2020 Ching Hou Biotechnology Award

Bioprocess development for novel functional lipid production: screening and analysis of microbial metabolisms and enzymes

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Kyoto, Japan

Bioprocess development for novel functional lipid production: screening and analysis of microbial metabolisms and enzymes

1) Polyunsaturated fatty acid production and modification
2) Gut microbial fatty acid metabolism

1) Polyunsaturated fatty acid production and modification

Various physiological activity

- Cardiovascular fitness
- Cerebral function development
- Anticancer
- Lipid metabolism control
- Immune and inflammation control

Precursor of active metabolites

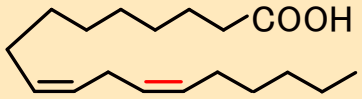
- prostaglandin, leukotriene, thromboxane
- Anti-inflammatory mediators: resolvins etc.

Current source…fish oil, plant oil

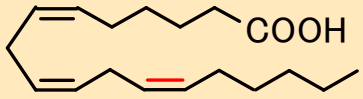
- unstable supply
- low purity
- contamination of unhealthy compounds

Microbial oil
as alternative source

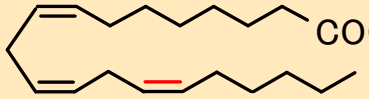
ω6 (n-6) PUFAs



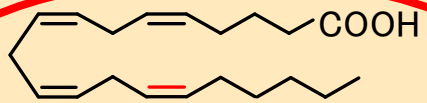
Linoleic acid
LA, 18:2ω6



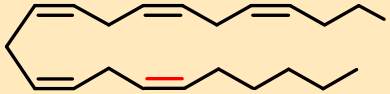
γ-Linolenic acid
GLA, 18:3ω6



Dihomo-γ-linolenic acid
DGLA, 20:3ω6

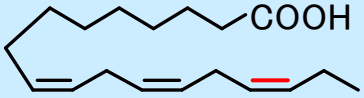


Arachidonic acid
AA, 20:4ω6



n-6 Docosapentaenoic acid
n-6 DPA, 22:5ω6

ω3 (n-3) PUFAs



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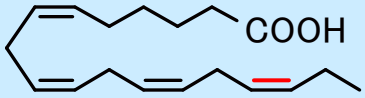

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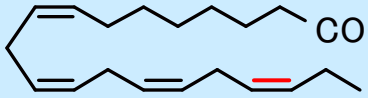
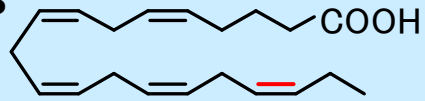


Eicosatetraenoic acid
ETA, 20:4ω3



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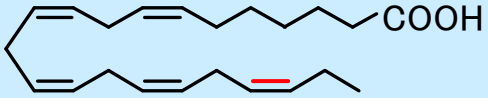
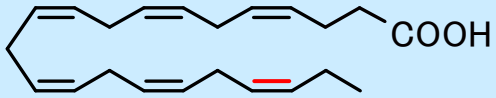
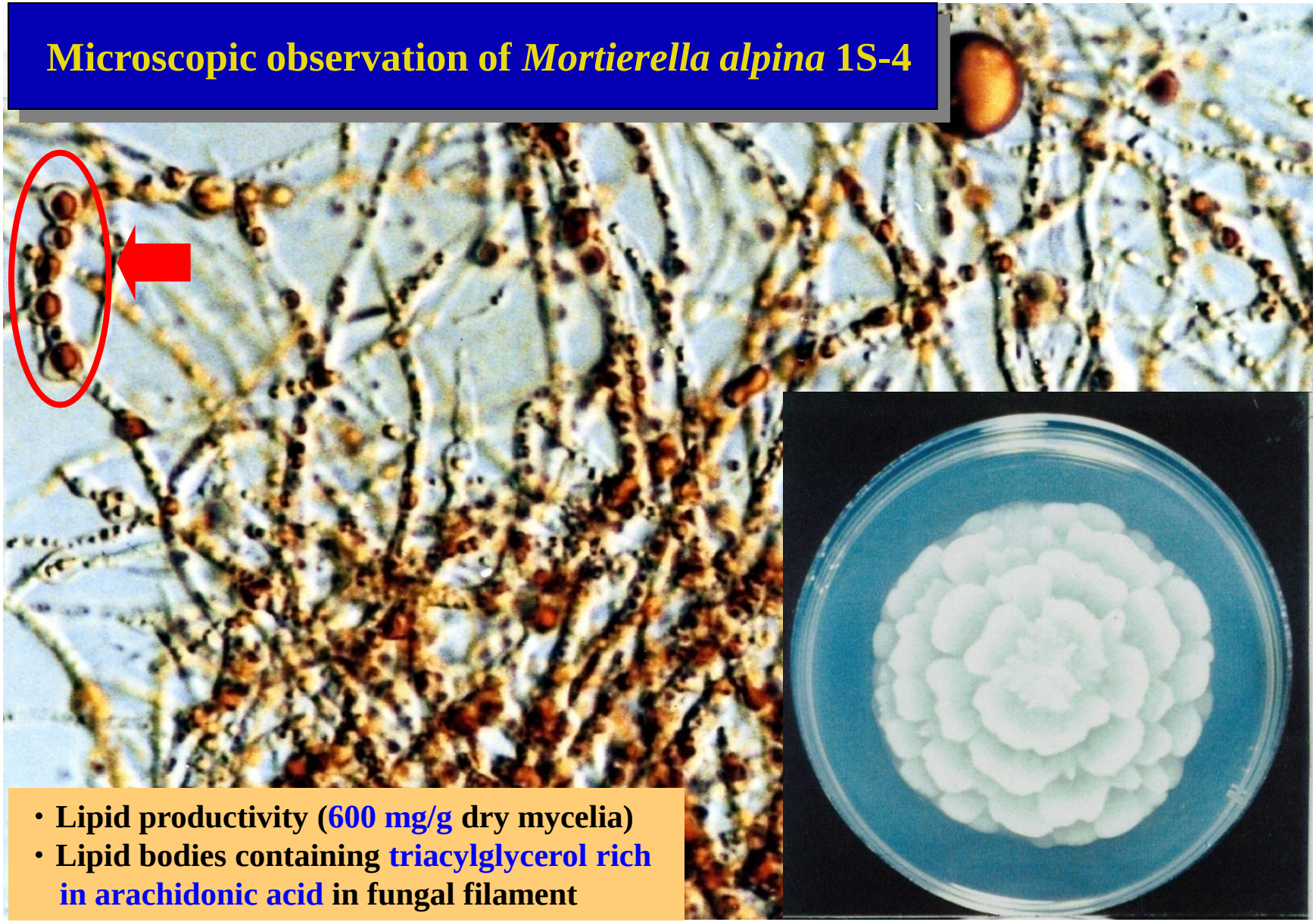


n-3 Docosapentaenoic acid
n-3 DPA, 22:5ω3



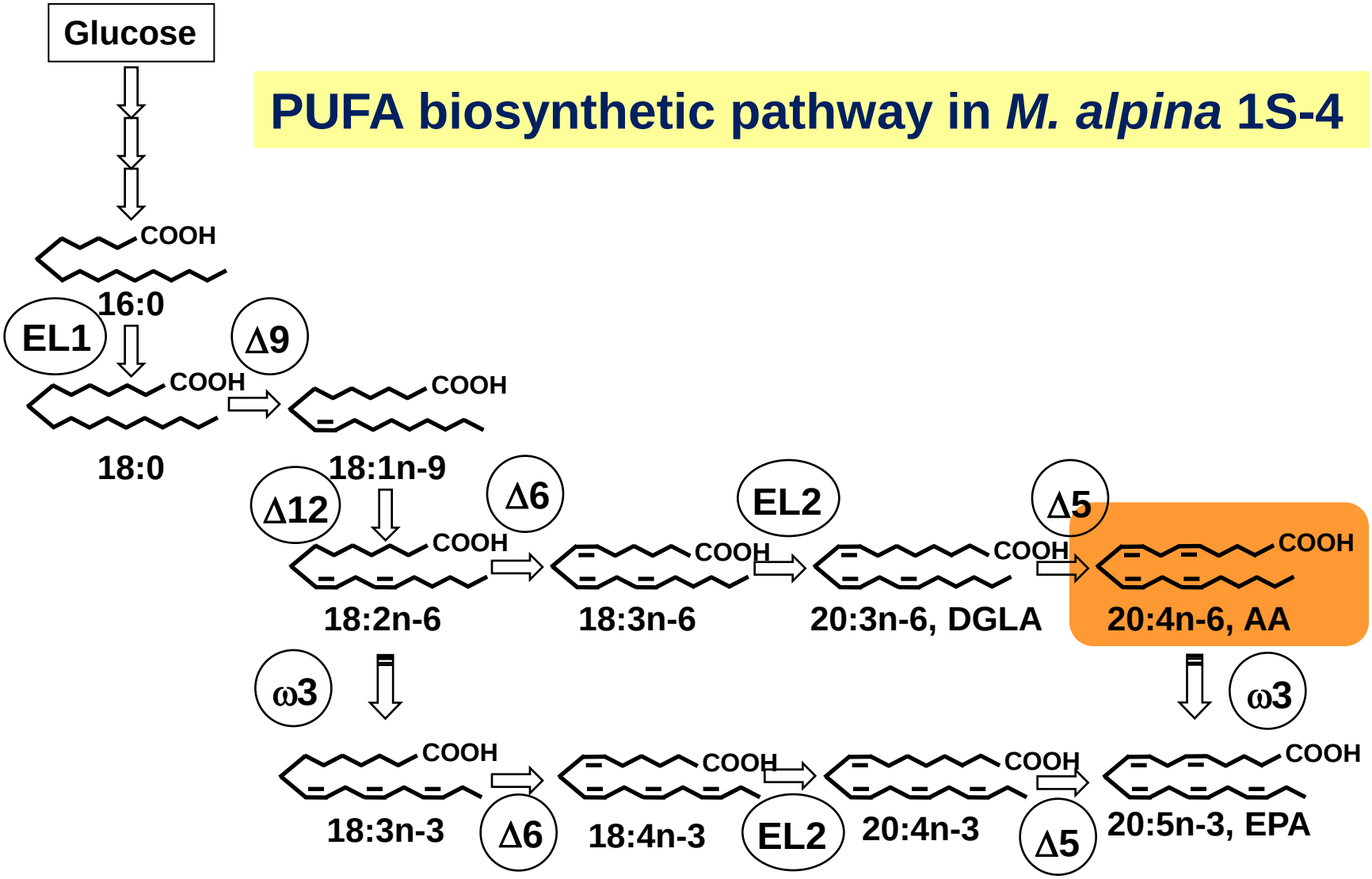
Docosahexaenoic acid
DHA, 22:6ω3

Microscopic observation of *Mortierella alpina* 1S-4

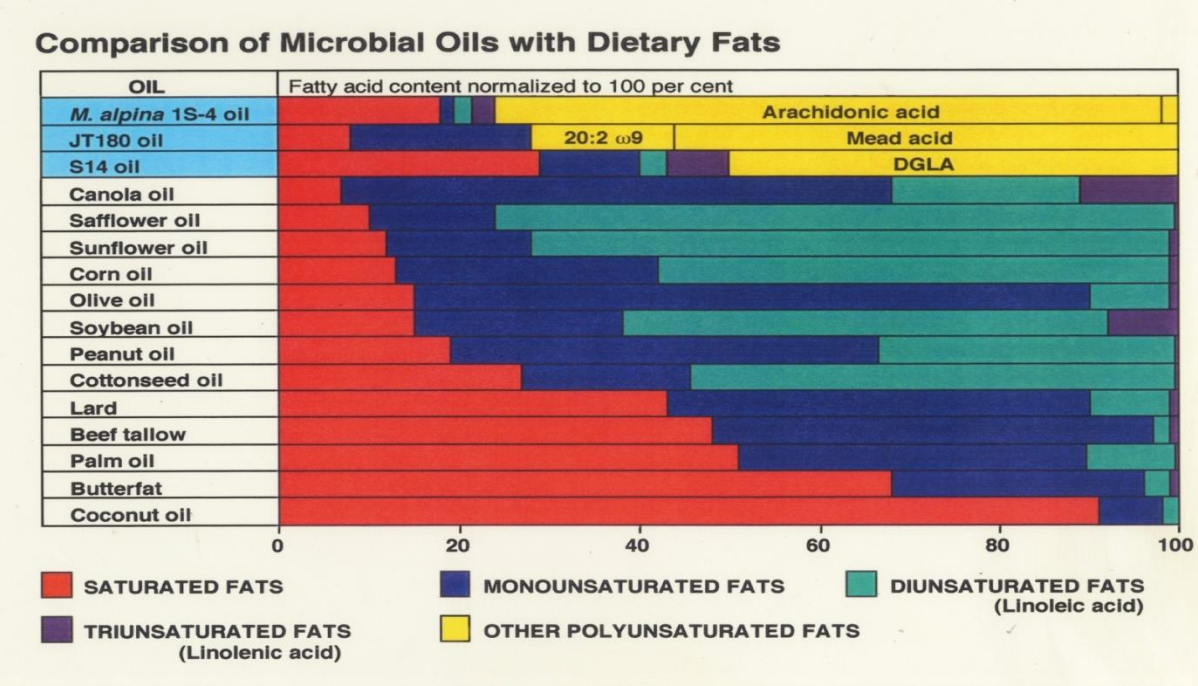


- **Lipid productivity (600 mg/g dry mycelia)**
- **Lipid bodies containing triacylglycerol rich in arachidonic acid in fungal filament**

PUFA biosynthetic pathway in *M. alpina* 1S-4

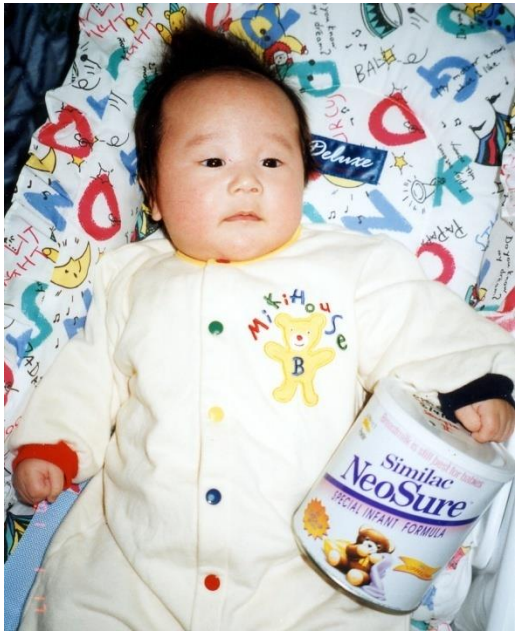


Fatty acid profile of "Single Cell Oil" produced by *M. alpina* is quite different from common edible oils and is used as an ingredient for infant formula in the world.



