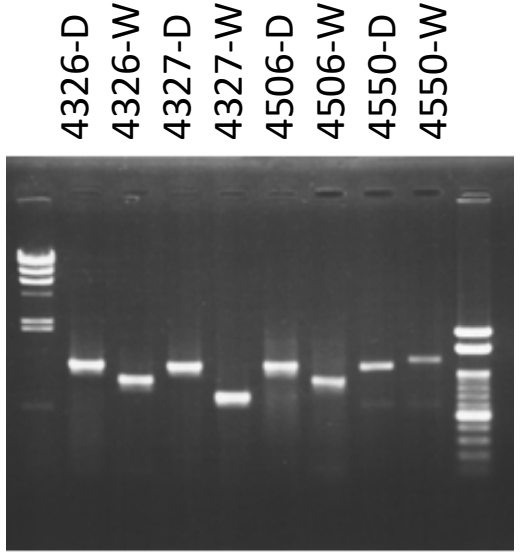


Disruption of candidate genes



PCR analyses of genomic DNAs of the candidate gene-disrupted strains





W: wild strain
D: disruptant

Phenotype of disruptant

Left; Wild strain
Right; SCO4550 disruptant

+
MK

-
MK

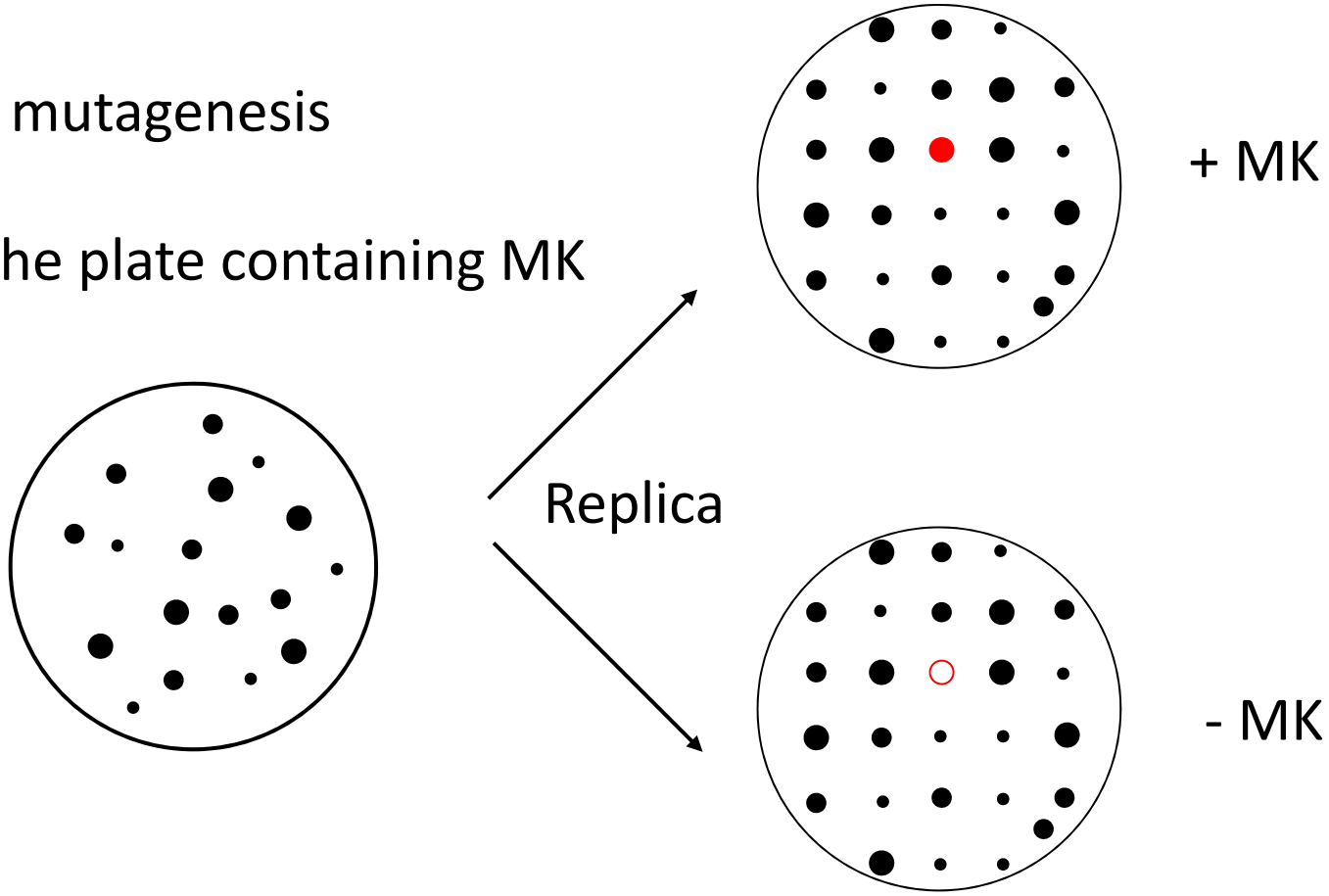
SCO4506, SCO4326 and SCO4327-disruptants also required MK for their growth

Isolation of mutants requiring MK for their growth by mutagenesis

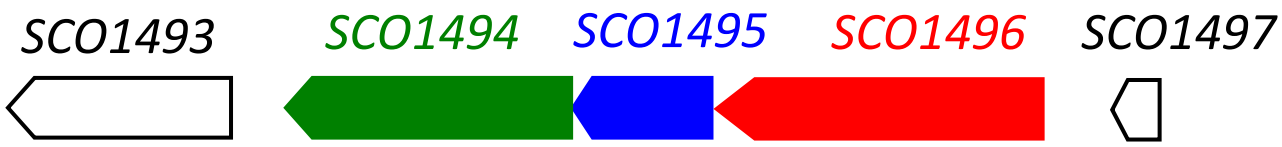
Spores of *Streptomyces coelicolor*

NTG mutagenesis

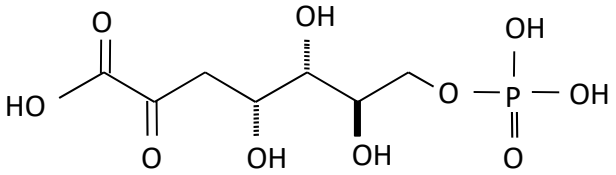
Spread on the plate containing MK



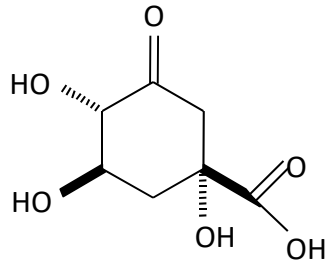
Some mutants were complemented with *SCO1494*, *SCO1495* and *SCO1496* genes



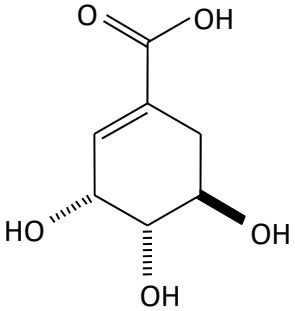
3-Dehydroquinate synthase — Shikimate kinase I — Chorismate synthase



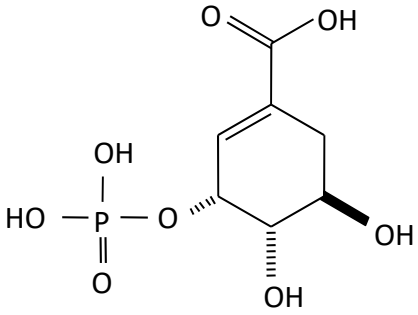
2-Dehydro-3-deoxy-D-arabino-heptonate 7-phosphate



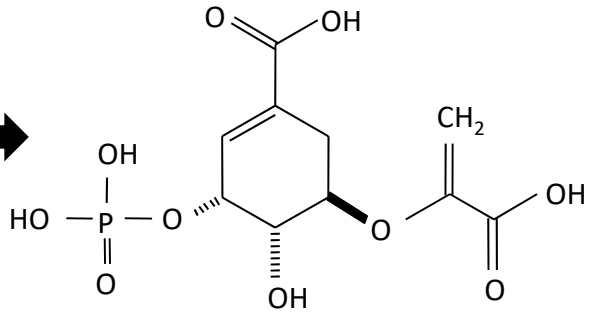
3-Dehydroquinate

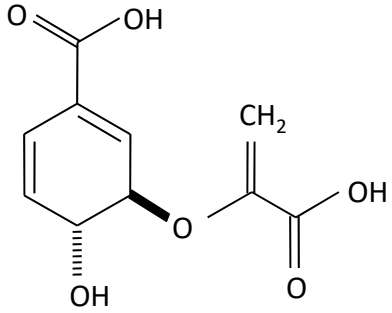


Shikimate



Shikimate 3-phosphate

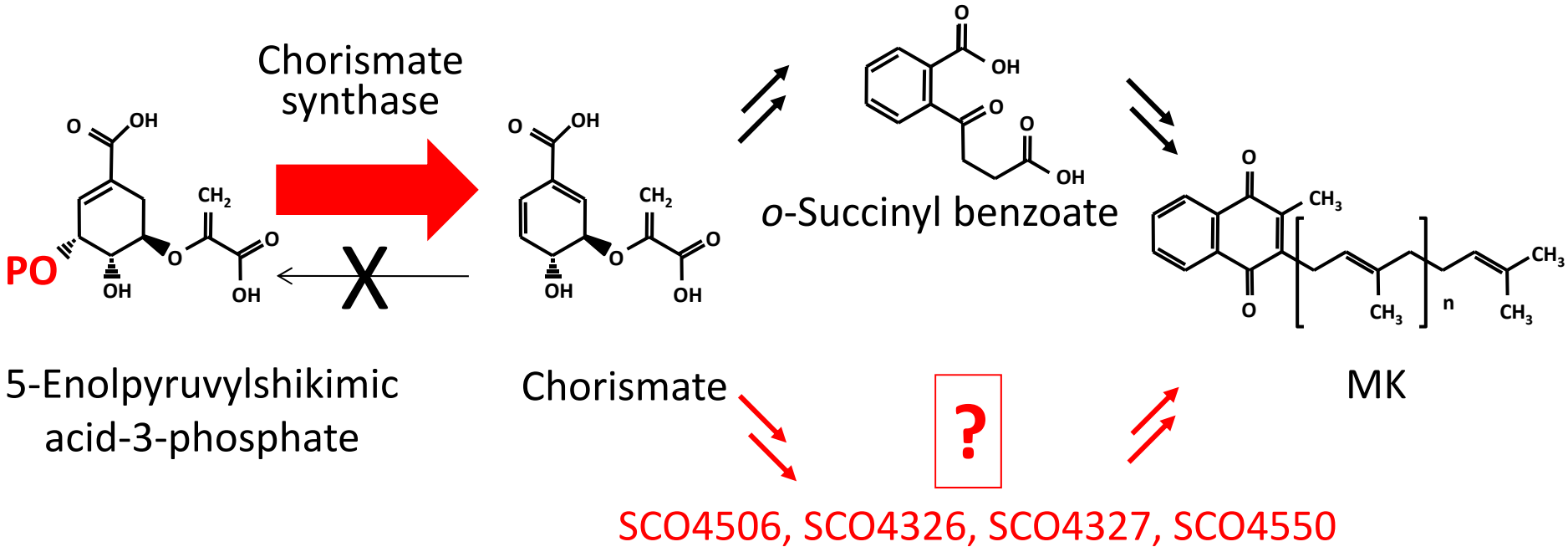




Chorismate

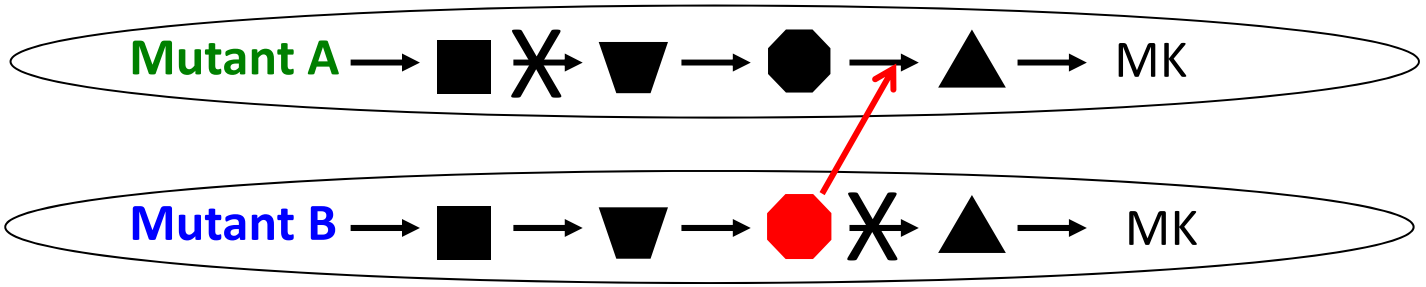
Characterization of the mutant

One mutant can grow in the presence of chorismate



Assay method to isolate intermediates

Cultivation of two disruptants independently in the presence of MK



Accumulated intermediate

↓

Removal of MK from culture broth of **mutant B**

↓

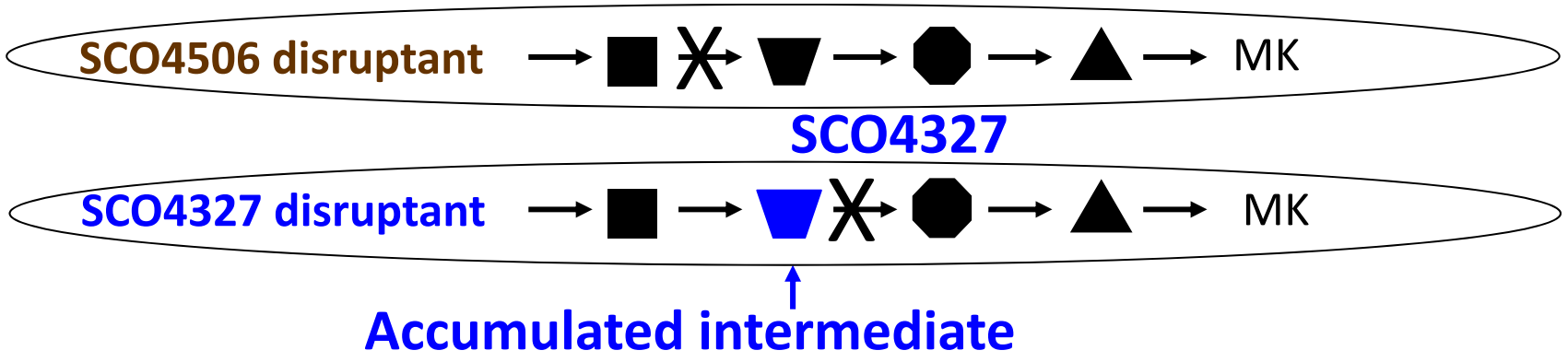
Examination if **mutant A** can grow on the plate containing 