Used for Infant milk ingredients

Looks like plant oils for diet, but the fatty acid profile is quite different and rich C20 n-6 PUFA



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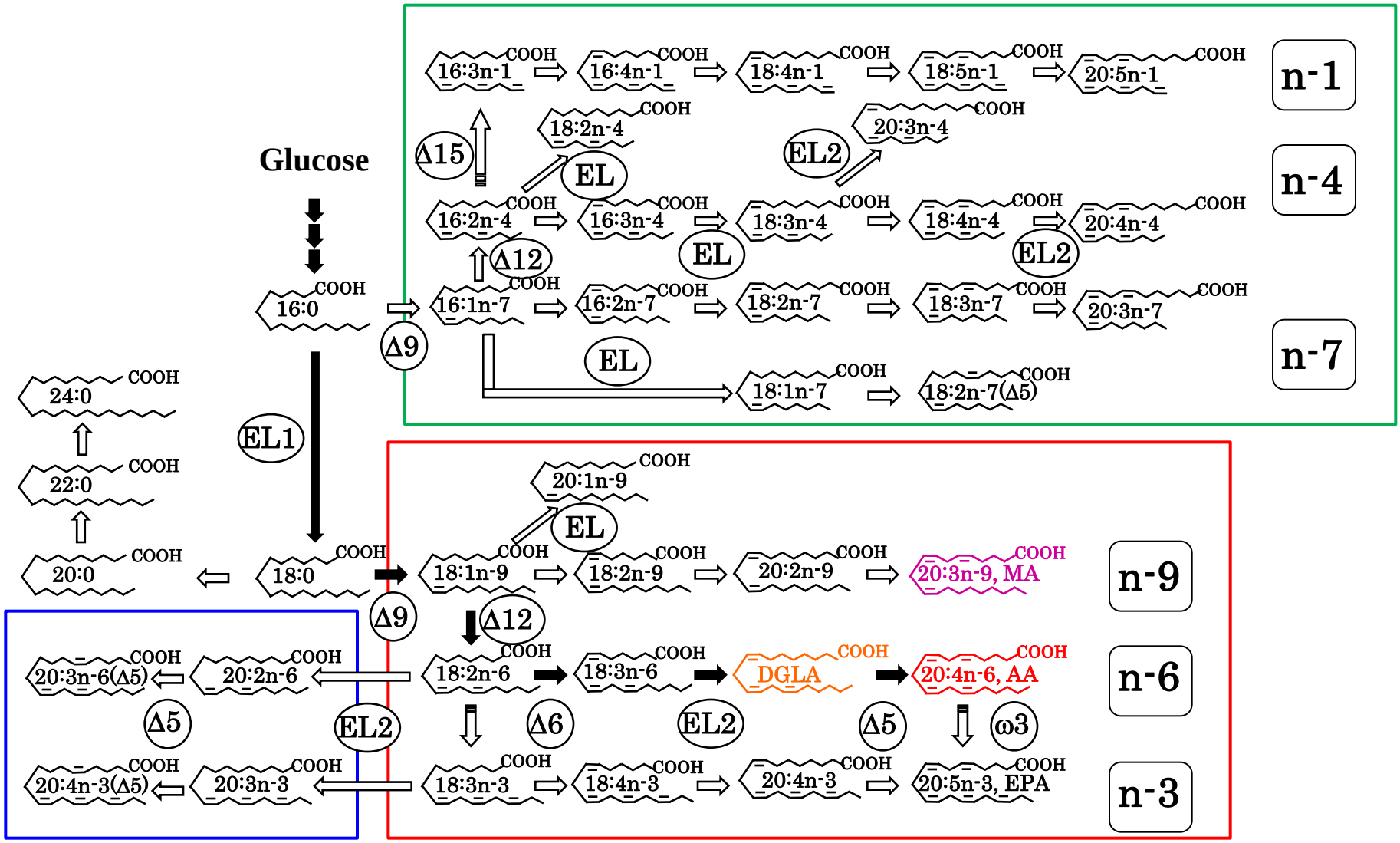

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Various physiological activity

- Cardiovascular fitness (**Medical use**)
- Cerebral function development
- Anticancer
- Lipid metabolism control
- Immune and inflammation control

Precursor of active metabolites

- prostaglandin, leukotriene, thromboxane
- Anti-inflammatory mediators: resolvins etc.

Current source···fish oil, plant oil

- unstable supply
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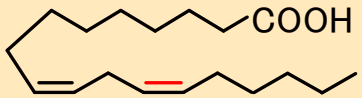
Microbial oil
as alternative source
Molecular breeding for efficient production

ω6 (n-6) PUFAs



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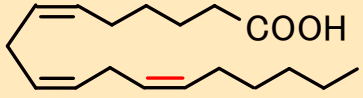

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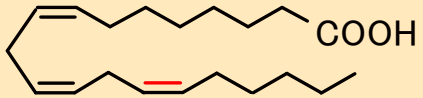

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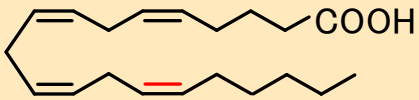

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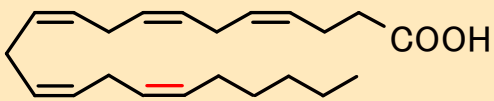

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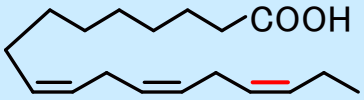

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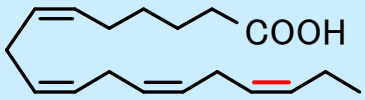

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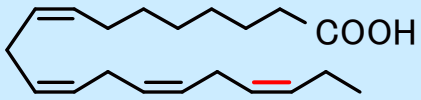

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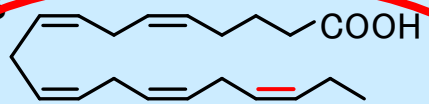

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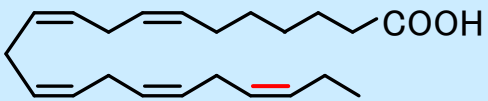

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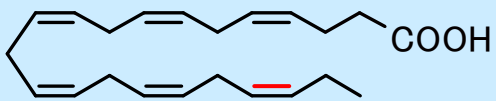

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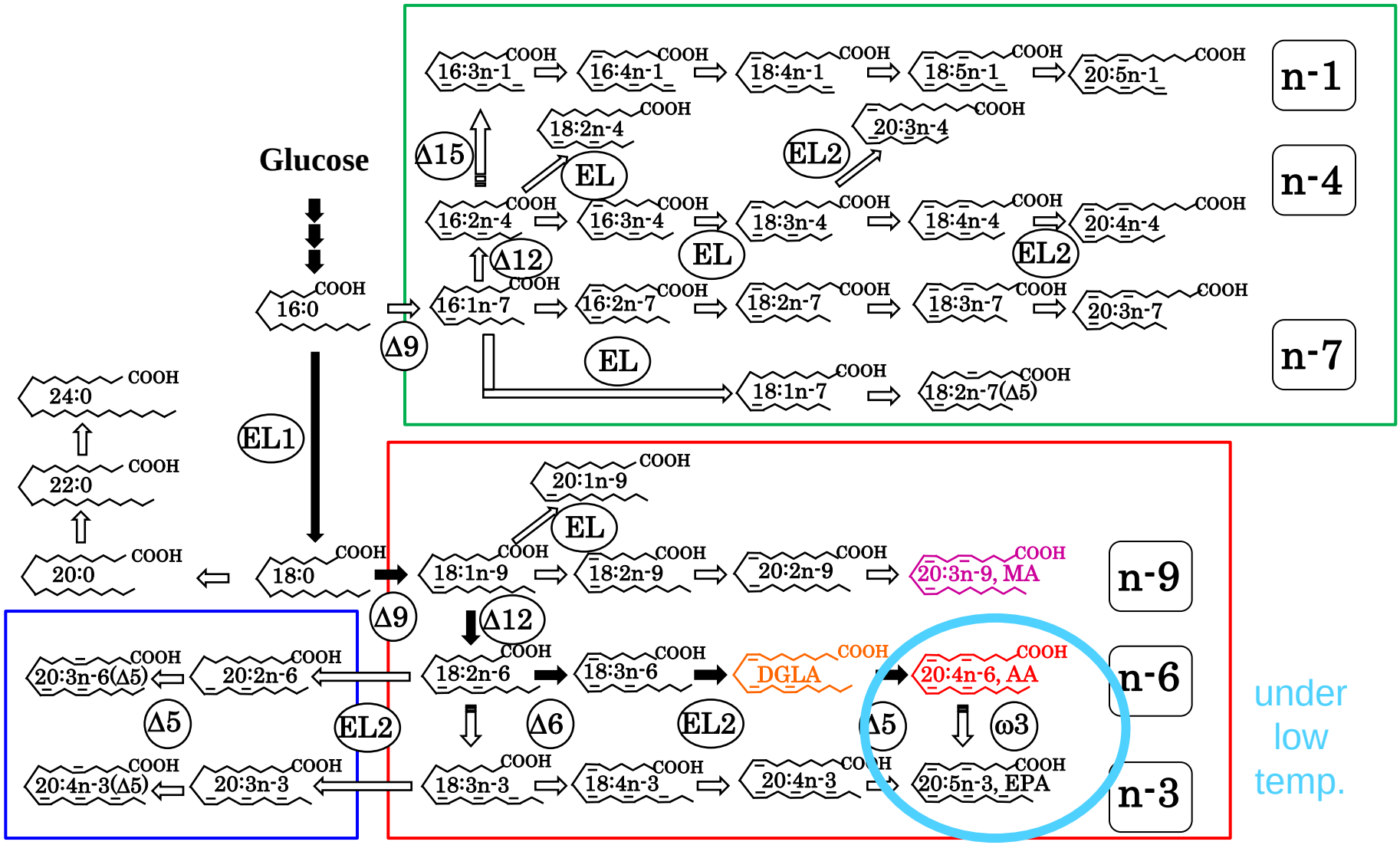


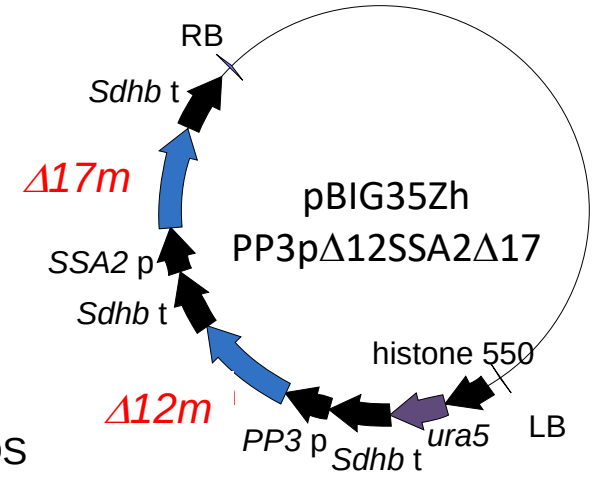
EPA production by expression of heterologous desaturase genes in *M. alpina* 1S-4 under **ordinary temperature**

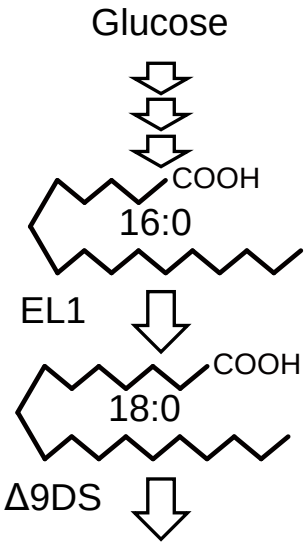
EPA production by expression of heterologous desaturase genes in *M. alpina* 1S-4 under ordinary temperature

gene: Δ17 desaturase (*Δ17m*) gene and Δ12 desaturase gene (*Δ12m*)

promoter: SSA2 promoter and PP3 promoter

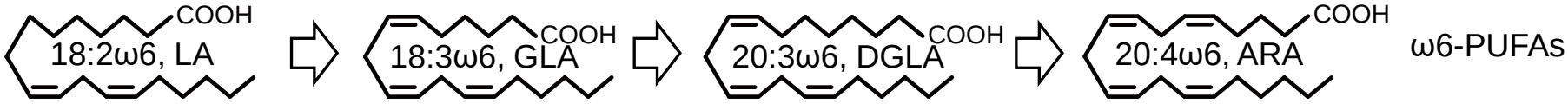
host: *Mortierella alpina*（uracil auxotroph）