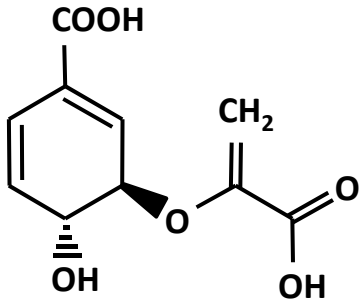
Estimated relative blocked points

SCO4506 → SCO4327 → SCO4550 → SCO4326 → MK

Isolation of an intermediate accumulated in SCO4327 disruptant

Cultivation of two disruptants independently in the presence of MK





Chorismate

SCO4327-disruptant accumulated futalosine

Cultivate SCO4327 disruptant with MK (5 L)

⇓

Extract MK by ethyl acetate

⇓

Dowex column chromatography

⇓

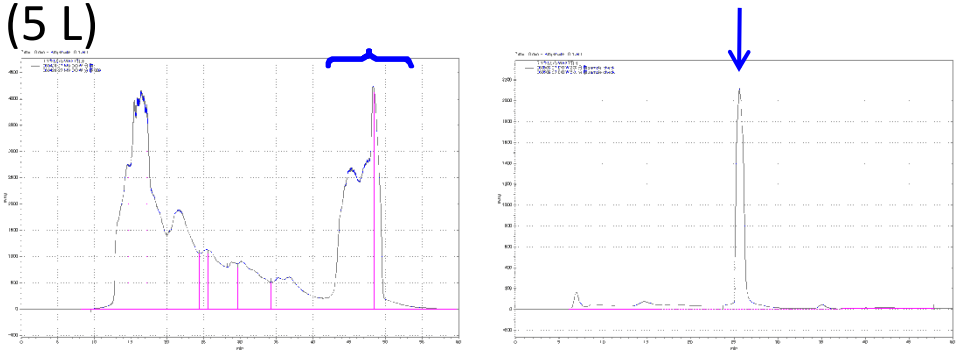
HP20 column chromatography

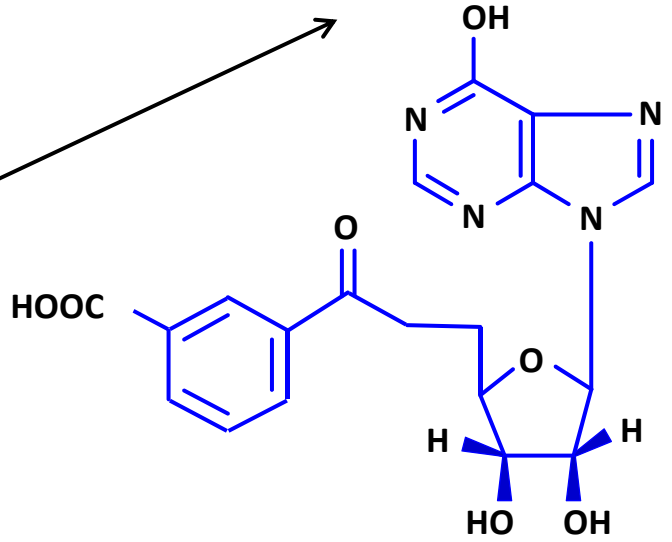
⇓

Preparative HPLC (twice)

⇓

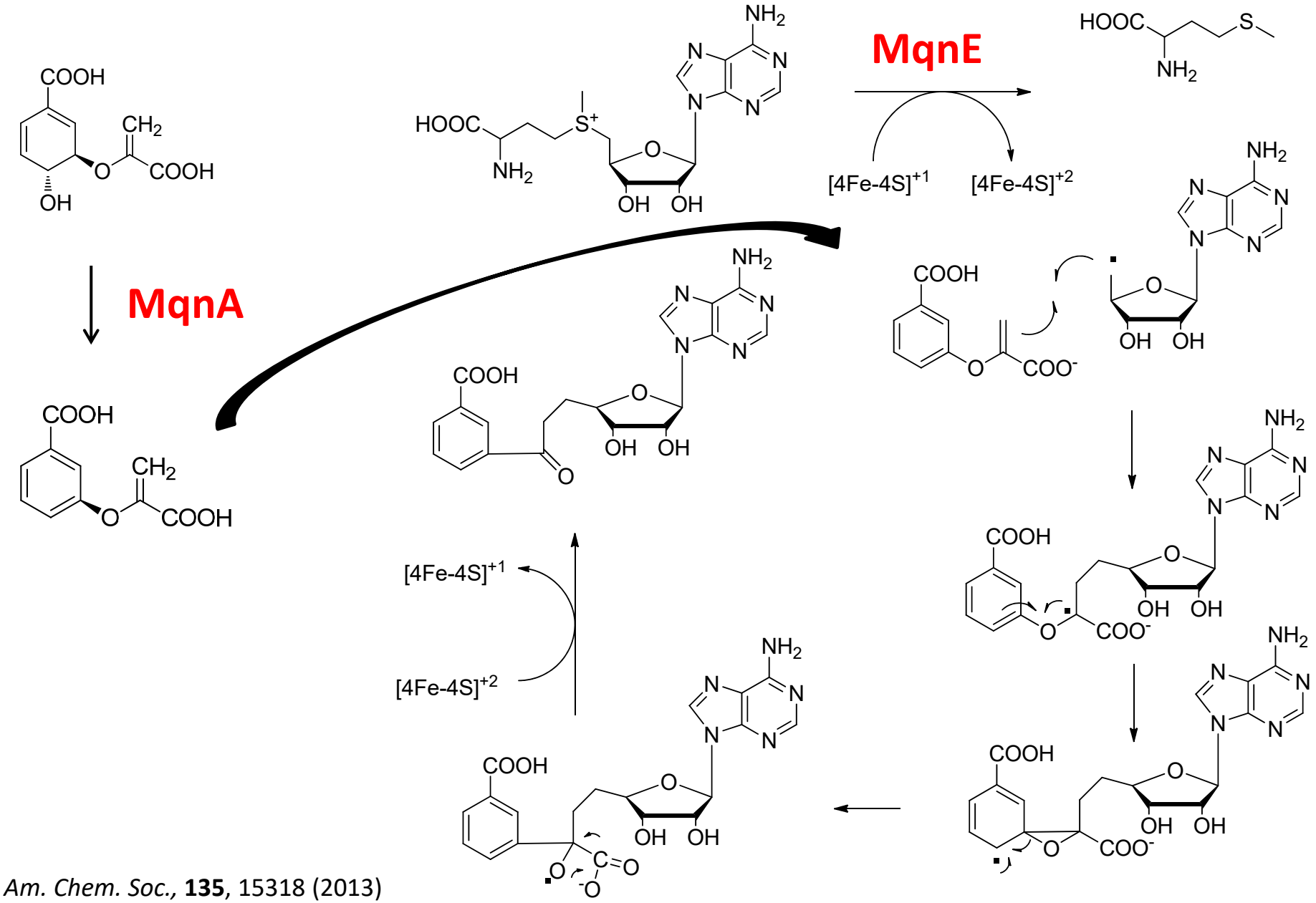
NMR, MS analyses





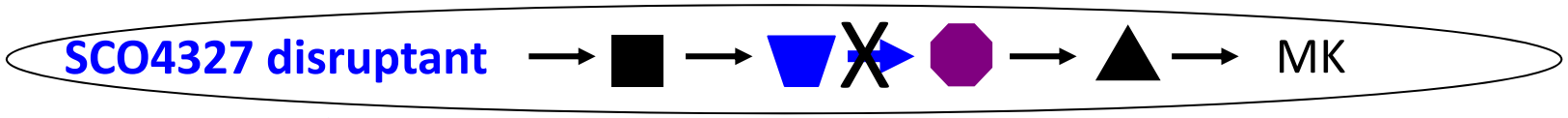
Futalosine
previously isolated from *Streptomyces*
(*Chem. Pharm. Bull*., **47,** 1032-1034, 1999)

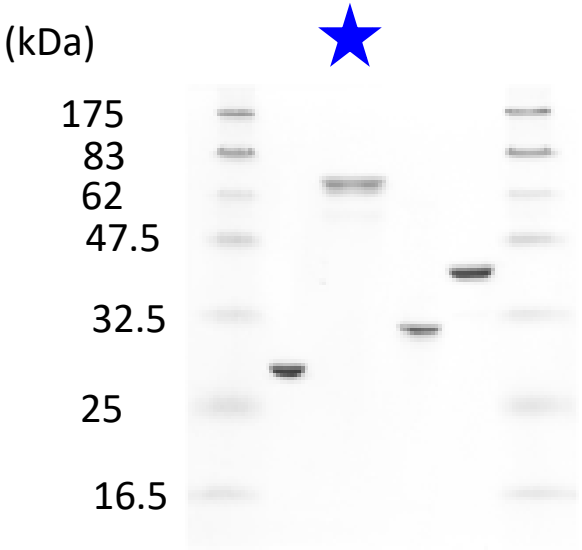
Reactions catalyzed by MqnA&E



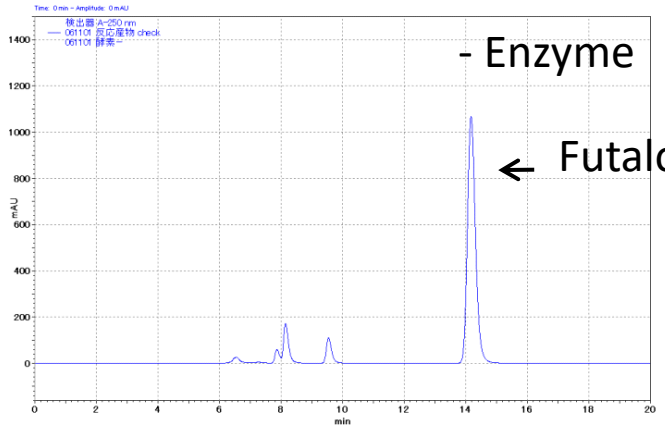
J. Am. Chem. Soc., **135**, 15318 (2013)

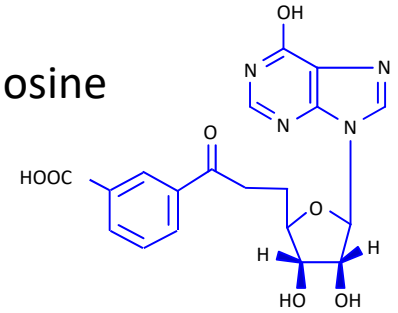
Recombinant TTHA0556 (SCO4327) converted futalosine into the next one

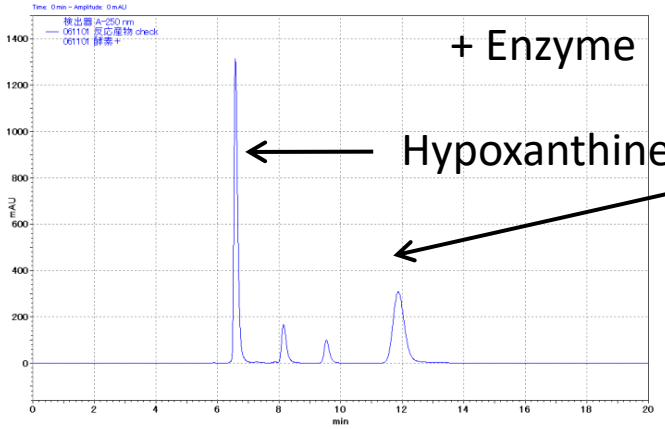


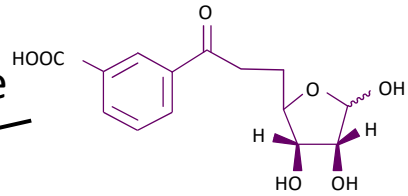


★ MBP-fused recombinant TTHA0556 (An orthologue of SCO4327 in *Thermus thermophilus* HB8)



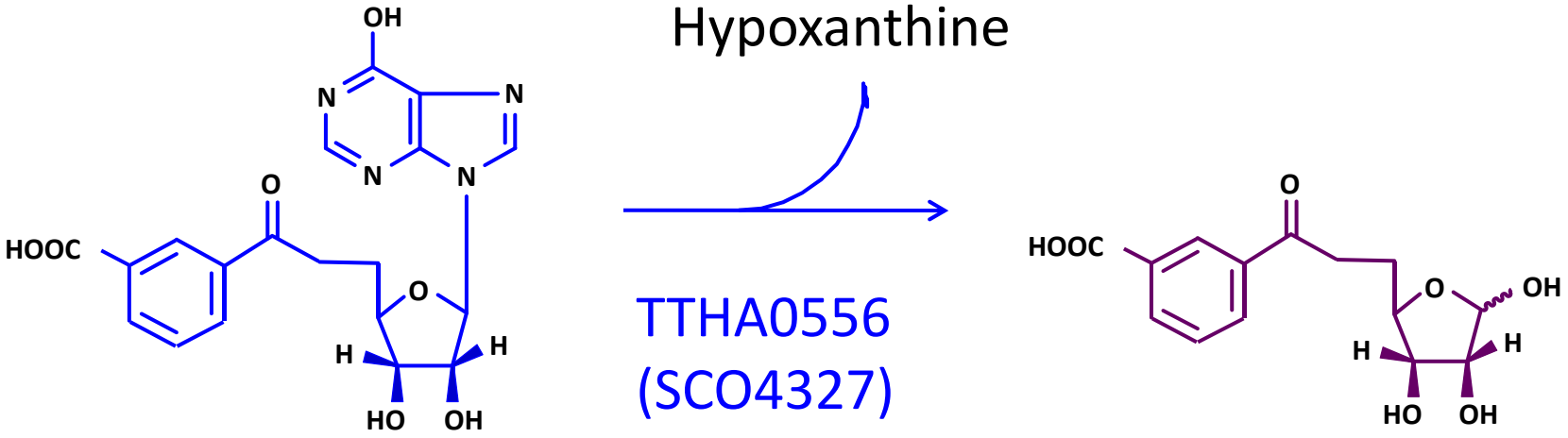






DHFL

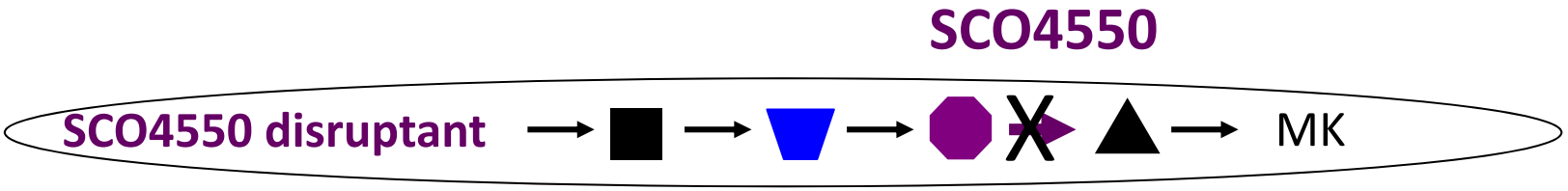
SCO4327 catalyzed the reaction to release hypoxanthine

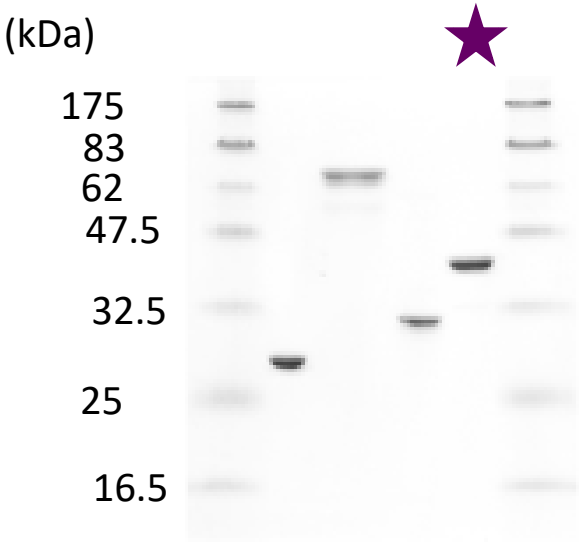


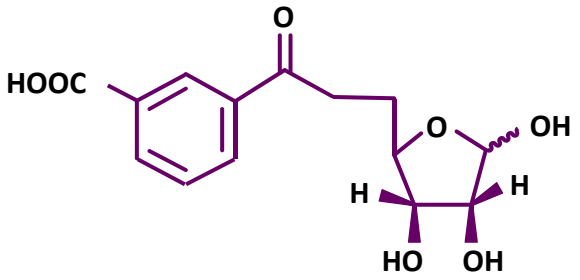
Futalosine

De-hypoxanthine futalosine (DHFL)

An attempt to convert DHFL to the next one by recombinant TTHA1092 (SCO4550)







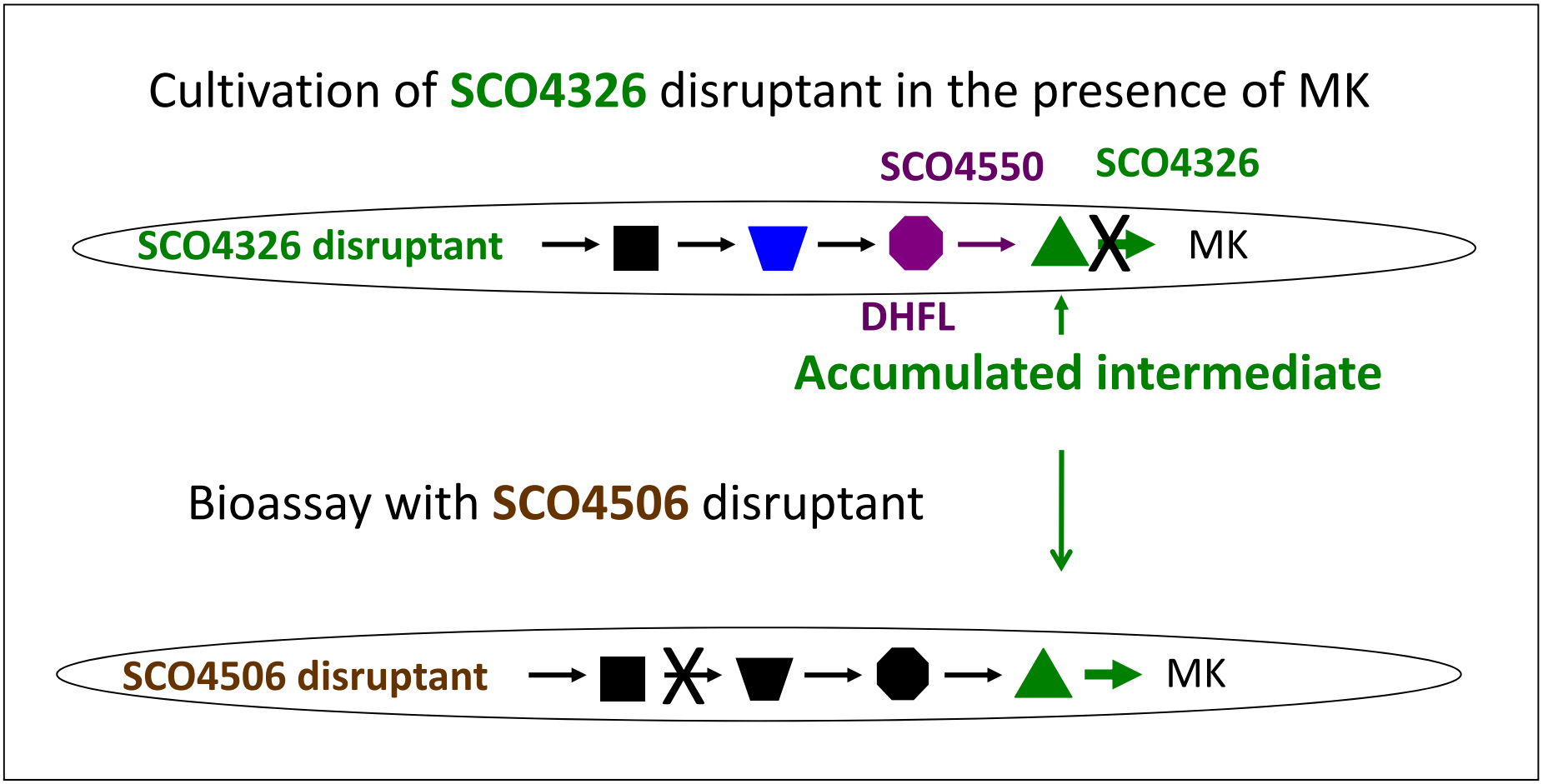
DHFL

TTHA1092
(SCO45507)

No products

★ MBP-fused recombinant TTHA1092
(An orthologue of SCO4550 in *Thermus thermophilus* HB8)

Isolation of an intermediate accumulated in SCO4326 disruptant



An intermediate accumulated in SCO4326 disruptant

Cultivate SCO4326 disruptant with MK (5 L)

⇓

Extract MK by ethyl acetate

⇓

Dowex column chromatography

⇓

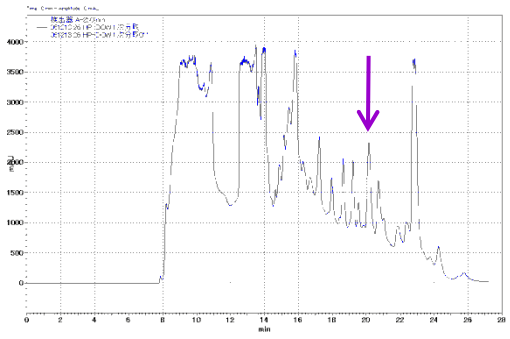
HP20 column chromatography

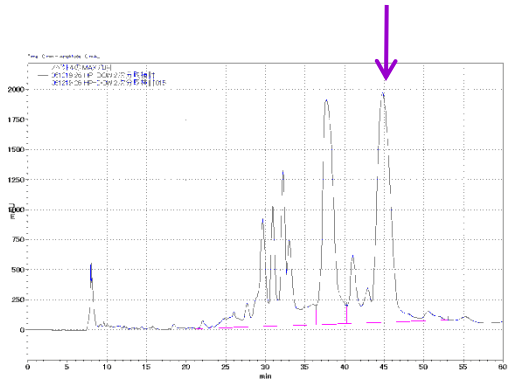
⇓

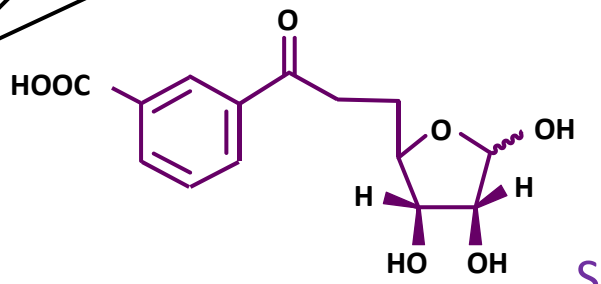
Preparative HPLC (twice)