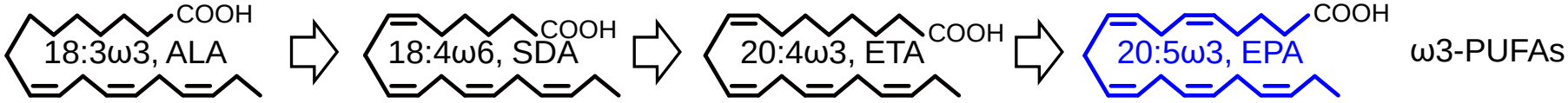


Δ6DS　　EL2　　Δ5DS

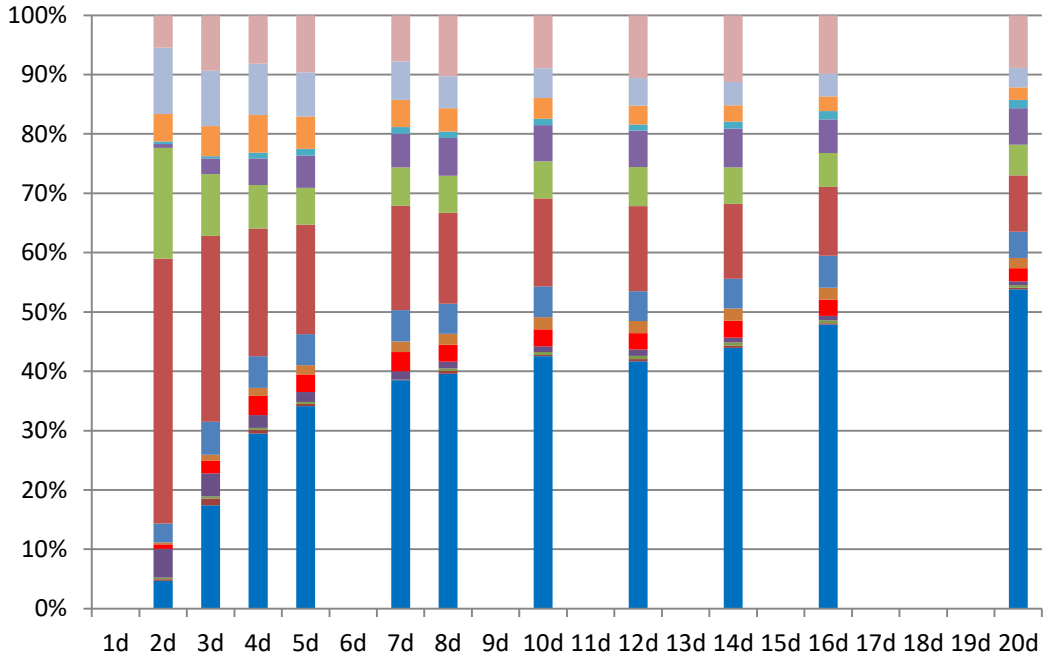
Δ12 desaturase *Δ12m*



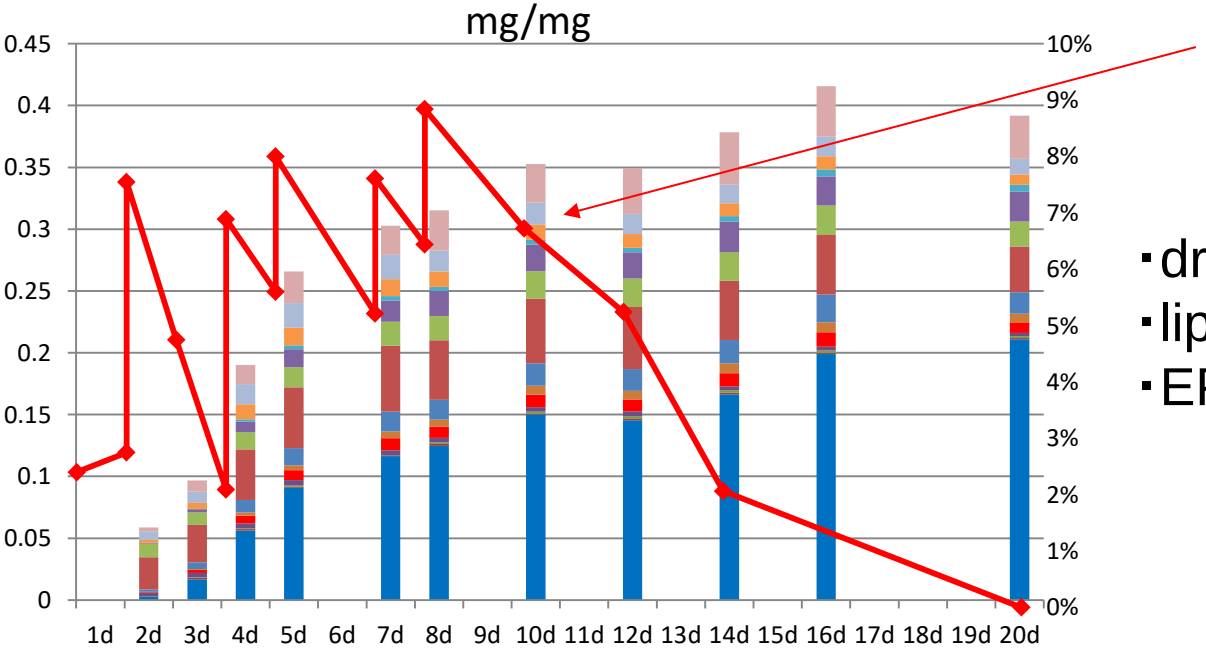
Δ17 desaturase *Δ17m*



5L jar fermentor scale EPA production (26ºC, 20 days cultivation)



- other fatty acid
- 16:0
- 18:0
- 22:0
- 24:0
- 18:1n-9 oleic acid
- 18:2n-6 linoleic acid
- 18:3n-6 γ-linolenic acid
- DGLA
- AA
- 18:3n-3α-linolenic acid
- 18:4n-3 SDA
- ETA
- EPA



Glucose (%)

- dry cell weight 70 g/L
- lipids production 28 g/L
- EPA production 52 % 14.4g/L

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Current source…fish oil, plant oil

- unstable supply
- low purity
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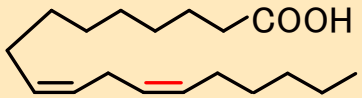
Microbial oil as alternative source
Production of rare PUFAs for future development

ω6 (n-6) PUFAs



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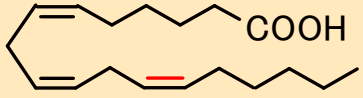

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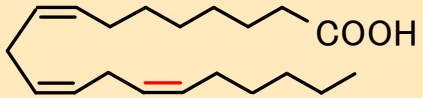

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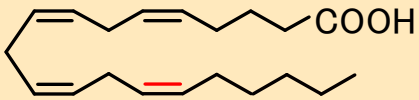

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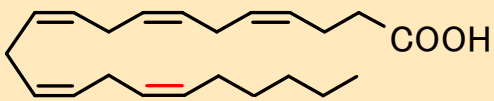

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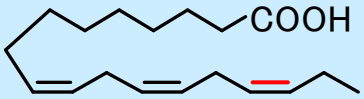
n-6 DPA, 22:5ω6

ω3 (n-3) PUFAs



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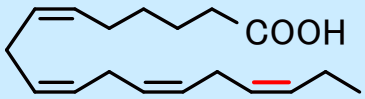

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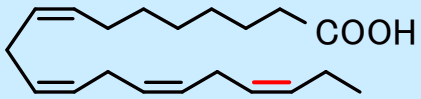

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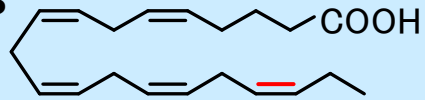

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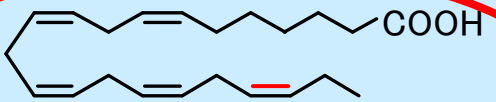

n-3 Docosapentaenoic acid
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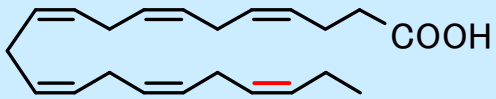
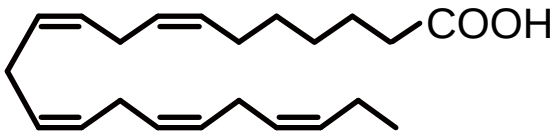


Docosahexaenoic acid
DHA, 22:6ω3

Introduction

n-3 DPA (n-3 docosapentaenoic acid)

- Preventive effect of arteriosclerosis (~10 times more effective than EPA)
- Inhibitory effect of angiogenesis



n-3 DPA
(22:5n-3)

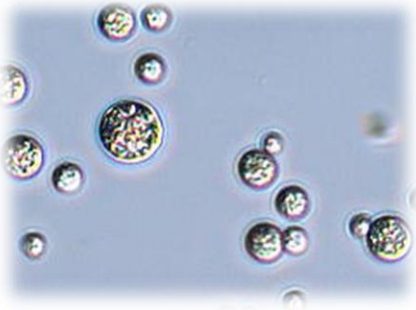
Current n-3 DPA source : **harp seal oil**

- Low DPA content (< 5%)
- difficulty of stable and large supply

Alternative source such as microorganisms is required.

Labyrinthulomycetes

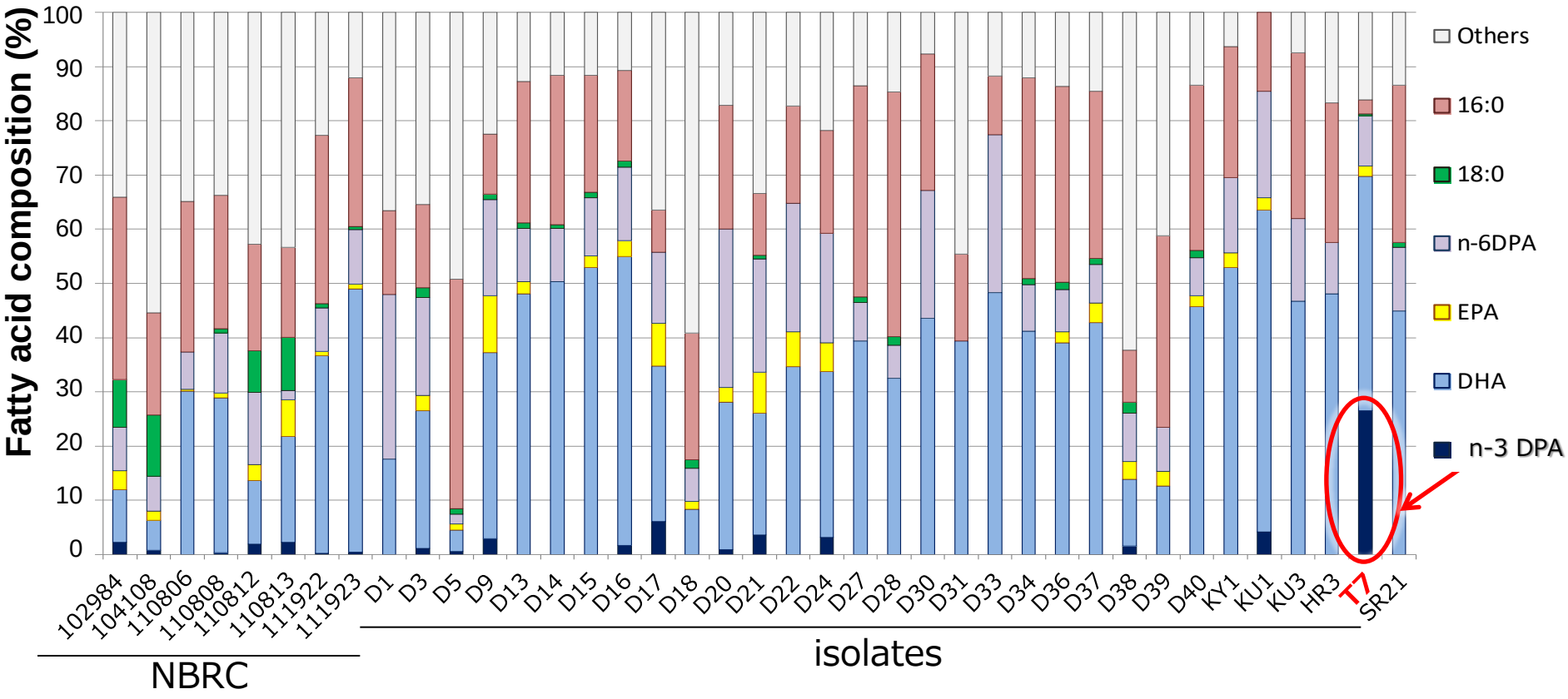
- marine eucaryotic microorganism
- highly accumulate PUFA (including DHA and EPA), astaxanthin, etc.



Isolation of n-3 DPA producing microorganism

—— fatty acid compositions of isolates and NBRC strains

31 isolates and 8 NBRC strains were analyzed of fatty acid profiles.



Isolated strain T7 highly accumulated n-3 DPA (26% of total fatty acids).
The strain T7 was identified as *Aurantiochytrium* sp.

1) Polyunsaturated fatty acid production and modification

Various physiological activity

- Cardiovascular fitness
- Cerebral function development
- Anticancer
- Lipid metabolism control
- Immune and inflammation control

Precursor of active metabolites

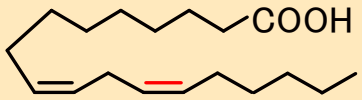
- **prostaglandin**, leukotriene, thromboxane
- Anti-inflammatory mediators: resolvins etc.