⇓

NMR, MS analyses

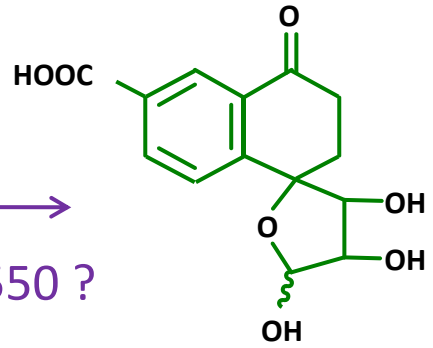






DHFL

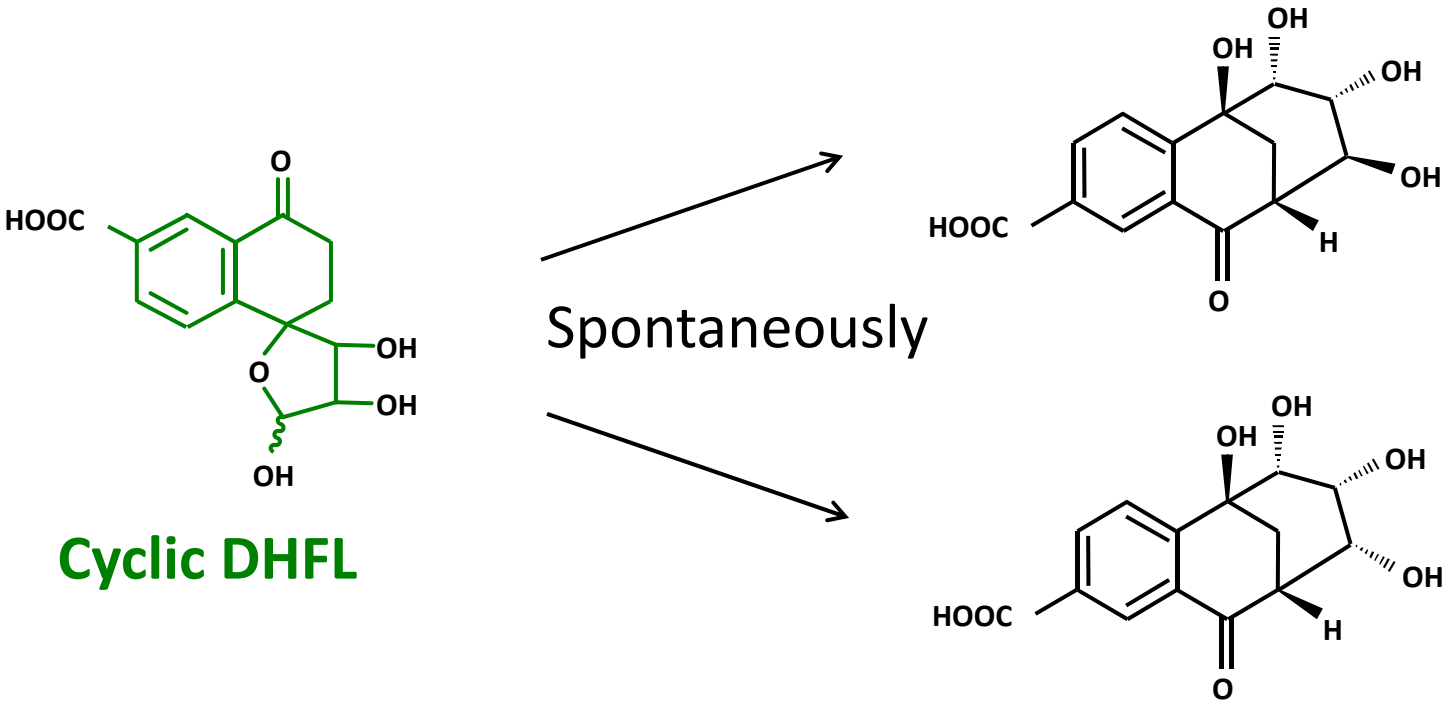
SCO4550 ?



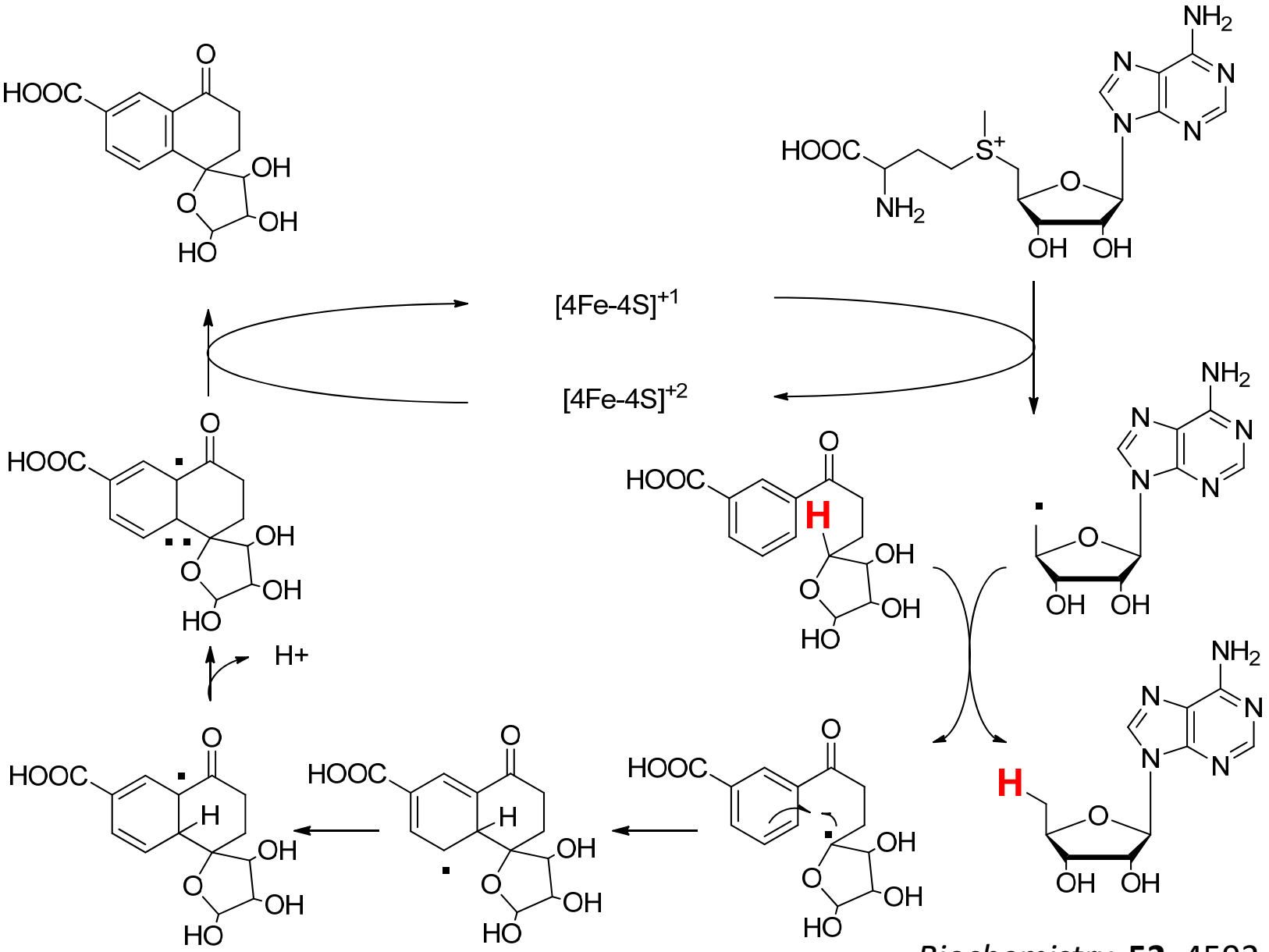
Cyclic DHFL

Cyclic DHFL was easily converted to shunt products

1 year to purify **cyclic DHFL**

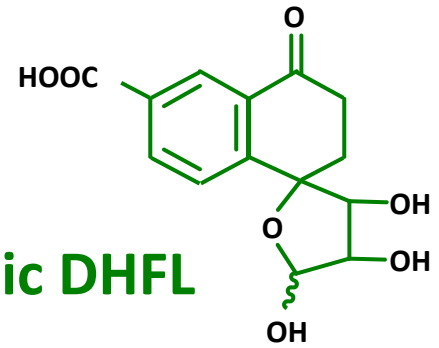


Reaction catalyzed by MqnC



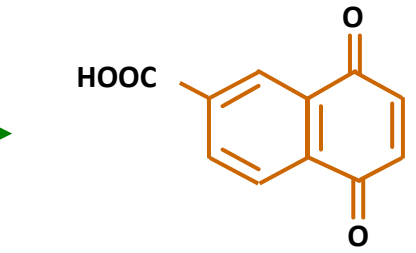
Biochemistry, **52**, 4592 (2013)

Reaction catalyzed by TTHA1568 (SCO4326)



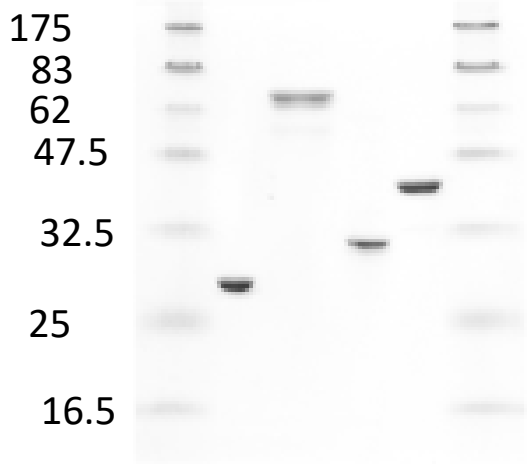
Cyclic DHFL

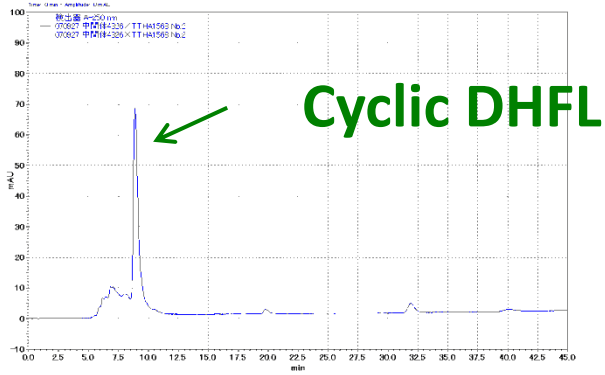
TTHA1568 (SCO4326)

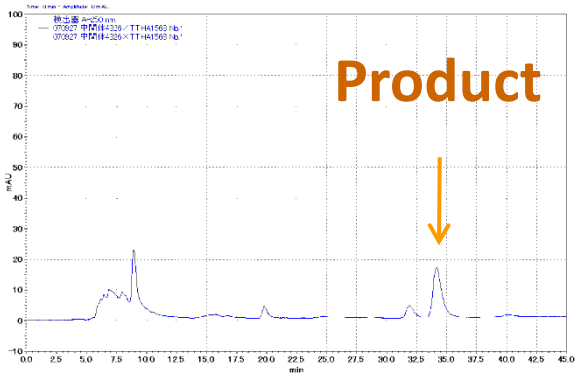


1,4-Naphthoquinone-6-carboxylic acid

(kDa) ★

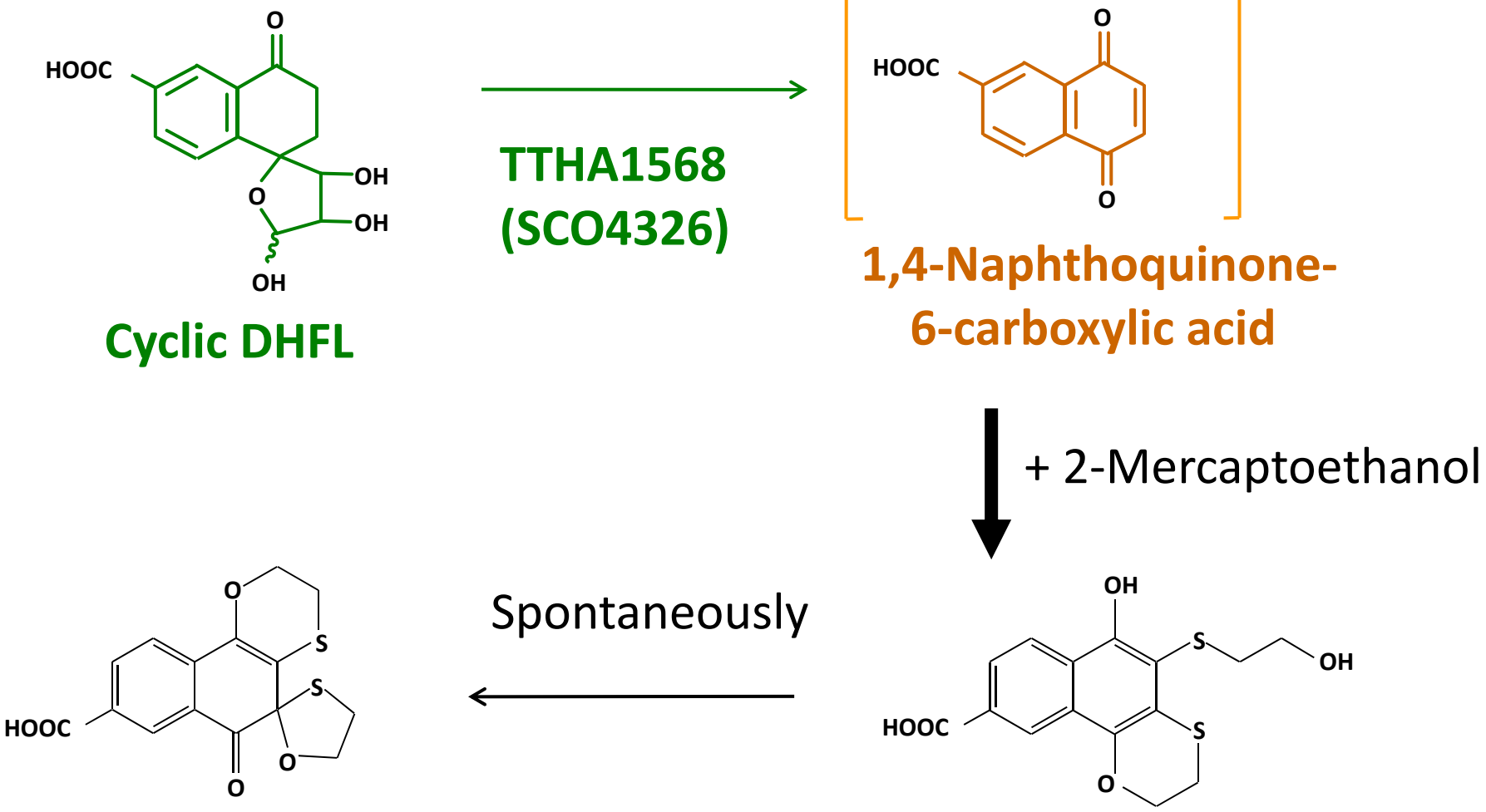




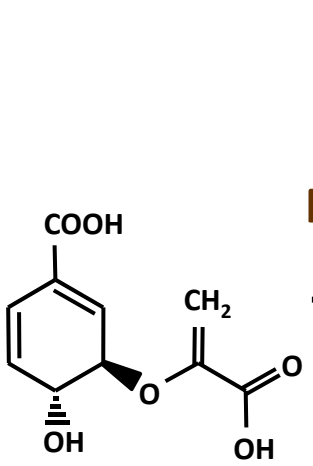


★His-tagged recombinant TTHA1568 (an orthologue of SCO4326 in *Thermus thermophilus* HB8)