Current source…fish oil, plant oil

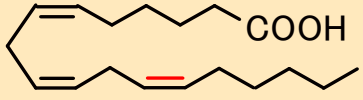
- unstable supply
- low purity
- contamination of unhealthy compounds

Microbial oil as alternative source
Modification of PUFAs to active metabolites

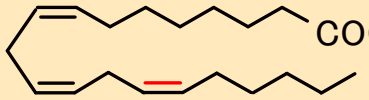
ω6 (n-6) PUFAs



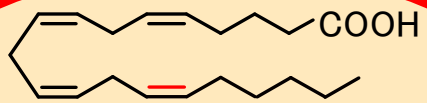
Linoleic acid
LA, 18:2ω6



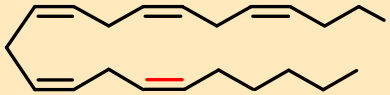
γ-Linolenic acid
GLA, 18:3ω6



Dihomo-γ-linolenic acid
DGLA, 20:3ω6

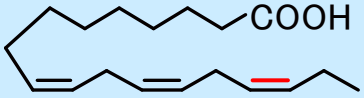


Arachidonic acid
AA, 20:4ω6

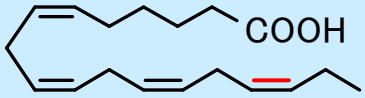


n-6 Docosapentaenoic acid
n-6 DPA, 22:5ω6

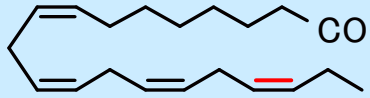
ω3 (n-3) PUFAs



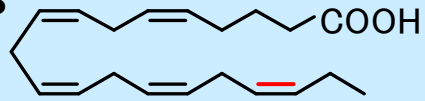
α-Linolenic acid
ALA, 18:3ω3



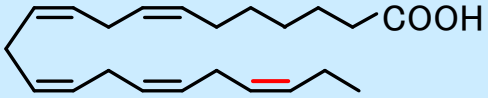
Stearidonic acid
SDA, 18:4ω3



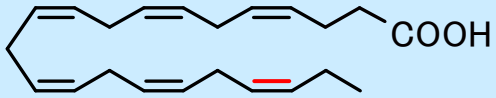
Eicosatetraenoic acid
ETA, 20:4ω3



Eicosapentaenoic acid
EPA, 20:5ω3



n-3 Docosapentaenoic acid
n-3 DPA, 22:5ω3



Docosahexaenoic acid
DHA, 22:6ω3

Prostaglandins (PG)

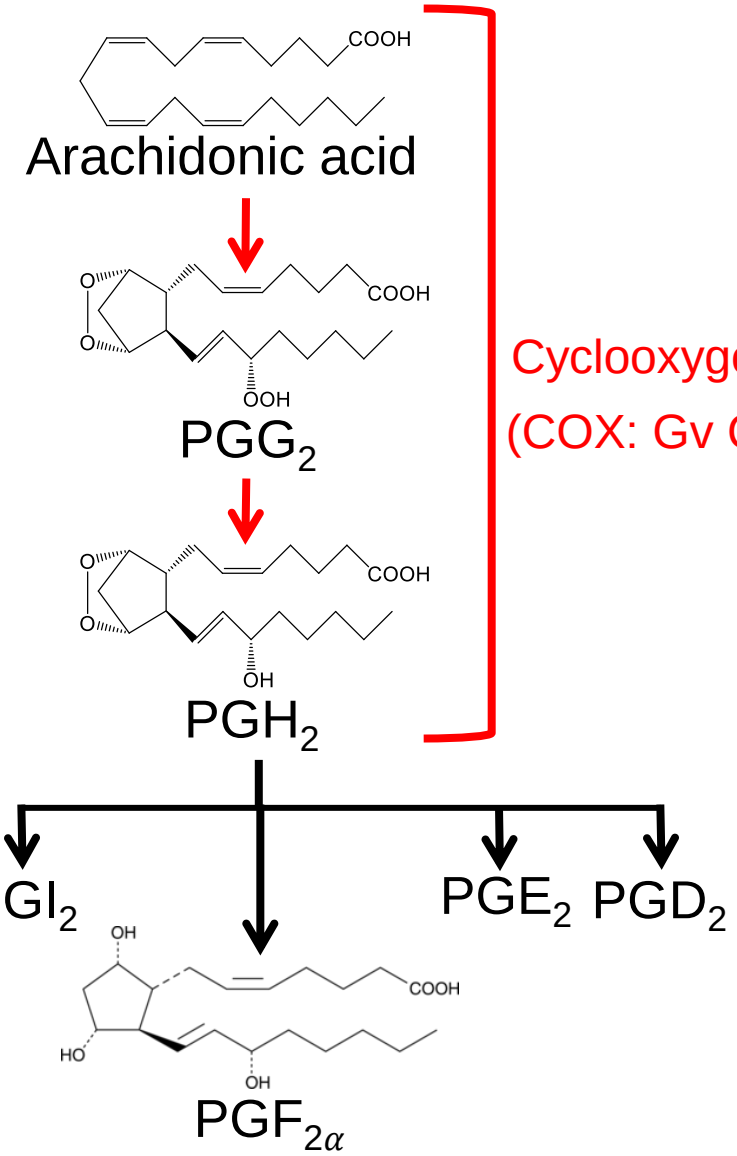
Bioactive substance

PGE_2, $PGF_{2\alpha}$ etc.

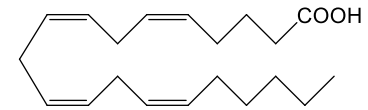
- ✓ Vasodilation
- ✓ Inhibition of platelet aggregation
- ✓ Constriction of smooth muscle

Pharmaceuticals

Biosynthesis pathway of PGs

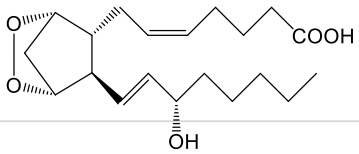


Time course Analysis of PGF2α Production by *Mortierella alpina* 1S-4 transformants



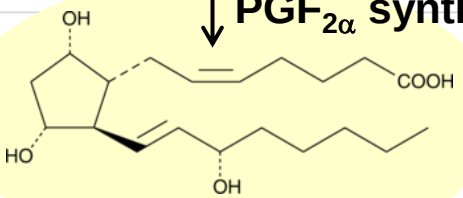
Arachidonic acid

↓ GvCOXm

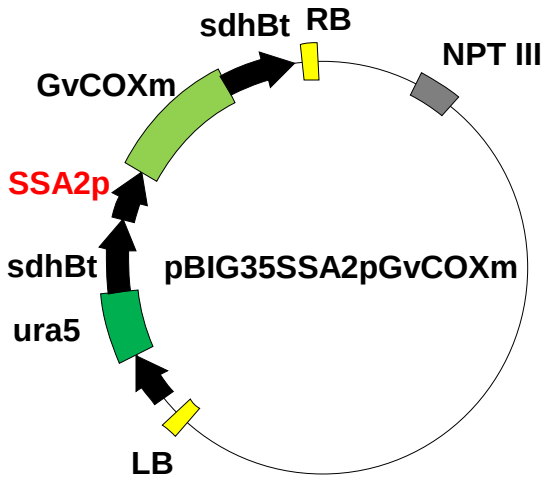


PGH_2

↓ $PGF_{2\alpha}$ synthetase



$PGF_{2\alpha}$



GY Medium (glucose 2%, YE 1%) , 28℃, 12 days

1) Polyunsaturated fatty acid production and modification

Various physiological activity

- Cardiovascular fitness
- Cerebral function development
- Anticancer
- Lipid metabolism control
- Immune and inflammation control

Precursor of active metabolites

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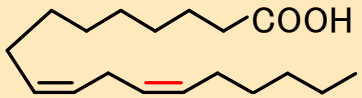
- **Anti-inflammatory mediators**: resolvins etc.

Current source･･･fish oil, plant oil

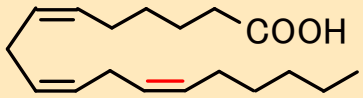
- unstable supply
- low purity
- contamination of unhealthy compounds

Microbial oil as alternative source
Modification of PUFAs to active metabolites

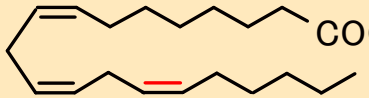
ω6 (n-6) PUFAs



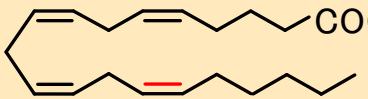
Linoleic acid
LA, 18:2ω6



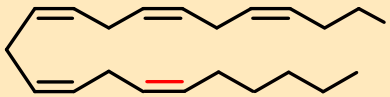
γ-Linolenic acid
GLA, 18:3ω6



Dihomo-γ-linolenic acid
DGLA, 20:3ω6

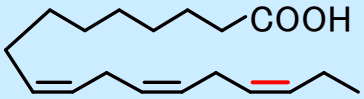


Arachidonic acid
AA, 20:4ω6

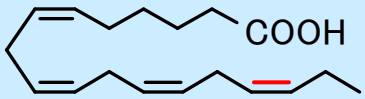


n-6 Docosapentaenoic acid
n-6 DPA, 22:5ω6

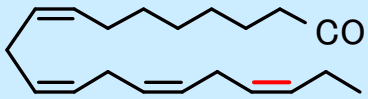
ω3 (n-3) PUFAs



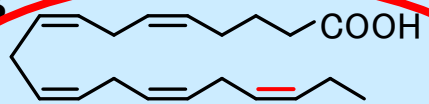
α-Linolenic acid
ALA, 18:3ω3



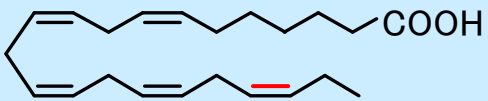
Stearidonic acid
SDA, 18:4ω3



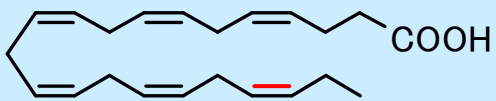
Eicosatetraenoic acid
ETA, 20:4ω3



Eicosapentaenoic acid
EPA, 20:5ω3

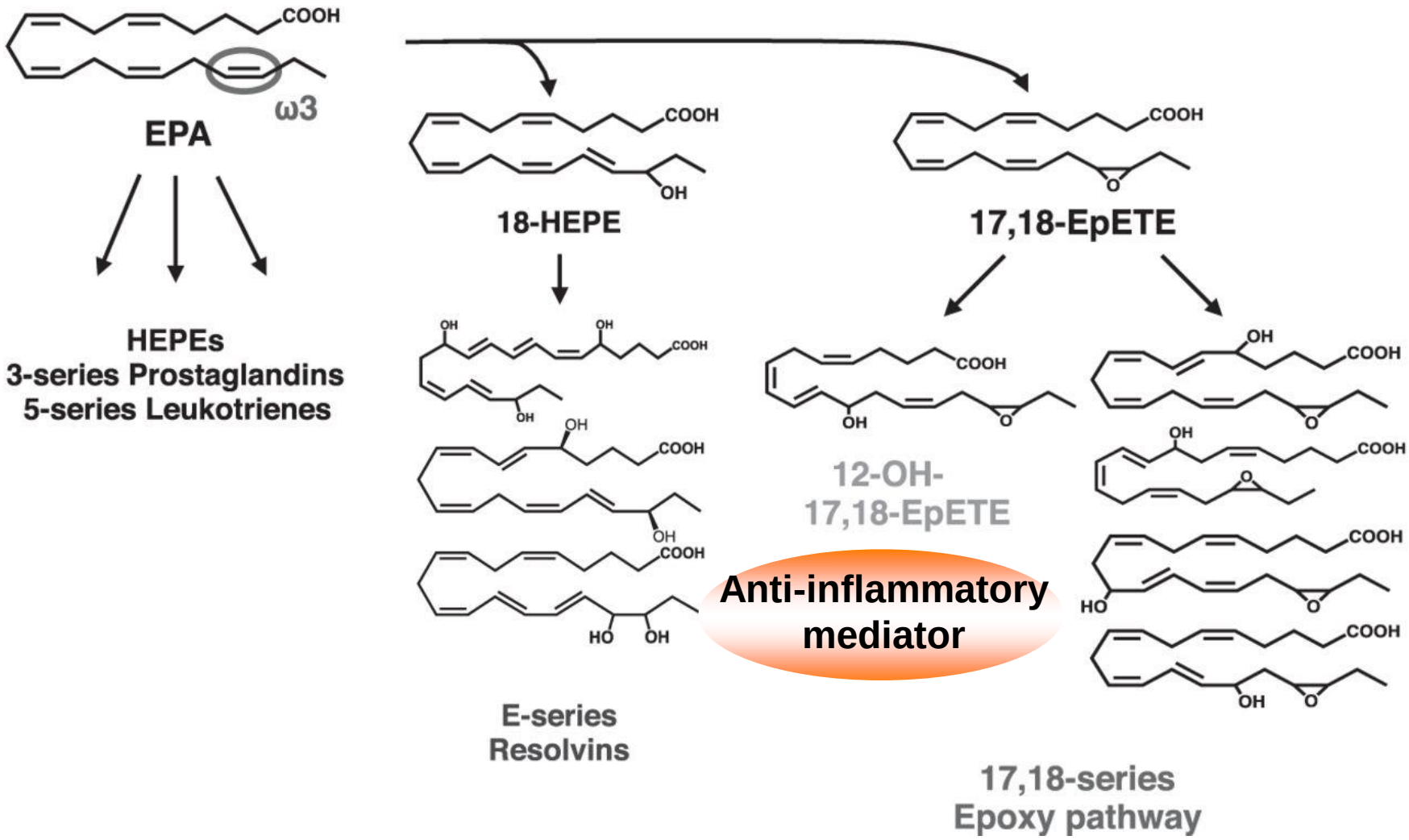


n-3 Docosapentaenoic acid
n-3 DPA, 22:5ω3

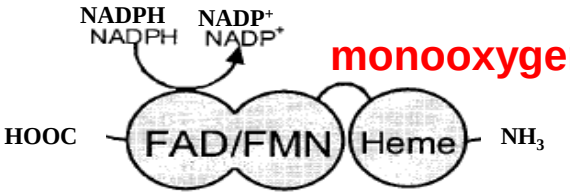


Docosahexaenoic acid
DHA, 22:6ω3

EPA metabolome in the body

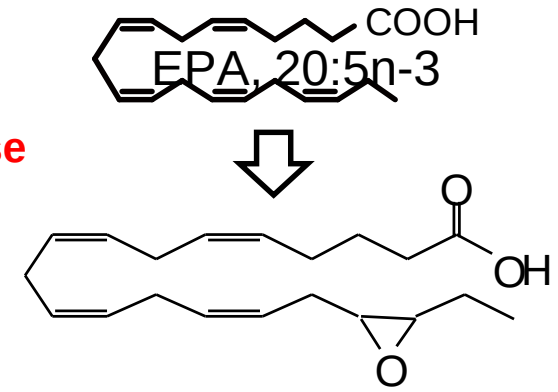


Kubota T et al. FASEB J
2014;28:586-593



P450

monooxygenase



17,18-EpETE

Developing new Natto (soy been fermentaion)
containing anti-inflammatory lipids
using *Bacillus* sp. showing P450 monooxygenase activity

Summary

1) Polyunsaturated fatty acid production and modification

Various physiological activity

- Cardiovascular fitness
- Cerebral function development
- Anticancer
- Lipid metabolism control
- Immune and inflammation control

Precursor of active metabolites

- **PGF_{2a} (6 mg/ L) by *M. alpina* transformant**
- **17,18-EpETE (0.4 mg/mL) by *Bacillus* P450 MO**

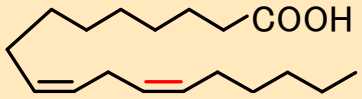
Current source…fish oil, plant oil

- unstable supply
- low purity
- contamination of unhealthy compounds

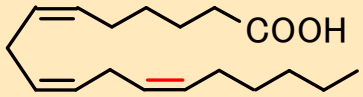
EPA: 52 % 14.4g/L
(*M. alpina* transformant)

n-3 DPA: 165 mg/L
(*Aurantiochytrium* sp. T7)

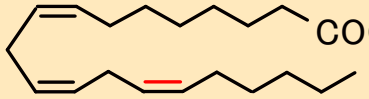
ω6 (n-6) PUFAs



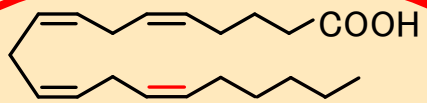
Linoleic acid
LA, 18:2ω6



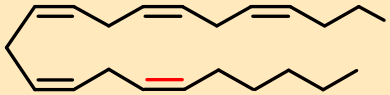
γ-Linolenic acid
GLA, 18:3ω6



Dihomo-γ-linolenic acid
DGLA, 20:3ω6

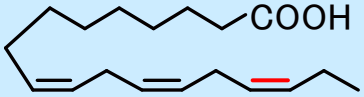


Arachidonic acid
AA, 20:4ω6

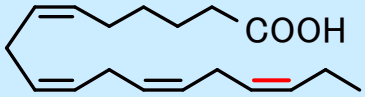


n-6 Docosapentaenoic acid
n-6 DPA, 22:5ω6

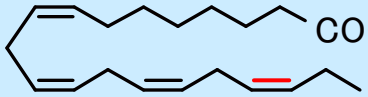
ω3 (n-3) PUFAs



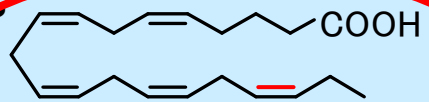
α-Linolenic acid
ALA, 18:3ω3



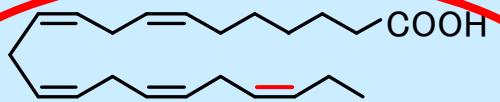
Stearidonic acid
SDA, 18:4ω3



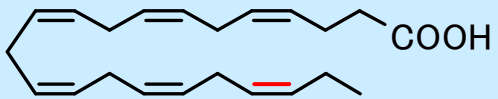
Eicosatetraenoic acid
ETA, 20:4ω3



Eicosapentaenoic acid
EPA, 20:5ω3

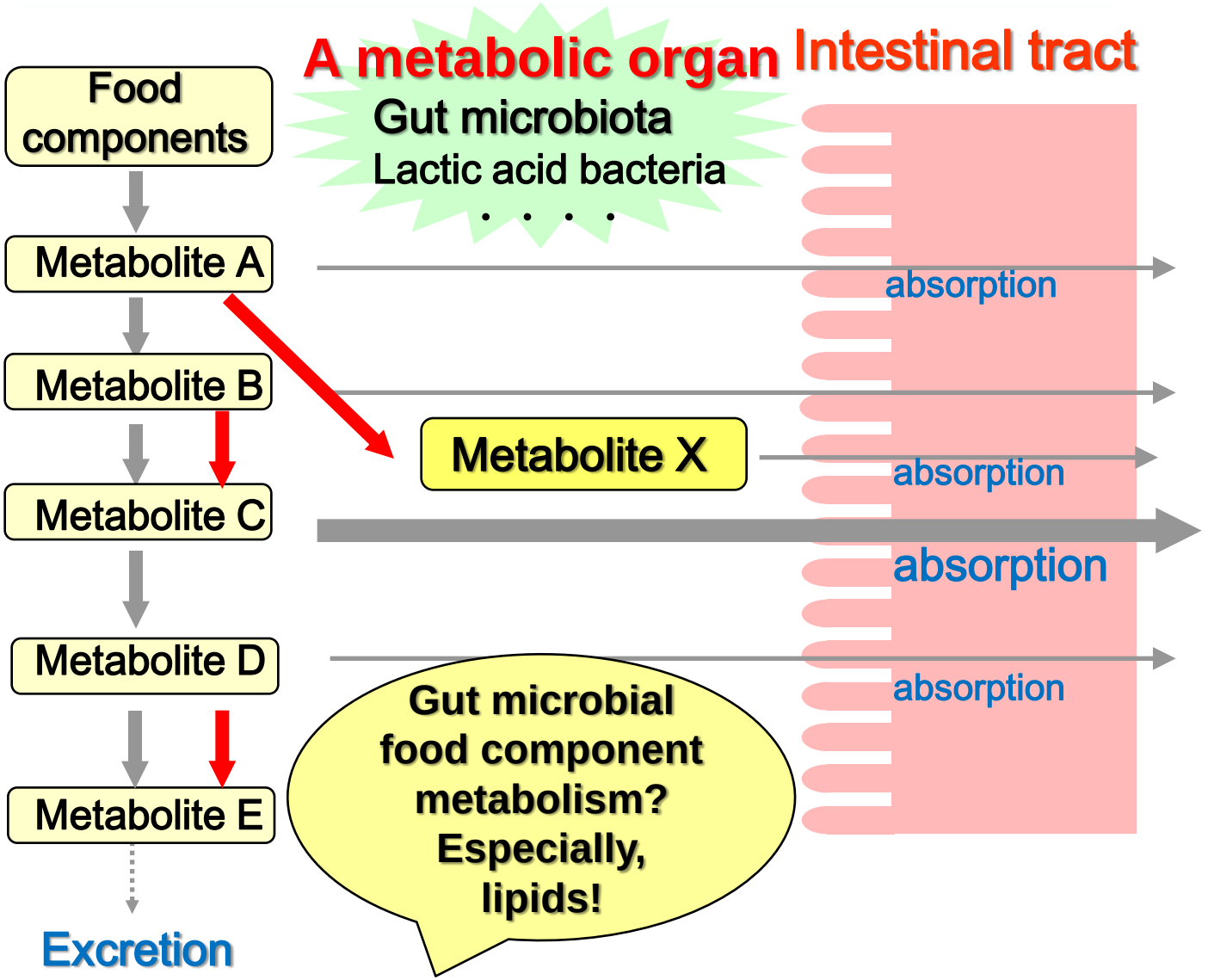


n-3 Docosapentaenoic acid
n-3 DPA, 22:5ω3



Docosahexaenoic acid
DHA, 22:6ω3

2) Gut microbial fatty acid metabolism



What kind molecular species of fat, especially bioactive PUFAs, are generated by our gut microbes and what kind of function they have in relation with our health?

Novel dietary fatty acid metabolism of gut microorganisms producing health-promoting functional lipids

- Gut microbial PUFA saturation metabolism
- Distribution of gut microbial PUFA metabolites in the host
- Physiological activities of gut microbial PUFA metabolites
- Preparation of gut microbial PUFA metabolites

Novel PUFA-saturation metabolism found in *Lactobacillus plantarum*

HOOC 9 12 **LA**

HOOC 10 12 ***t*10, *c*12-CLA**

HOOC 9 11 ***c*9, *t*11-CLA**

HOOC 9 11 ***t*9, *t*11-CLA**

HOOC 9 oleic acid

HOOC 10 **10(*E*)-octadecenoic acid**

CLA-HY

CLA-HY

CLA-HY

HOOC OH 12 10 10-hydroxy-12(*Z*)-octadecenoic acid (HYA)

HYA

HOOC OH 10 11 10-hydroxy-11(*E*)-octadecenoic acid

HYC

HOOC OH 10 10-hydroxy octadecanoic acid

HYB

CLA-DH

CLA-DH

CLA-DH

HOOC O 12 10 10-oxo-12(*Z*)-octadecenoic acid

KetoA

CLA-DC

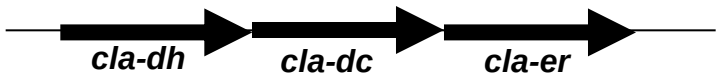
HOOC O 10 11 10-oxo-11(*E*)-octadecenoic acid

KetoC

CLA-ER

HOOC O 10 10-oxo-octadecanoic acid

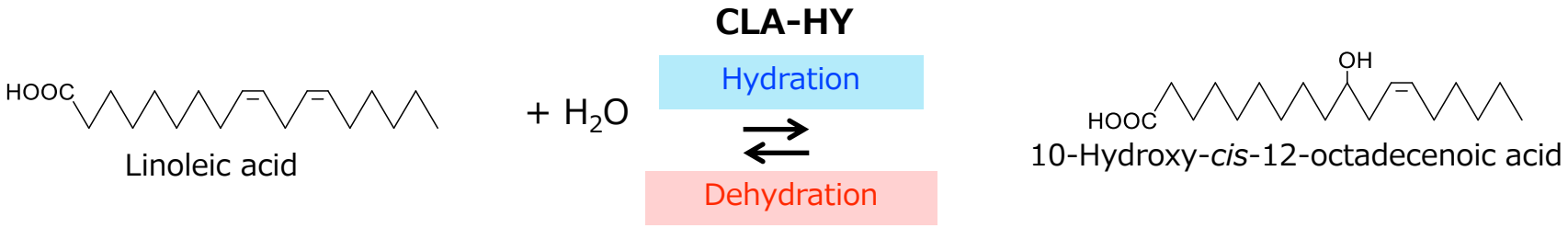
KetoB



cla-dh, Short-chain dehydrogenase/oxidreductase (861 bp)
cla-dc, Acetoacetate decarboxylase (846 bp)
cla-er, Nitroreductase (645 bp)

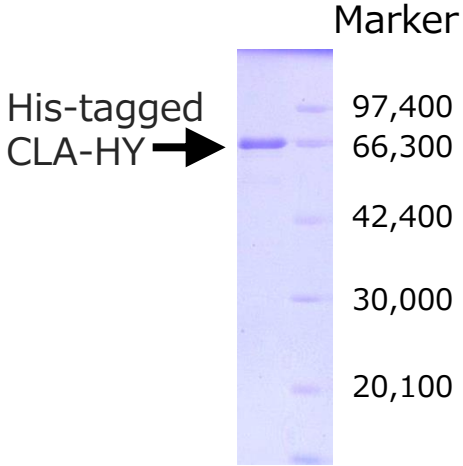
Kishino, S. et al, *Proc. Natl. Acad. Sci. USA,* 110, 17808-17813 (2013)

CLA-HY -Summary-



Native M_r	52	kDa
Number of subunit	1	
Cofactor	FAD	
Activator	NADH	
Optimal reaction temperature	40℃	
Optimal reaction pH	5.5	
Thermal stability	~28℃	
pH stability	5~7	

(For hydration and dehydration)



(For linoleic acid)

$appK_m$	92	μM
k_{cat}	2.6×10^{-2}	sec^{-1}
$k_{cat}/appK_m$	2.8×10^{-4}	$sec^{-1} \cdot \mu M^{-1}$
Hill coefficient	3.3	
V_{max}	3.1×10^{-2}	$\mu mol \cdot sec^{-1} \cdot mg^{-1}$

*Sodium succinate buffer pH 5.5, 20 mM;
FAD, 0.1 mM; without NADH; reaction temperature, 37℃