Reaction product reacts on 2-mercaptoethanol

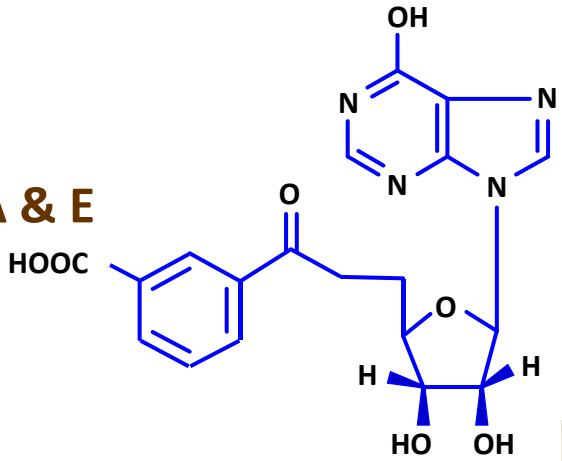


Novel pathway for MK biosynthesis



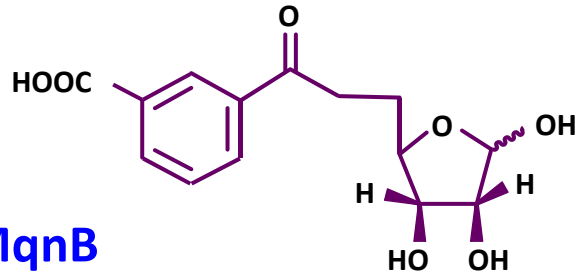
Chorismate

MqnA & E



Futalosine

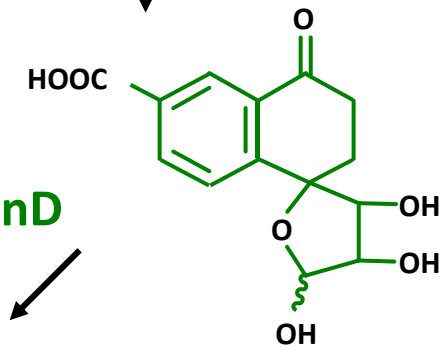
MqnB



DHFL

(A novel compound)

MqnC

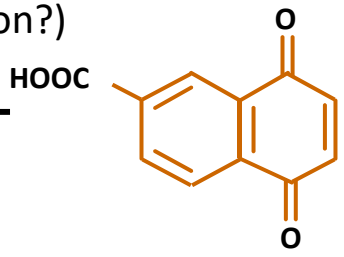


Cyclic DHFL

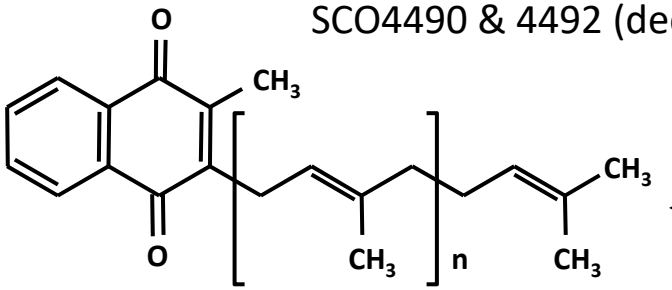
(A novel compound)

MqnD

SCO4491 (prenylation?)
SCO4556 (methylation?)
SCO4490 & 4492 (decarboxylation?)



1,4-Naphthoquinone-
6-carboxylic acid



MK

Science, **321,** 1670-1673 (2008).

Distribution of the pathway

At least three orthologs were identified in the following microorganisms:

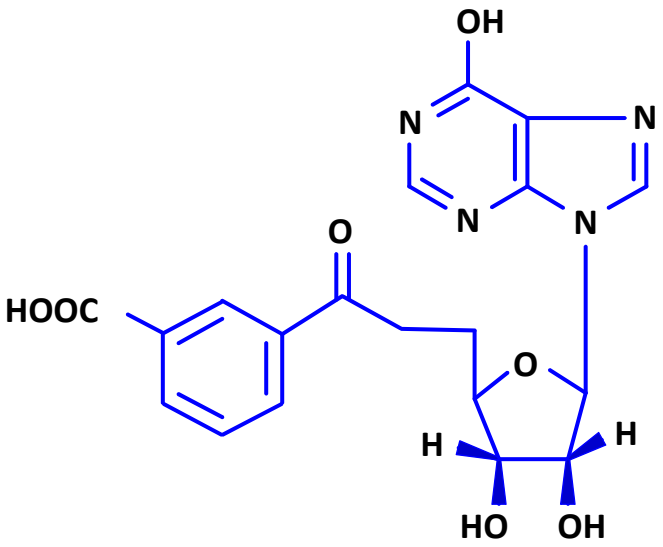
- Epsilon category (Gram positive) (*Helicobacter*, *Wolinella*, *Thiomicrospira*, *Campylobacter*, *Arcobacter*, *Nitratiruptor*, *Sulfurovum*)
- Delta category (Gram negative) (*Geobacter*, *Pelobacter*, *Desulfovibrio*, *Desulfococcus*, *Anaeromyxobacter*, *Syntrophobacter*)
- Acidobacteria (Gram negative) (*Acidobacteria, Solibacter*)
- Synergistetes (Gram negative) (*Syntrophomonas*)
- Bacillales of Firmicutes (Gram positive) (*Bacillus halodurans Bacillus clausii*)
- Lactobacillus category of Firmicutes (Gram positive) (*Symbiobacterium*)
- Clostridia category of Firmicutes (Gram positive) (*Carboxydothermus*, *Desulfotomaculum*, *Pelotomaculum*, *Desulforudis*, *Heliobacterium*, *Moorella*)
- Actinobacteria (Gram positive) (*Streptomyces*, *Frankia*, *Acidothermus*, *Salinispora*)
- Planctomyces (Gram negative) (*Rhodopirellula*)
- Chlamydia (Gram negative) (*Chlamydia, Chlamydophila*)
- Spirochete (Gram negative) (*Leptospira*)
- Green nonsulfur bacteria (Gram positive) (*Herpetosiphon*)
- Deinococcus-Thermus (Gram positive) (*Deinococcus, Thermus*)
- Hyperthermophilic bacteria (Gram negative) (*Aquifex*)

No bacteria possessing both classical and alternative pathways

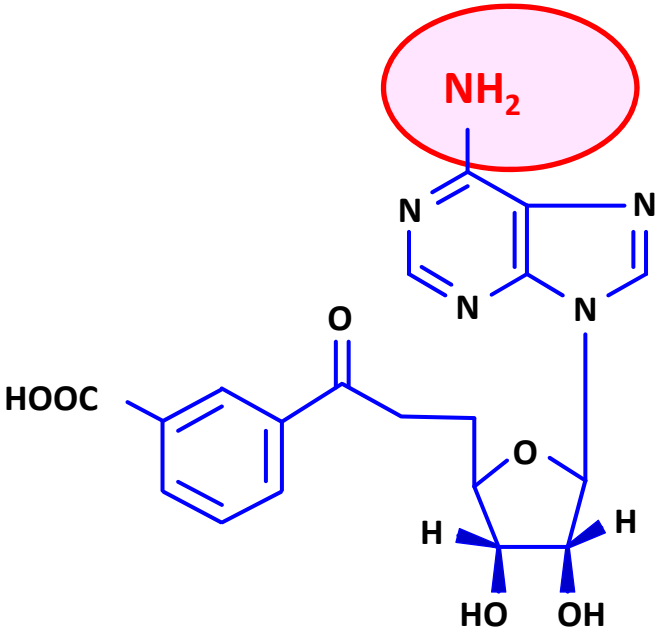
- Archaea: Euryarchaeota (*Archaeoglobus, Thermoplasma*)
- Crenarchaeota (*Ignicoccus*, *Pyrobaculum*, *Caldivirga*, *Thermoproteus*, *Nitrosopumilus*)

No bacteria possessing both ubiquinone and alternative pathways

ADF is an intermediate of futalosine pathway in some bacteria including *H. pylori*



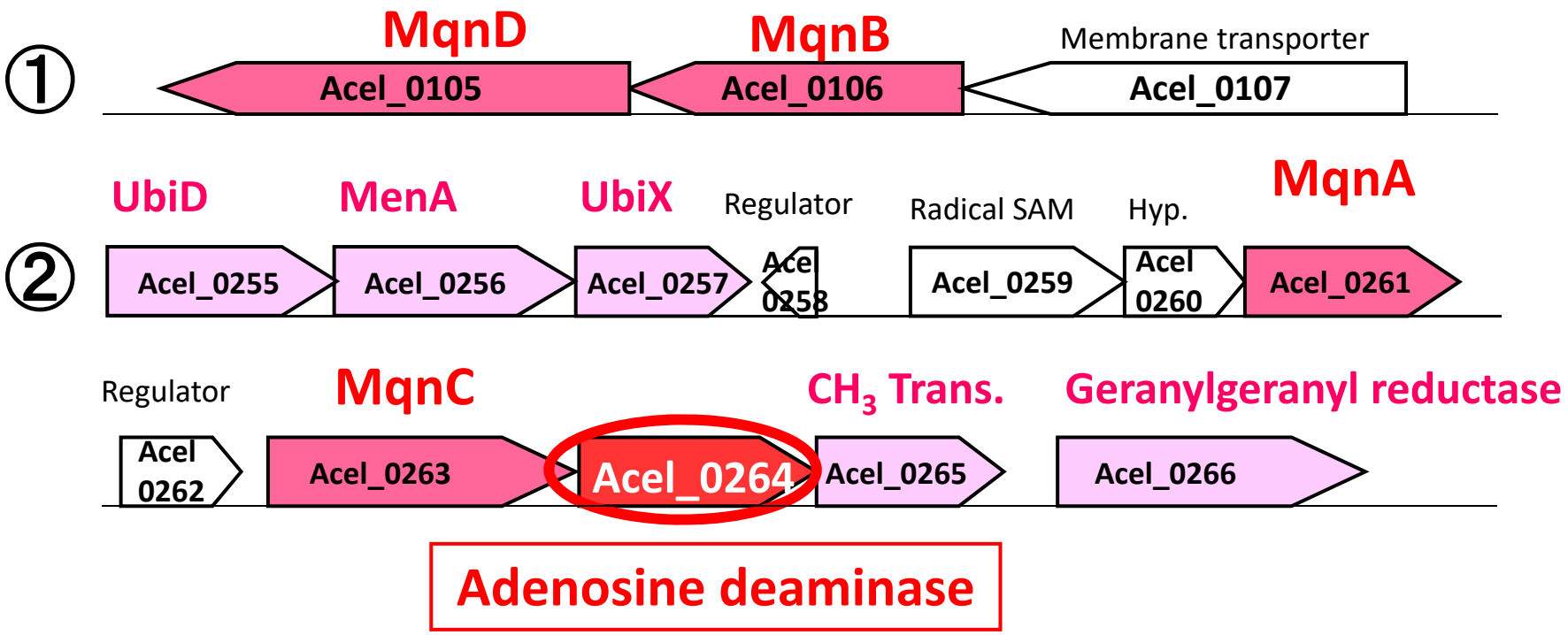
Futalosine



Aminodeoxyfutalosine (ADF)

Aminodeoxyfutalosine (ADF) is an intermediate ?