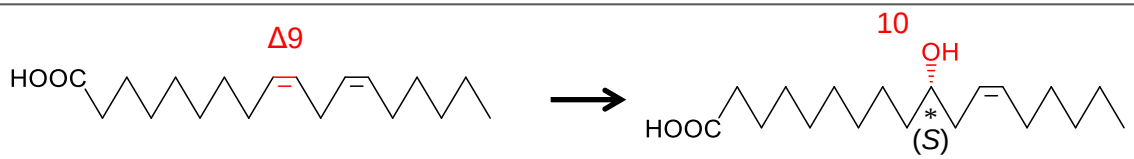
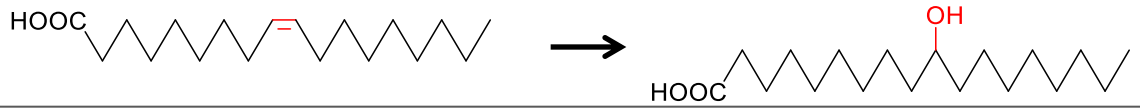
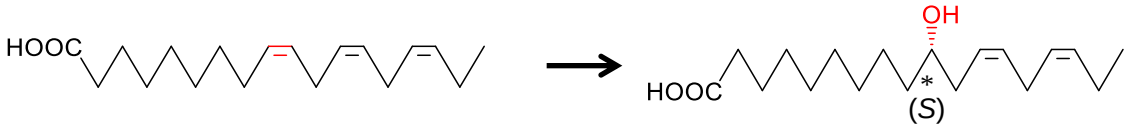
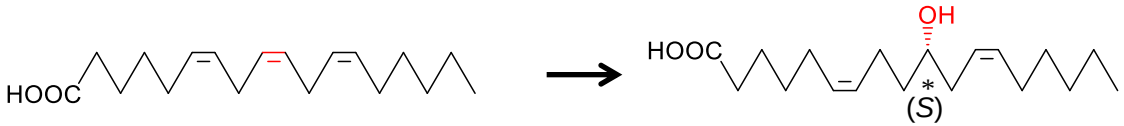
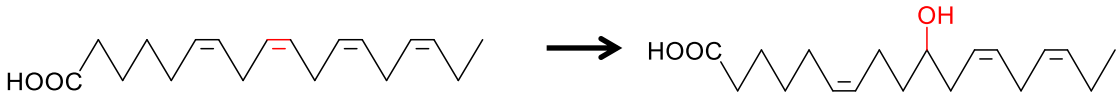
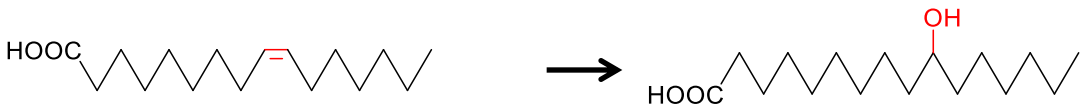
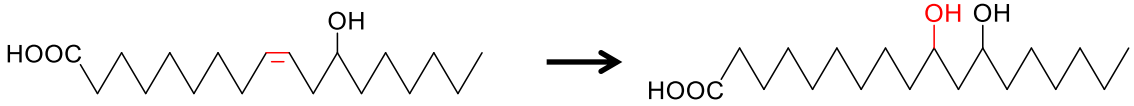
(For 10-hydroxy-*cis*-12-octadecenoic acid)

K_m	98	μM
k_{cat}	1.2×10^{-3}	sec^{-1}
k_{cat}/K_m	1.2×10^{-5}	$sec^{-1} \cdot \mu M^{-1}$
V_{max}	1.4×10^{-3}	$\mu mol \cdot sec^{-1} \cdot mg^{-1}$

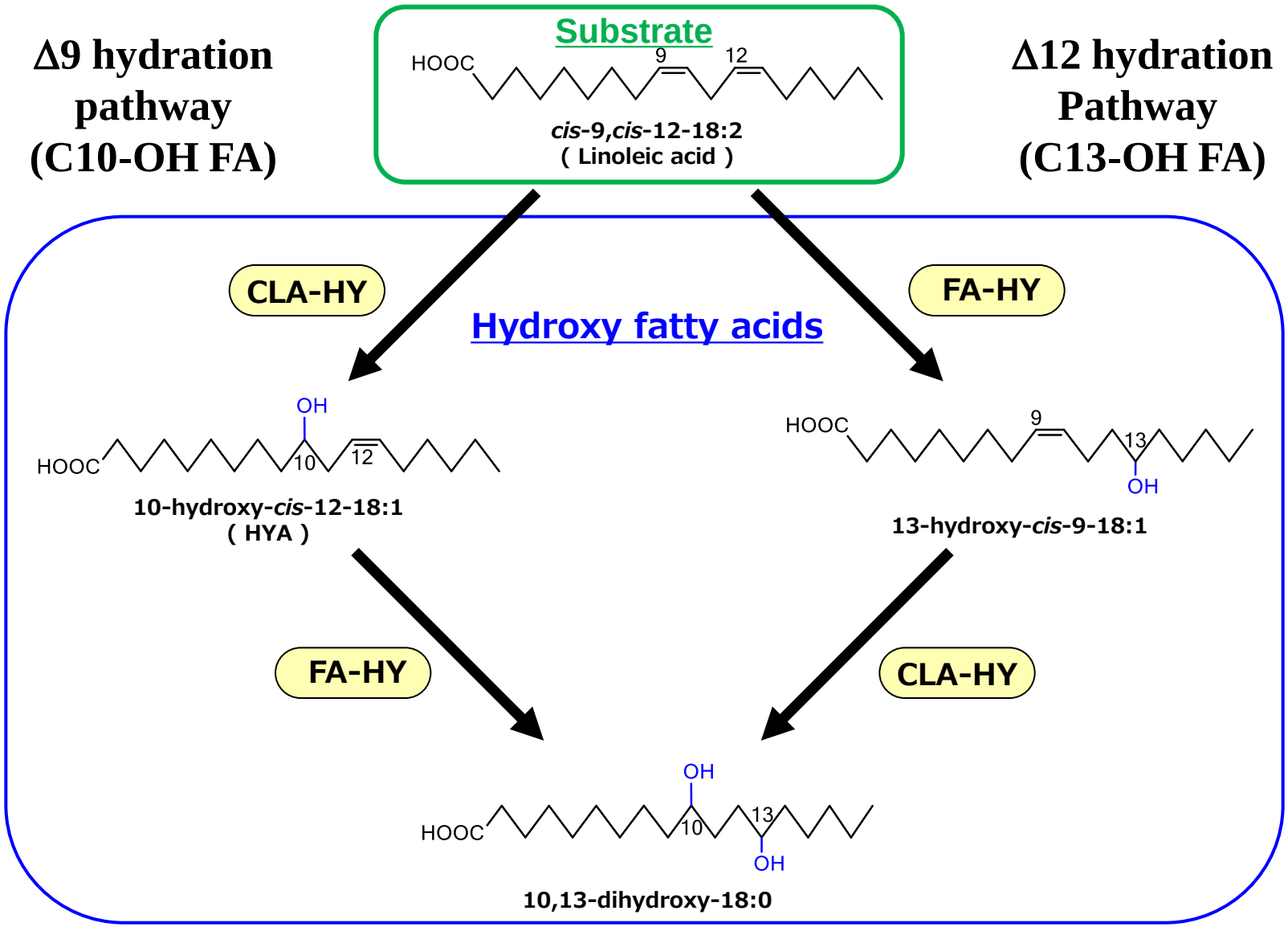
*Sodium succinate buffer pH 5.5, 20 mM;
FAD, 0.1 mM; without NADH; reaction temperature, 37℃

Takeuchi, M. et al, *J. Biosci. Bioeng.* 119, 636-641 (2015)

CLA-HY -Substrate specificity-

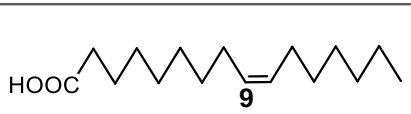
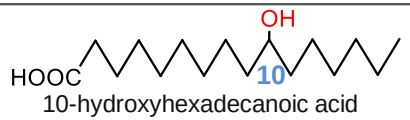
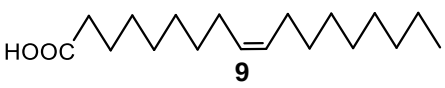
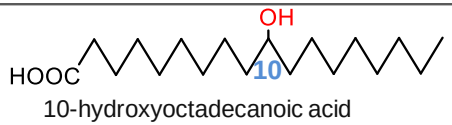
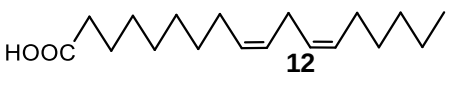
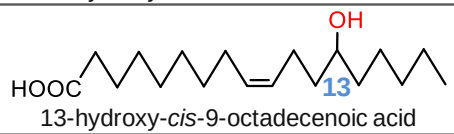
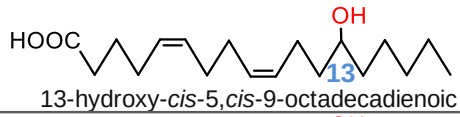
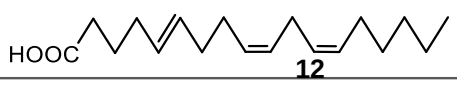
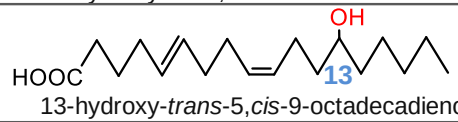
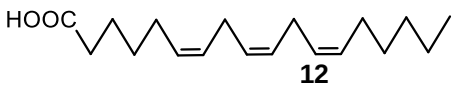
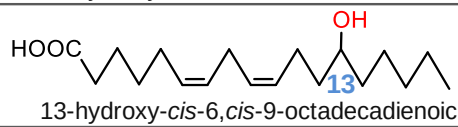
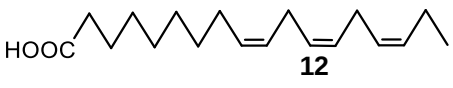
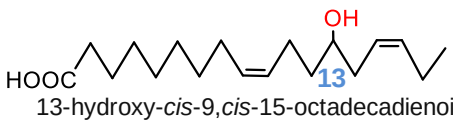
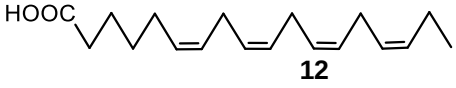
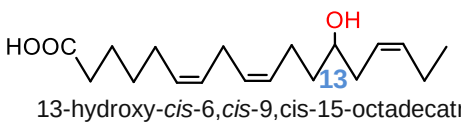
Substrates (for hydration)		Relative activity (%)
Linoleic acid (18:2)		100
Oleic acid (18:1)		335
α-Linolenic acid (18:3)		29
γ-Linolenic acid (18:3)		43
Stearidonic acid (18:4)		43
Palmitoleic acid (16:1)		44
Ricinoleic acid (12-OH 18:1)		0.5
cis-Vaccenic acid (*cis*-11-Octadecenoic acid) (18:1)		—
Elaidic acid (*trans*-9-Octadecenoic acid) (18:1)		—
Methyl linoleate (18:1 methyl ester)		—

Linoleic acid metabolism by *Pediococcus* sp. AKU 1080



Takeuchi, M. et al, *Eur. J. Lipid Sci. Technol*., 115, 386-393 (2013)

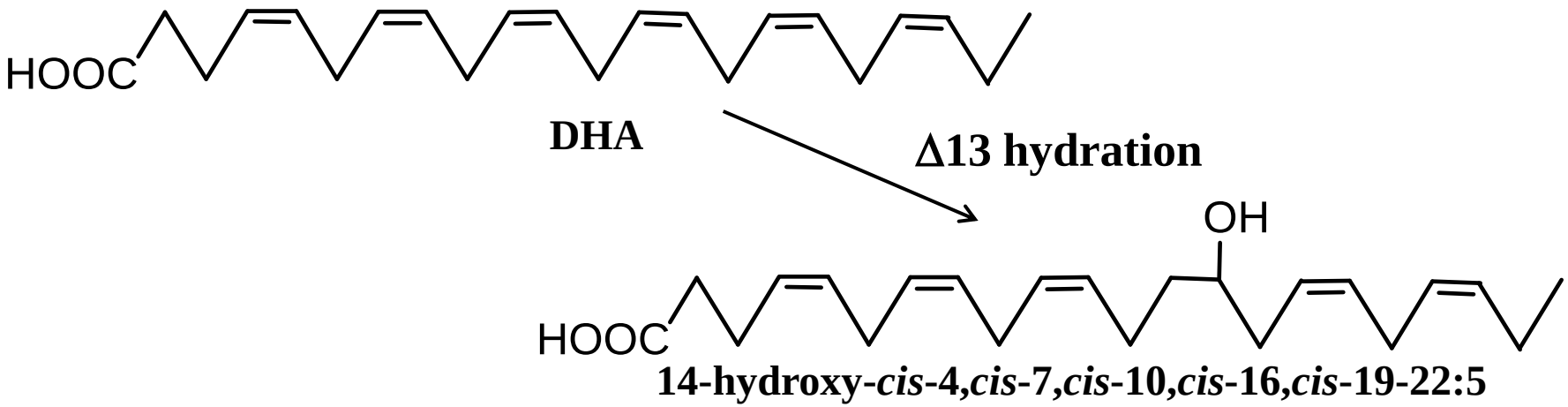
Hydration reactions catalyzed by *L. acidophilus* hydratase (FA-HY1)

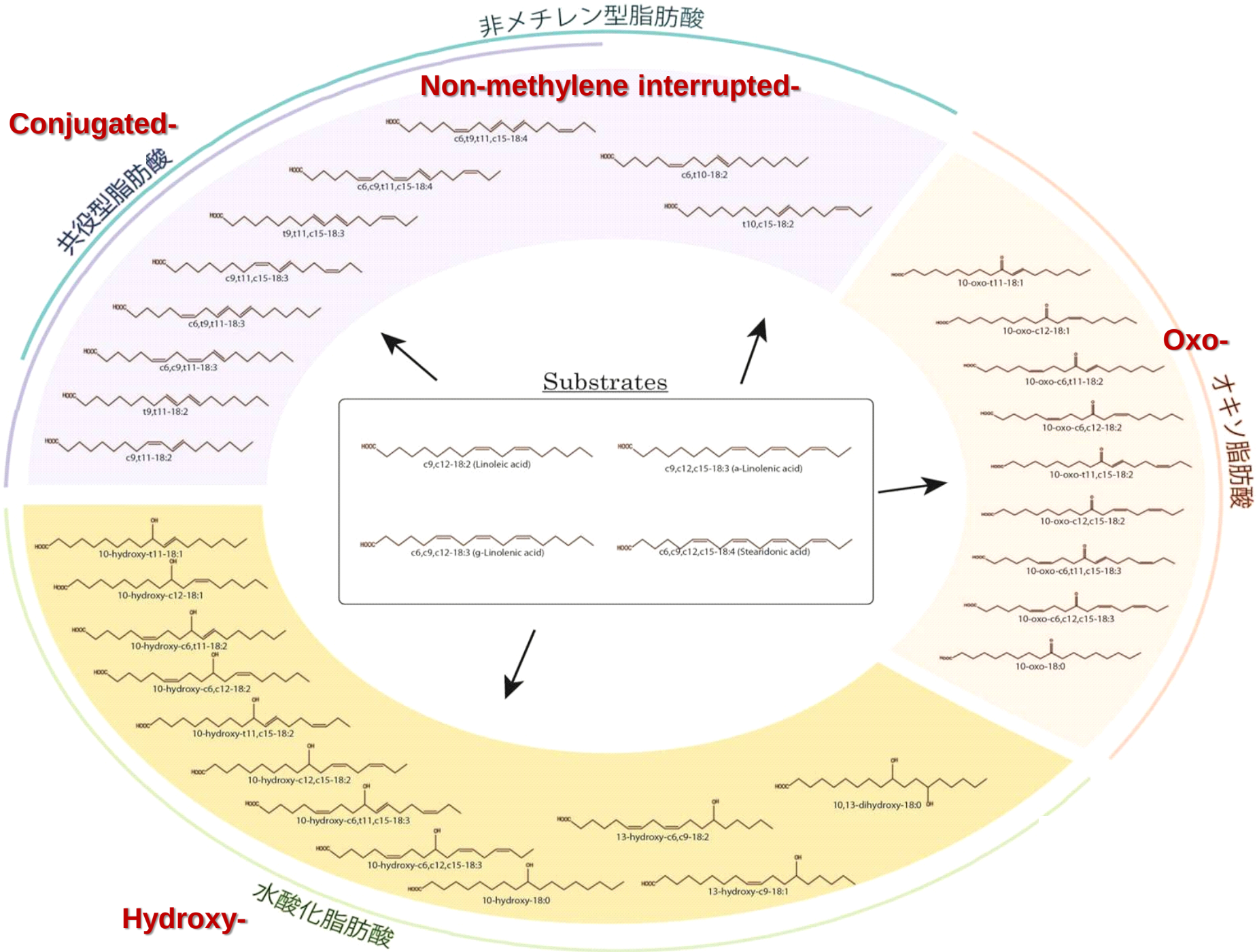
Substrate (10 mM)	Product	Yield (%) in 16 h reaction
Palmitoleic acid C16:1$^{\Delta 9cis}$		 10-hydroxyhexadecanoic acid	2
Oleic acid C18:1$^{\Delta 9cis}$		 10-hydroxyoctadecanoic acid	1
cis-Vaccenic acid C18:1$^{\Delta 11cis}$		 12-hydroxyoctadecanoic acid	59
Linoleic acid C18:2$^{\Delta 9cis, \Delta 12cis}$		 13-hydroxy-*cis*-9-octadecenoic acid	48
C18:2$^{\Delta 9trans, \Delta 12trans}$		Not hydrated	
Pinolenic acid C18:3$^{\Delta 5cis, \Delta 9cis, \Delta 12cis}$		 13-hydroxy-*cis*-5,*cis*-9-octadecadienoic acid	57
Columbinic acid C18:3$^{\Delta 5trans, \Delta 9cis, \Delta 12cis}$		 13-hydroxy-*trans*-5,*cis*-9-octadecadienoic acid	46
γ-Linolenic acid C18:3$^{\Delta 6cis, \Delta 9cis, \Delta 12cis}$		 13-hydroxy-*cis*-6,*cis*-9-octadecadienoic acid	57
α -Linolenic acid C18:3$^{\Delta 9cis, \Delta 12cis, \Delta 15cis}$		 13-hydroxy-*cis*-9,*cis*-15-octadecadienoic acid	54
Stearidonic acid C18:4$^{\Delta 6cis, \Delta 9cis, \Delta 12cis, \Delta 15cis}$		 13-hydroxy-*cis*-6,*cis*-9,cis-15-octadecatrienoic acid	12

Hirata, A. et al, *J. Lipid Res.*, 56, 1340-1350 (2015)

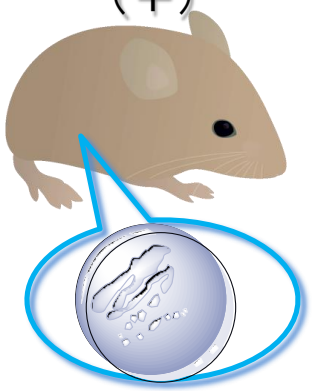
Substrate specificity of fatty acid hydratase (FA-HY1) from *Lactobacillus acidophilus* NTV001

Substrate	Product
20:2$^{\Delta 11cis,\ \Delta 14cis}$	15-hydroxy-*cis*-11-20:1
20:3$^{\Delta 5cis,\ \Delta 8cis,\ \Delta 11cis}$	12-hydroxy-*cis*-5,*cis*-8-20:2
20:3$^{\Delta 5cis,\ \Delta 11cis,\ \Delta 14cis}$	15-hydroxy-*cis*-5,*cis*-11-20:2
20:3$^{\Delta 8cis,\ \Delta 11cis,\ \Delta 14cis}$ (DGLA)	15-hydroxy-*cis*-8,*cis*-11-20:2
	12-hydroxy-*cis*-8,*cis*-14-20:2
20:3$^{\Delta 11cis,\ \Delta 14cis,\ \Delta 17cis}$	12-hydroxy-*cis*-14,*cis*-17-20:2
	15-hydroxy-*cis*-11,*cis*-17-20:2
20:4$^{\Delta 5cis,\ \Delta 8cis,\ \Delta 11cis,\ \Delta 14cis}$ (AA)	15-hydroxy-*cis*-5,*cis*-8,*cis*-11-20:3
20:4$^{\Delta 8cis,\ \Delta 11cis,\ \Delta 14cis,\ \Delta 17cis}$	12-hydroxy-*cis*-8,*cis*-14,*cis*-17-20:3
EPA	–
DHA	**14-hydroxy-*cis*-4,*cis*-7,*cis*-10,*cis*-16,*cis*-19-22:5**



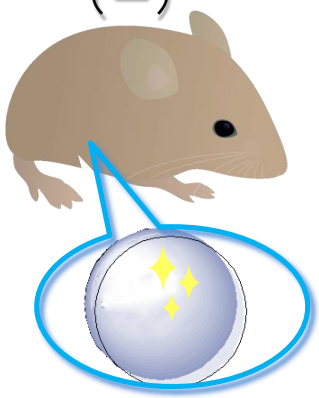

非メチレン型脂肪酸
Non-methylene interrupted-
Conjugated-
共役型脂肪酸
c6,t9,t11,c15-18:4
c6,t10-18:2
c6,c9,t11,c15-18:4
t10,c15-18:2
t9,t11,c15-18:3
c9,t11,c15-18:3
c6,t9,t11-18:3
c6,c9,t11-18:3
t9,t11-18:2
c9,t11-18:2
Substrates
c9,c12-18:2 (Linoleic acid)
c9,c12,c15-18:3 (a-Linolenic acid)
c6,c9,c12-18:3 (g-Linolenic acid)
c6,c9,c12,c15-18:4 (Stearidonic acid)
Oxo-
オキソ脂肪酸
10-oxo-t11-18:1
10-oxo-c12-18:1
10-oxo-c6,t11-18:2
10-oxo-c6,c12-18:2
10-oxo-t11,c15-18:2
10-oxo-c12,c15-18:2
10-oxo-c6,t11,c15-18:3
10-oxo-c6,c12,c15-18:3
10-oxo-18:0
10-hydroxy-t11-18:1
10-hydroxy-c12-18:1
10-hydroxy-c6,t11-18:2
10-hydroxy-c6,c12-18:2
10-hydroxy-t11,c15-18:2
10-hydroxy-c12,c15-18:2
10-hydroxy-c6,t11,c15-18:3
10-hydroxy-c6,c12,c15-18:3
10-hydroxy-18:0
13-hydroxy-c6,c9-18:2
10,13-dihydroxy-18:0
13-hydroxy-c9-18:1
Hydroxy-
水酸化脂肪酸

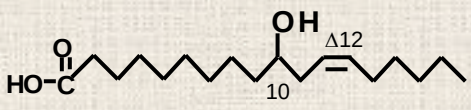
With gut microbes
(specific pathogen-free)
(+)

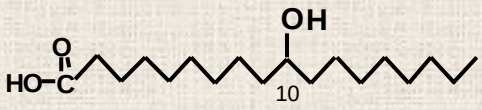


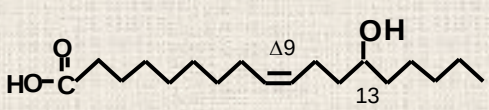
VS

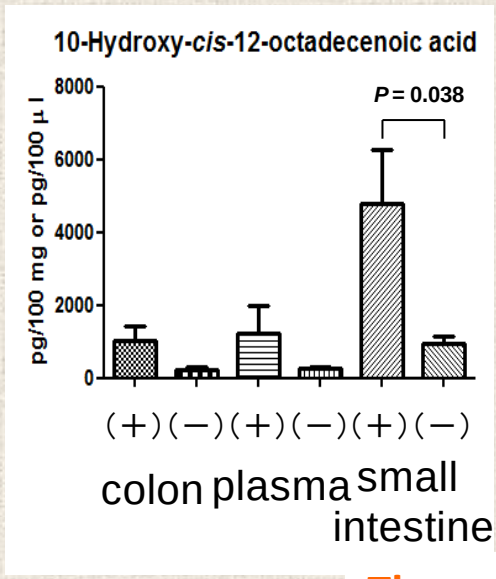
Without gut microbes
(germ-free)
(−)

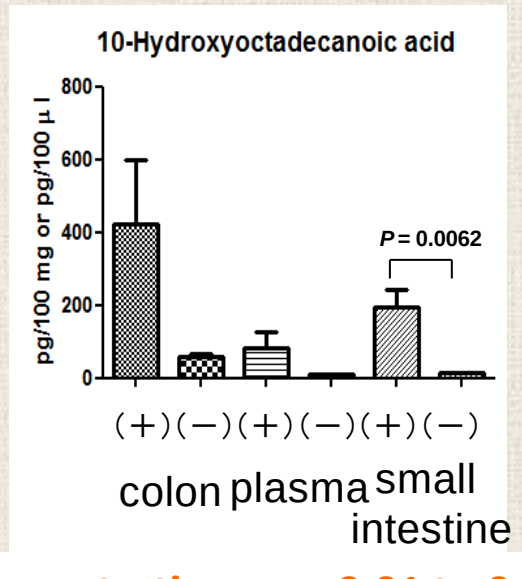


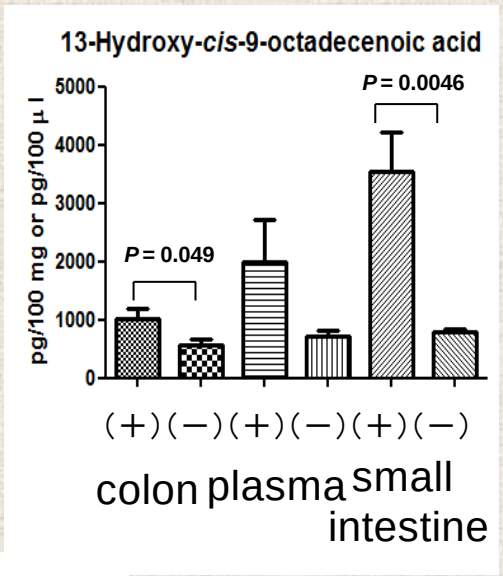












The concentrations are 0.01 to 0.1μ M

Kishino, S. et al, *Proc. Natl. Acad. Sci. USA*, 110, 17808-17813 (2013)

Enhancing gut hormone secretion & anti-diabetic activity

Goto J. et al, *Biochem. Biophys. Res. Commun.*, 459, 597-603 (2015)
Kim M. et al, *FASEB J.*, 31, 5036-5048 (2017)
Yonejima Y., et al, *Prog. Med.*, 37, 1105-1111 (2017)
Miyamoto J., et al, *Nat. Commun.*10, 4007 (2019)

Hypolipidemic effect

Nanthirudjanar T., et al, *Lipids*, 50, 1093-1102 (2015)

Anti-inflammatory and immune controlling effects

Bergamo P,. et al, *J. Funct. Foods*, 11, 192-202 (2014)
Ohue-Kitano R,. et al, *FASEB J.*, 32, 304-318 (2018)