MK biosynthetic genes were clustered in two loci in *Acidothermus cellulolyticus* (actinobacterium)

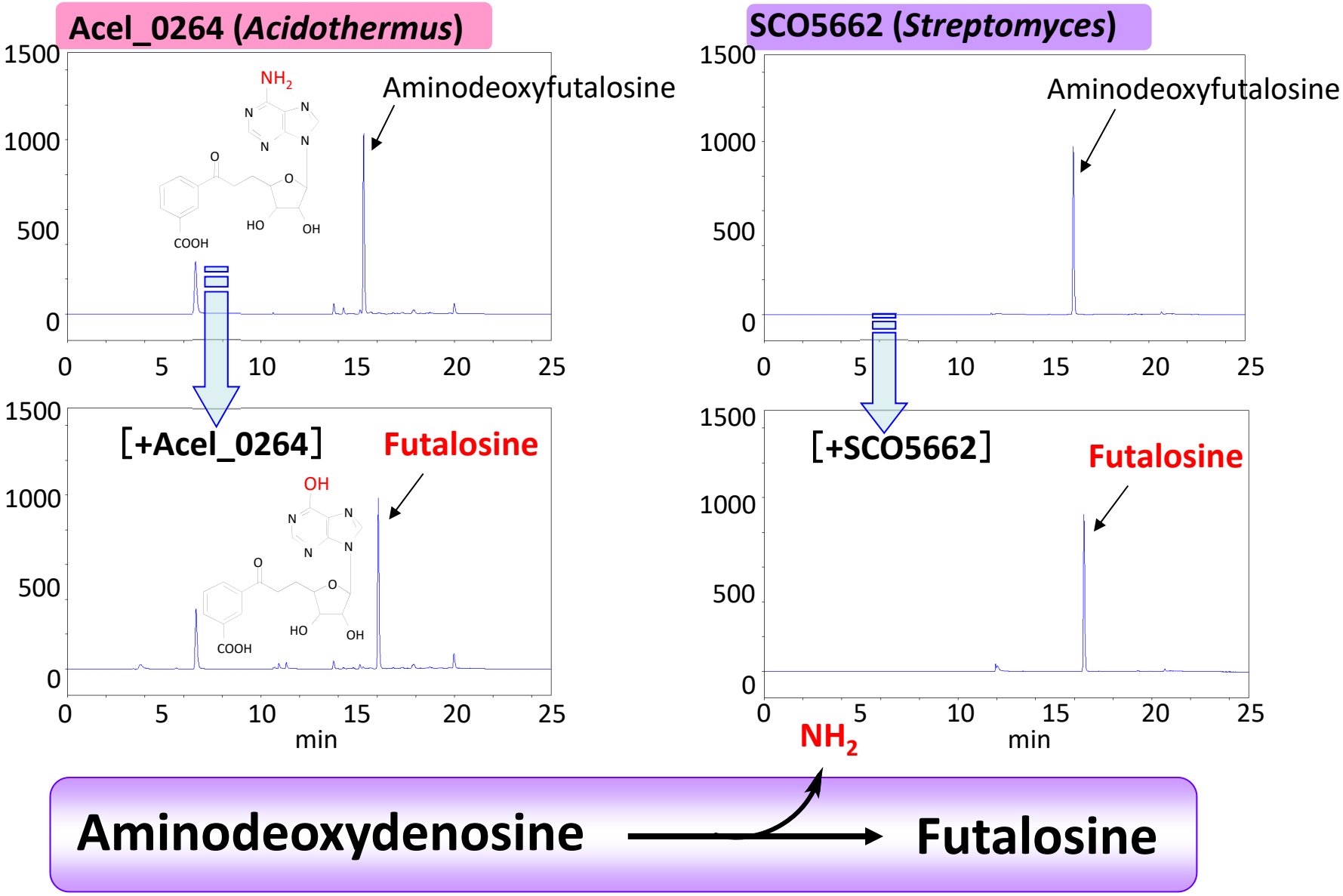


Adenosine deaminase

Adenine, Adenosine ≫ Hypoxanthine, Inosine in a cell

Functional analysis of adenosine deaminases

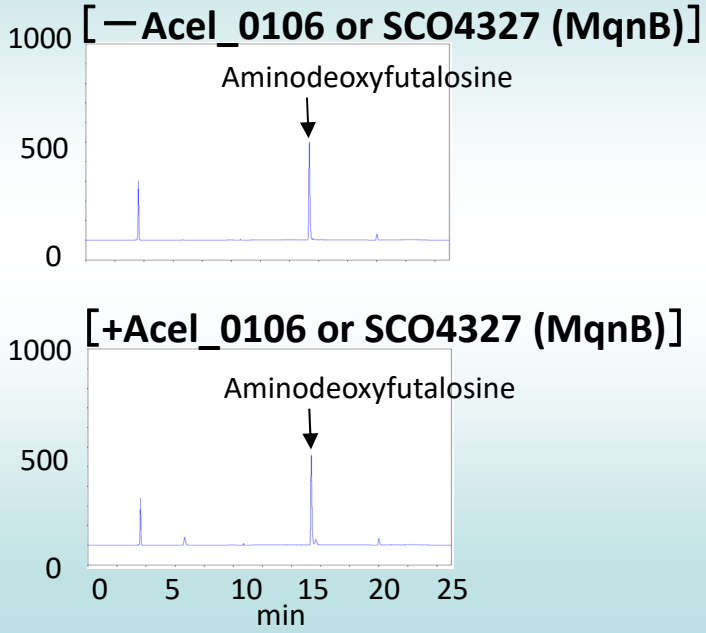
Substrate: Aminodeoxyfutalosine



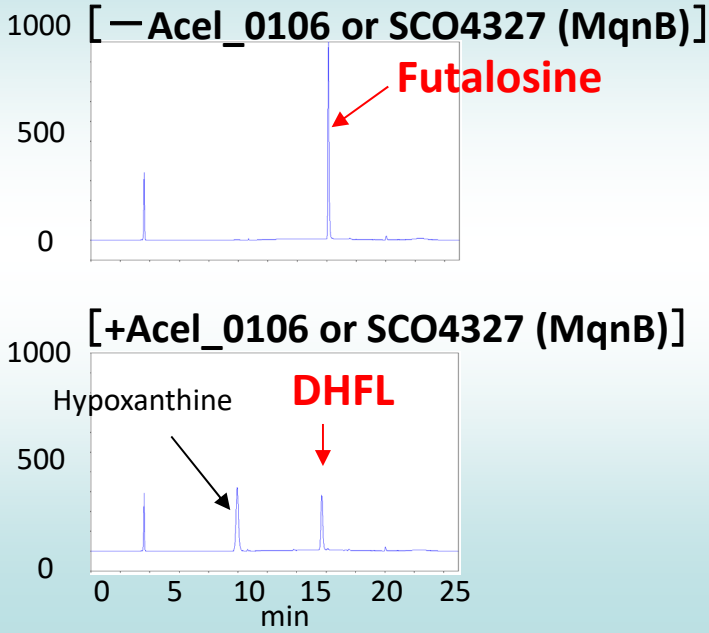
Aminodeoxydenosine → **Futalosine**

Functional analysis of futalosine hydrolase

Substrate: Aminodeoxyfutalosine

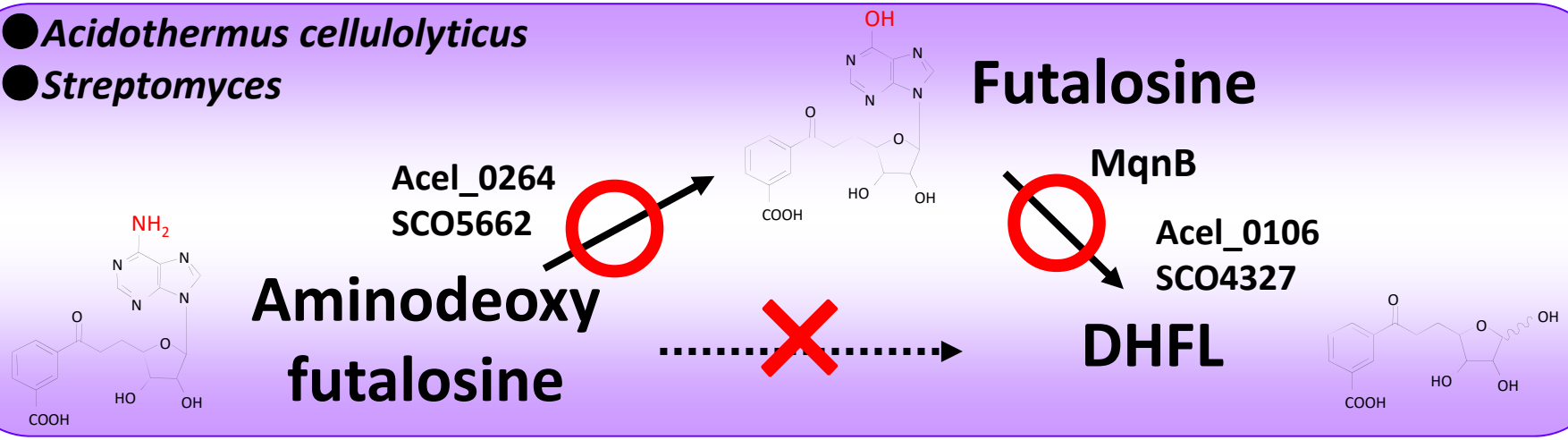


Substrate: Futalosine



● *Acidothermus cellulolyticus*

● *Streptomyces*

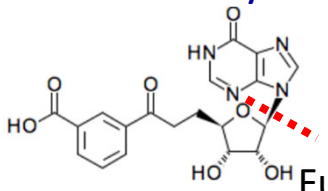


MqnB in *Helicobacter pylori*

Enzyme	*S. coelicolor*	*H. pylori*	E Value
MqnA	SCO4506	HP0778	$7 \times e^{-04}$
MqnB	SCO4327	HP0089	0.18
MqnC	SCO4550	HP0656	$1 \times e^{-84}$
MqnD	SCO4326	HP0152	$1 \times e^{-19}$

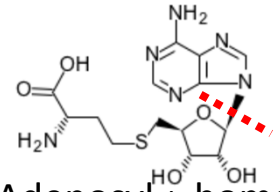
Low similarity

● MqnB（futalosine hydrolase）



Futalosine → Hypoxanthine + DHFL

● HP0089 (*S*-adenosylhomocysteine nucleosidase)

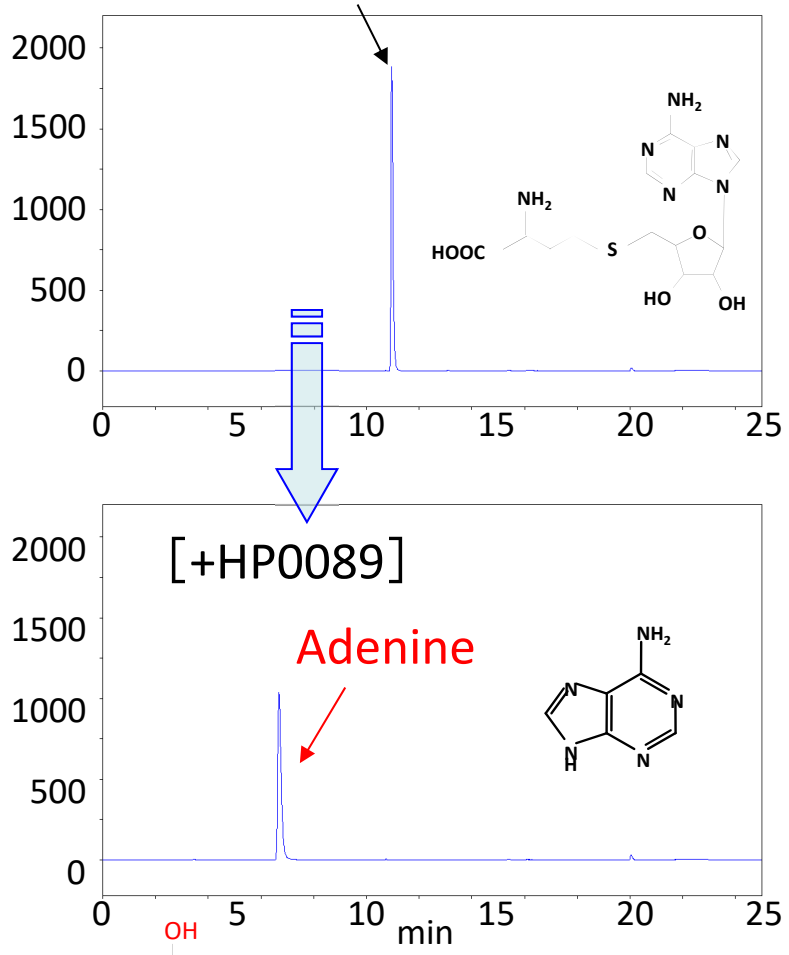


S-Adenosyl-L-homocysteine → Adenine + *S*-Ribosyl-L-homocysteine

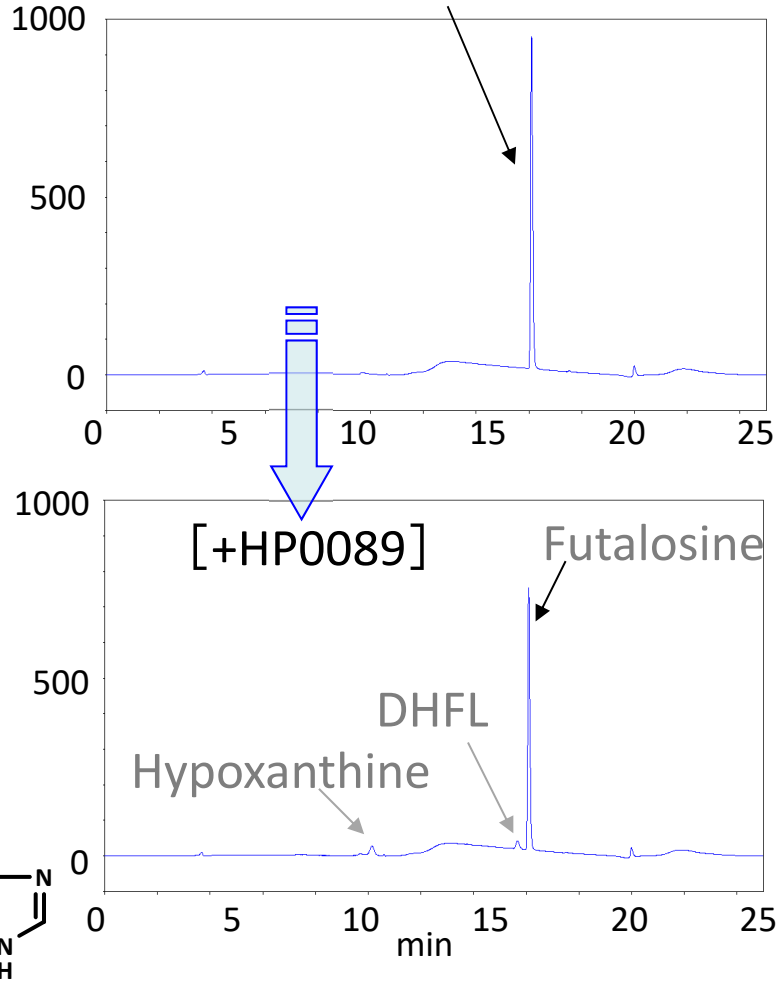
Does HP0089 function as MqnB ? ⇒ *in vitro* analysis

Functional analysis of HP0089 (futalosine hydrolase)

Substrate: *S*-adenosyl-L-homocysteine (control)



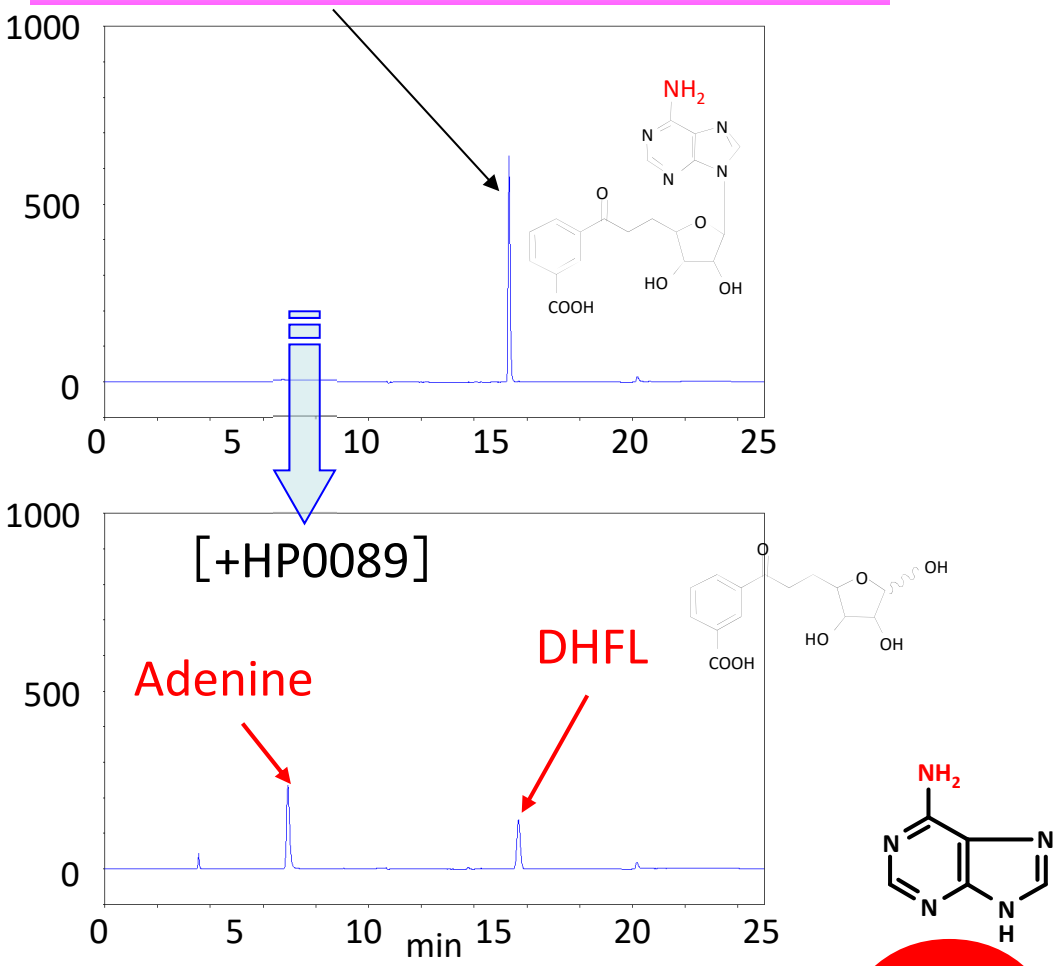
Substrate: Futalosine

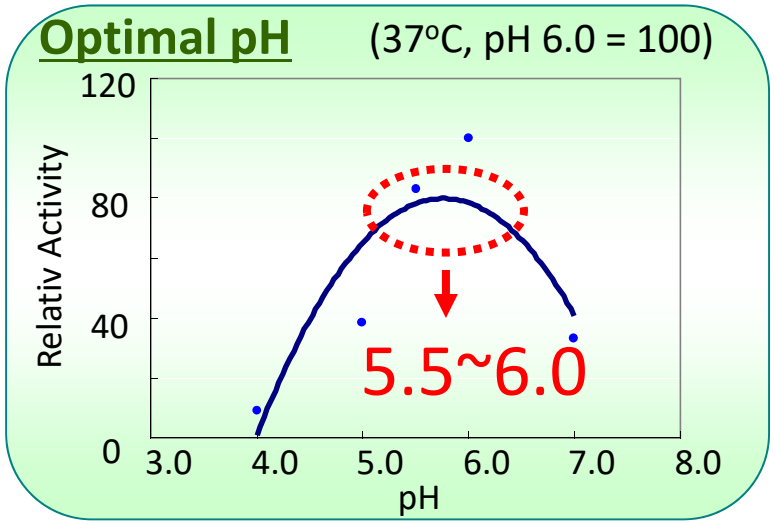


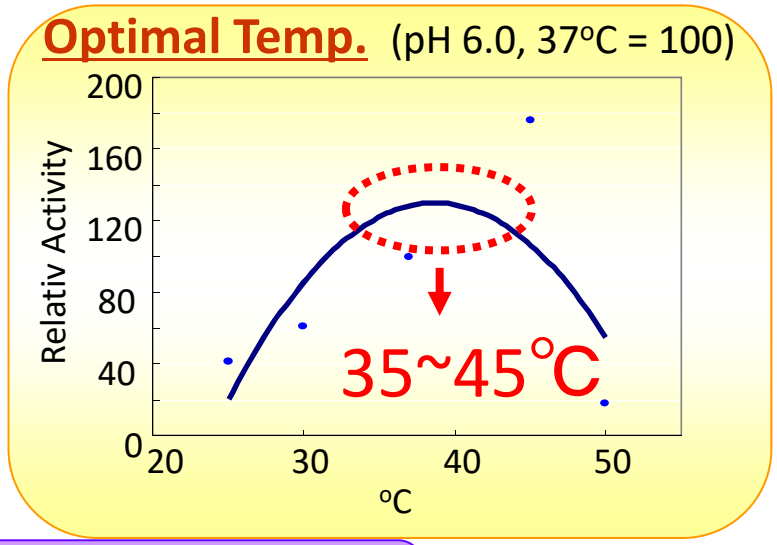
Futalosine ⋯✕⋯▶ **DHFL**

Functional analysis of HP0089 (futalosine hydrolase)

Substrate: Aminodeoxyutalosine



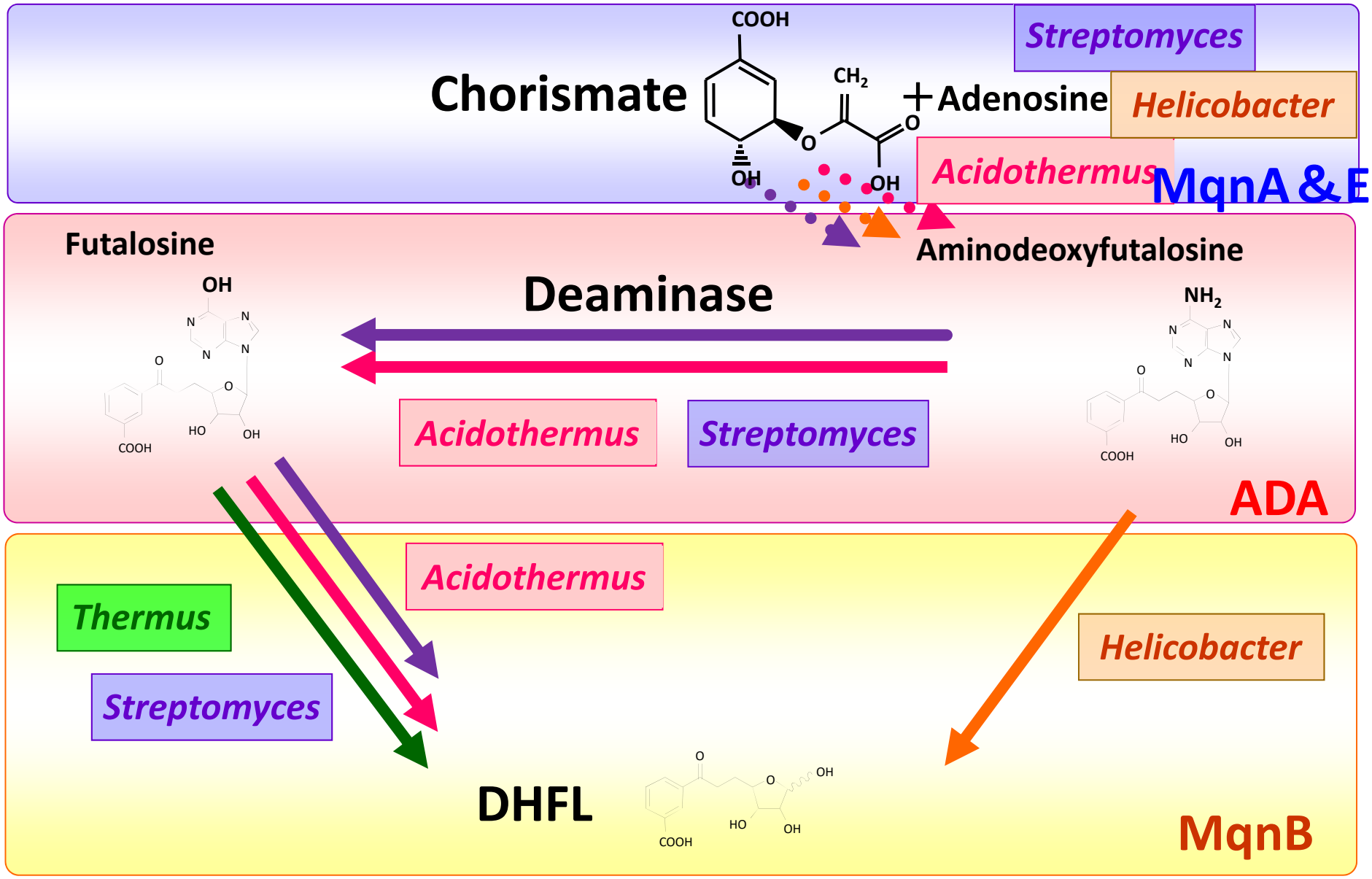




Aminodeoxyfutalosine → **DHFL**

Antimicrob. Agents Chemother., **55**, 913 (2011)

Diversity of the early step of MK pathway



Menaquinone is essential for growth

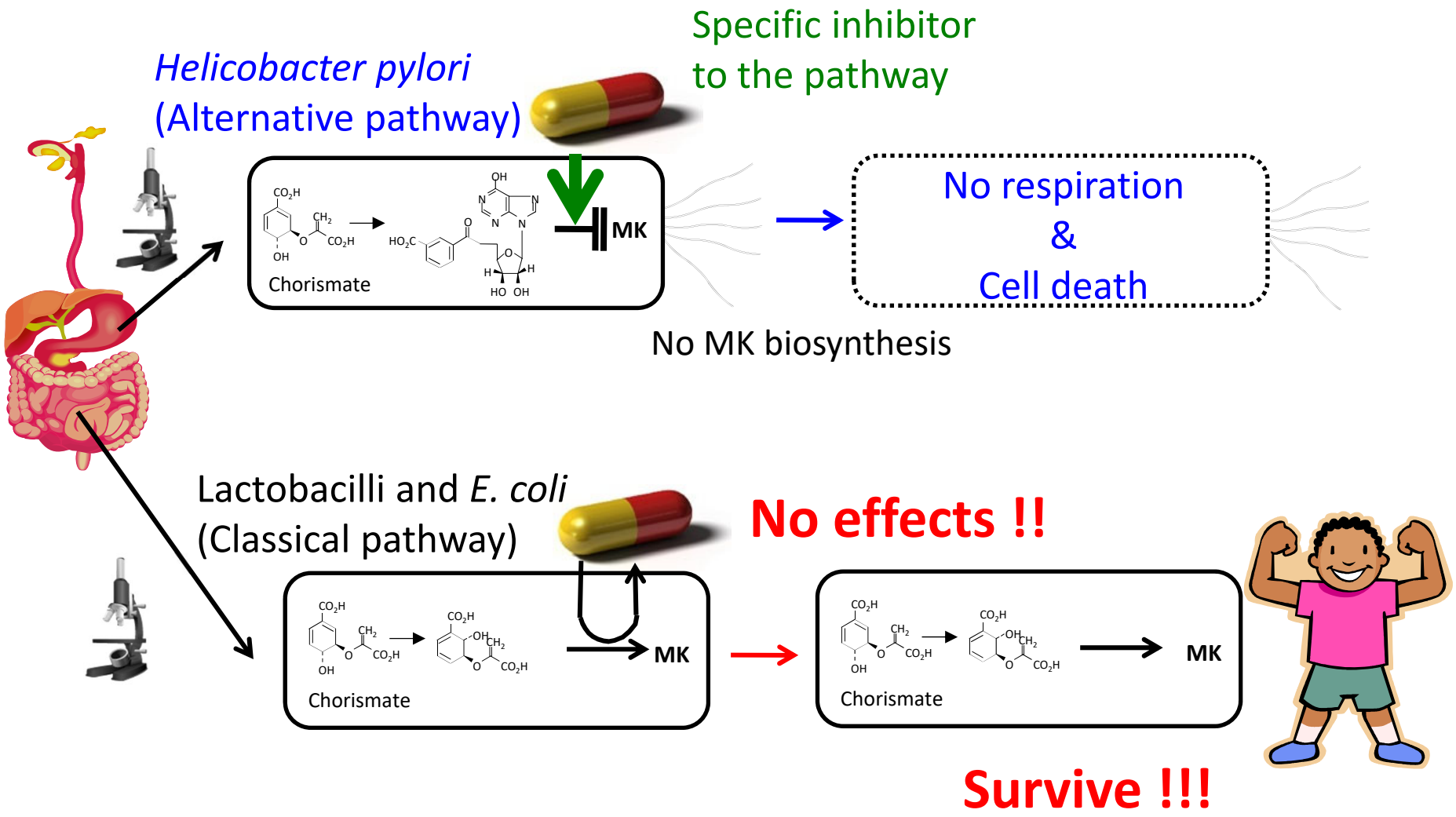
Left; Wild strain
Right; MqnD-disruptant

+ Menaquinone

- Menaquinone

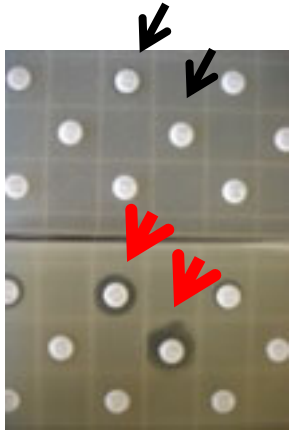
Mqn genes-disrupted mutants required menaquinoine (0.2 mg/mL) for their growth

An attractive target for the chemotherapeutics to *Helicobacter pylori*



An example for screening of inhibitors

Bioassay with paper disck



Bacillus subtilis
(+ classical pathway)

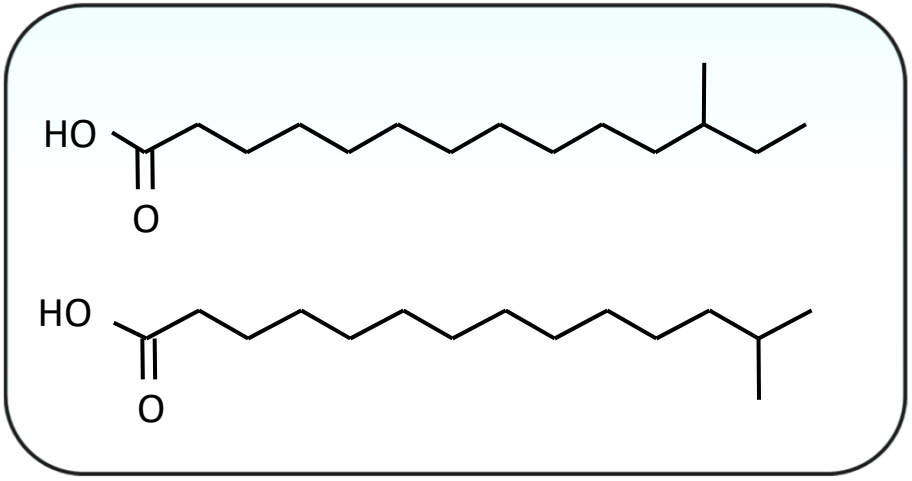
Bacillus halodurans
(+ new pathway)

Re-confirmation
by liquid cultivation

1 2 3

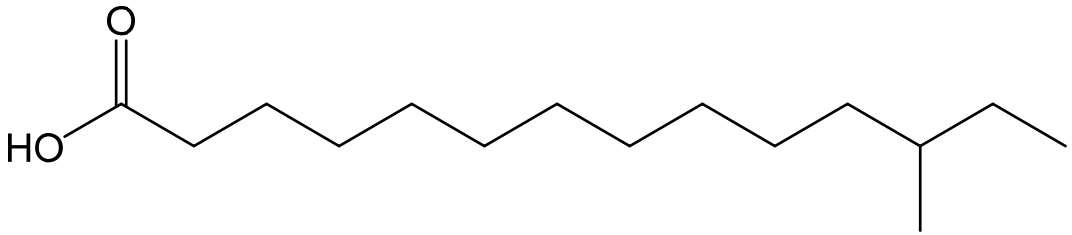
1, +solvent
2, +sample
3, +sample+MK

Purification
Structure



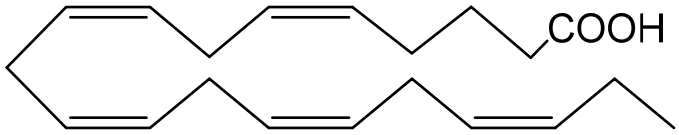
J. Antibiot., **64**, 151 (2011).

Identified Inhibitors

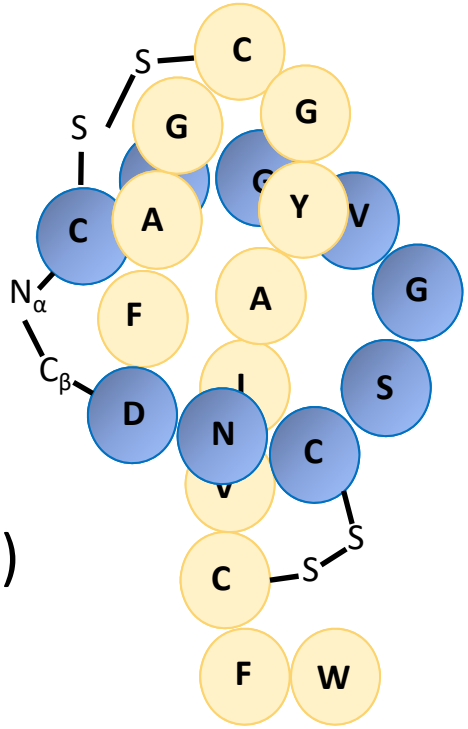


12-Methyltetradecanoic acid MIC 32 mg/ml

J. Antibiot., **64**, 151 (2011)



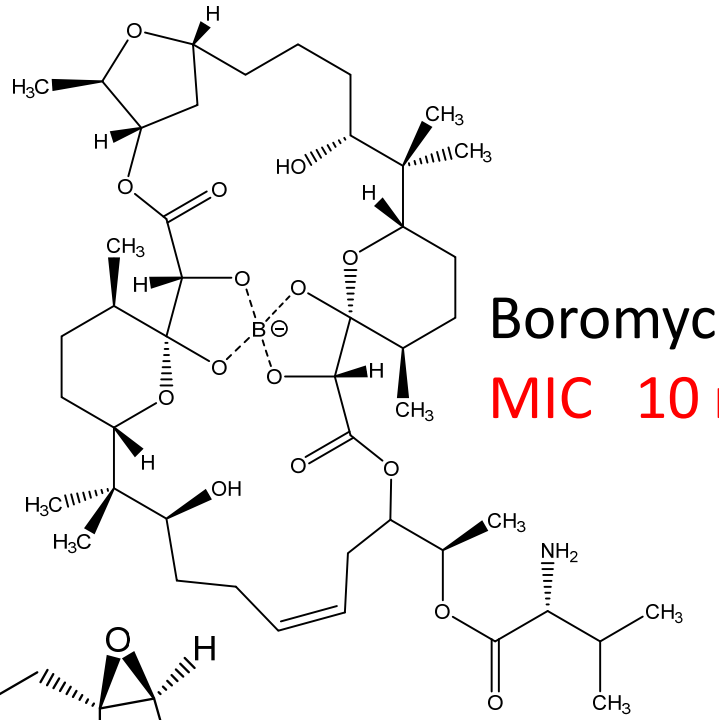
EPA MIC 30 mg/ml



J. Infect. Chemother., **22**, 587 (2016)

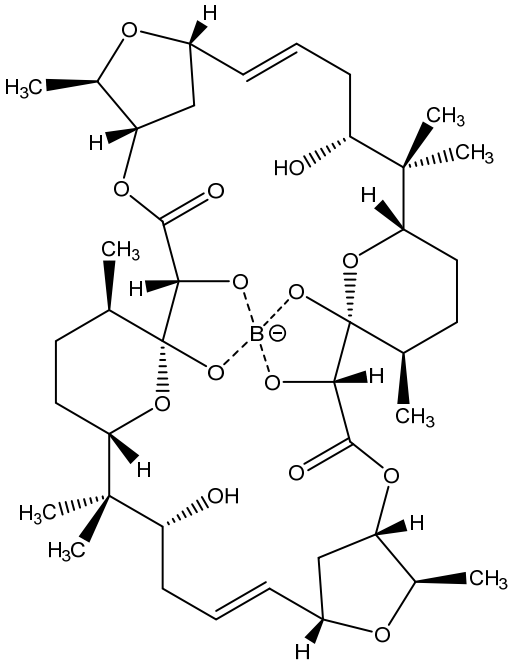
Siamycin I MIC 10 mg/ml

Identified Inhibitors



Boromycin

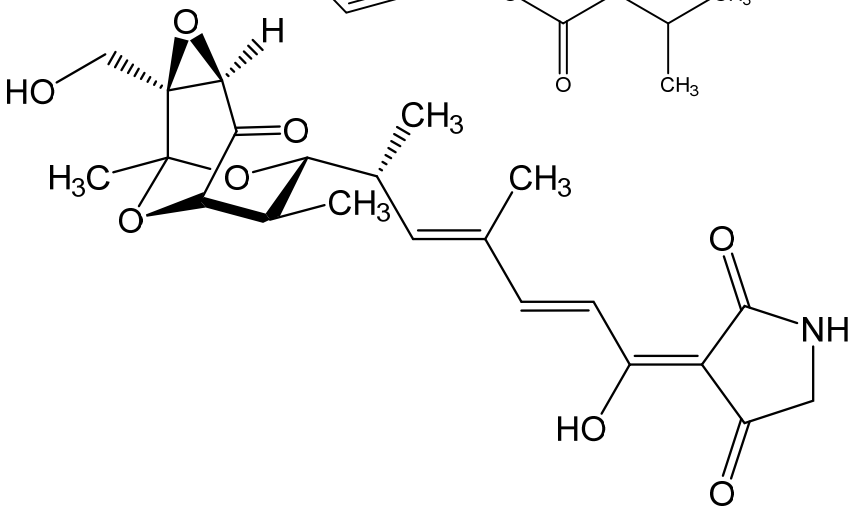
MIC 10 ng/ml



Aplasmomycin

MIC 40 ng/ml

J. Antibiot., **71**, 968 (2018)



Tirandamycin B MIC 1 mg/ml *J. Antibiot.*, **70**, 798 (2017)