Intestinal and gingival epithelial barrier protection

Miyamoto J. et al, *J. Biol. Chem.*, 290, 2902-2918 (2015)
Yamada M. et al., *Sci. Rep.*, 8, 9008 (2018)

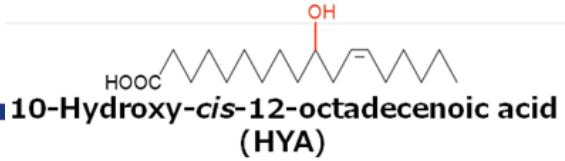
Anti-oxidative effects

Furumoto H., et al, *Toxicol. Appl. Pharmacol.*, 296, 1-9 (2016)

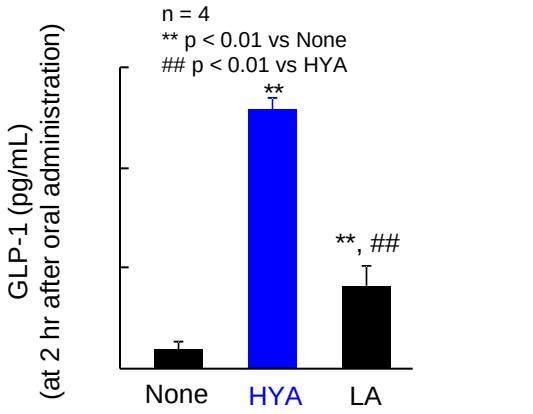
Anti *Helicobacter pylori* activity

Matsui H., et al, *Helicobacter*, 22, e12430 (2017)

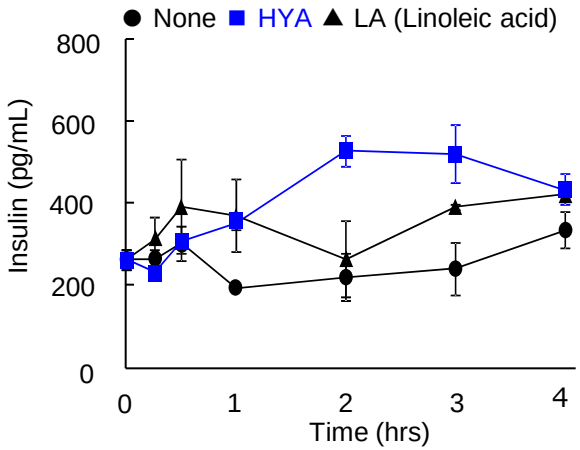
HYA induced gut hormone secretion



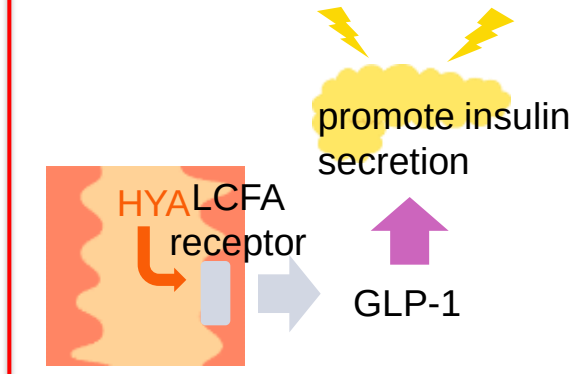
Oral administration of HYA (1000 mg/kg) induces insulin secretion by increasing GLP-1 level



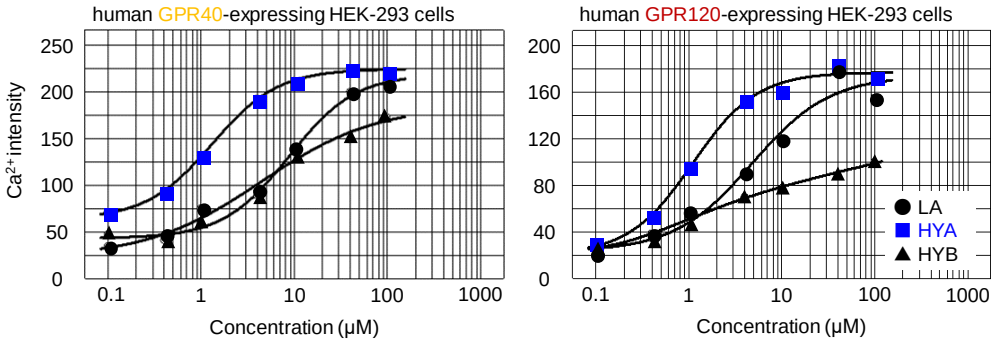
Blood GLP-1 concentration



Blood Insulin concentration



HYA may induce GLP-1 secretion through GPR40, and promote insulin secretion from pancreatic β cells.

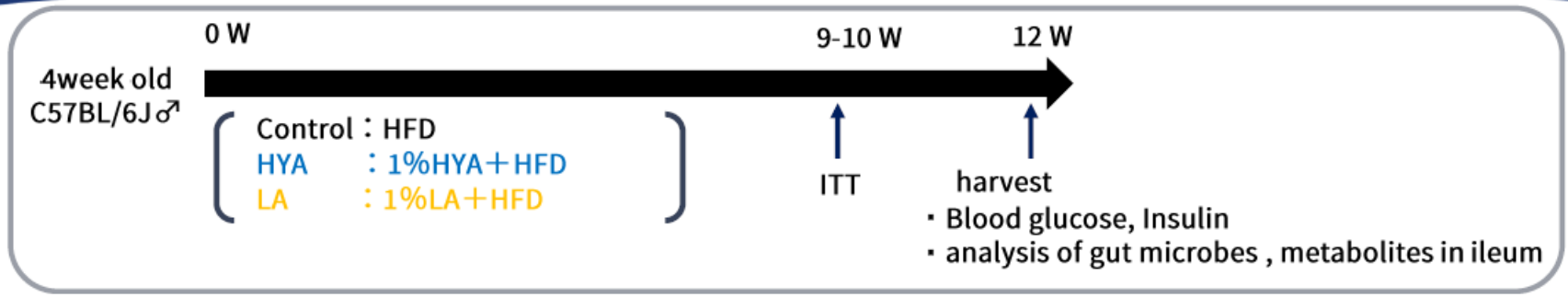


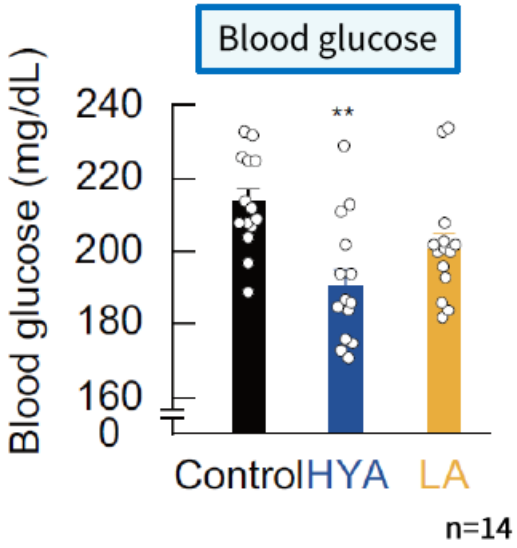
Affinity of GPR40 and GPR120

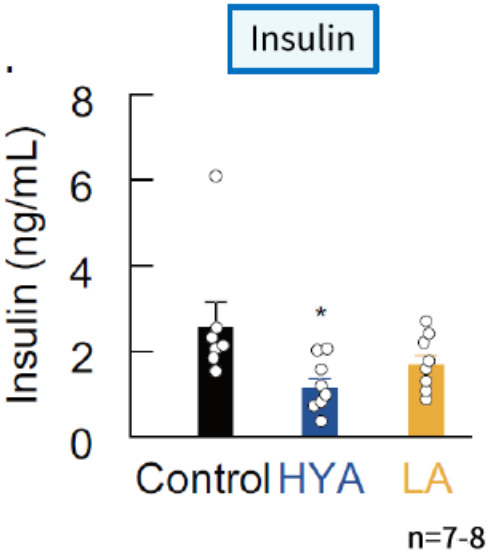
Miyamoto J. The 2016 annual conference of the Japan Society for Bioscience, Biotechnology and Agrochemistry.

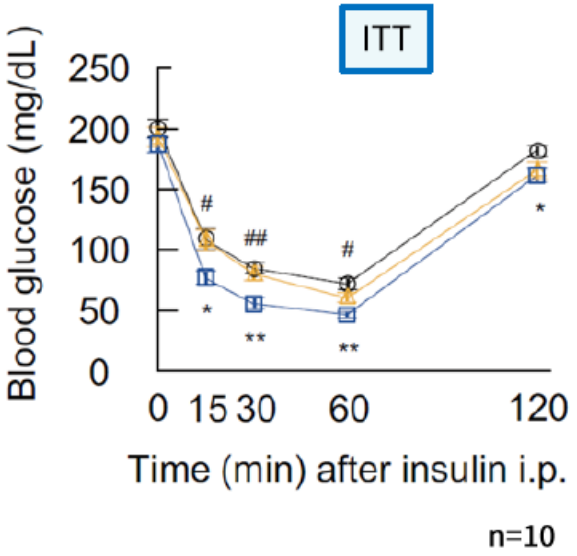
Miyamoto J., et al, NATURE COMMUNICATIONS 10:4007, 2019

HYA improves insulin resistance







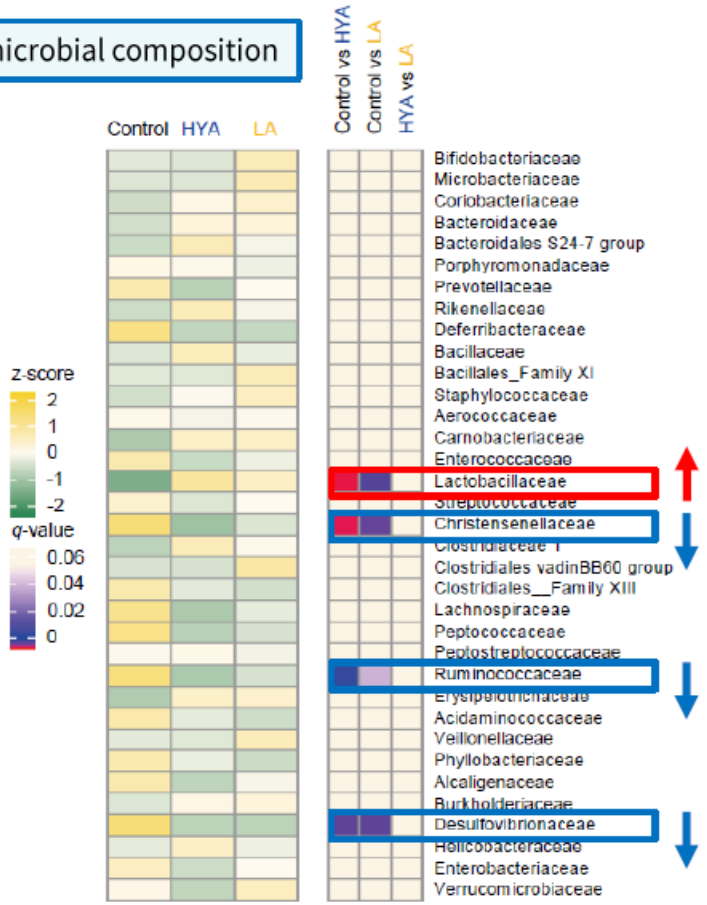


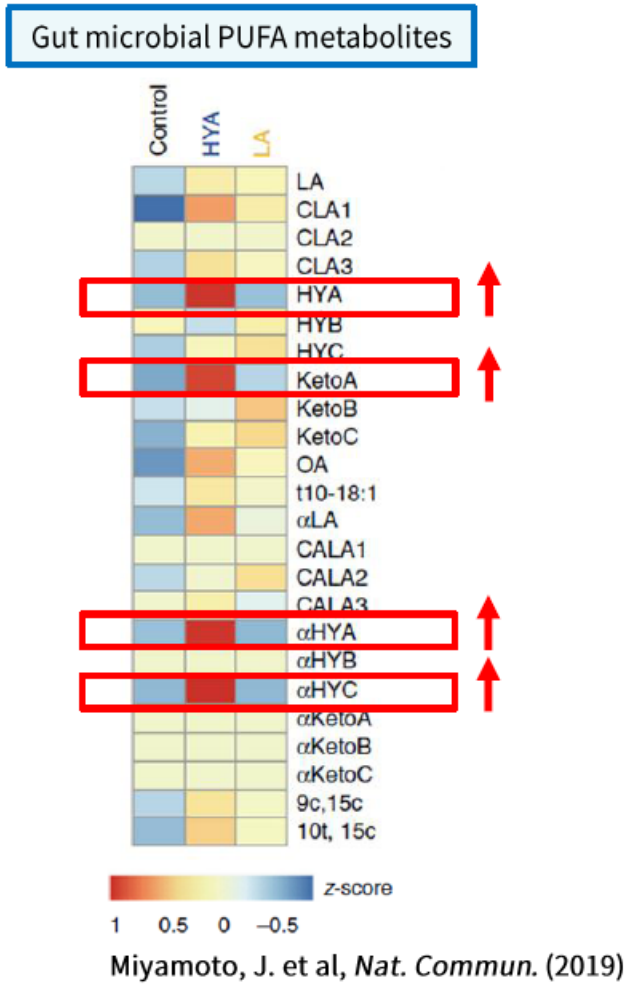
HYA improves HFD-induced insulin resistance by promoting GPR40 and 120-mediated GLP-1 secretion.

Miyamoto J., et al, NATURE COMMUNICATIONS 10:4007, 2019

HYA alters gut microbiome and their metabolites

12 weeks administration
Control：HFD
HYA　：1%HYA＋HFD
LA　：1%LA＋HFD

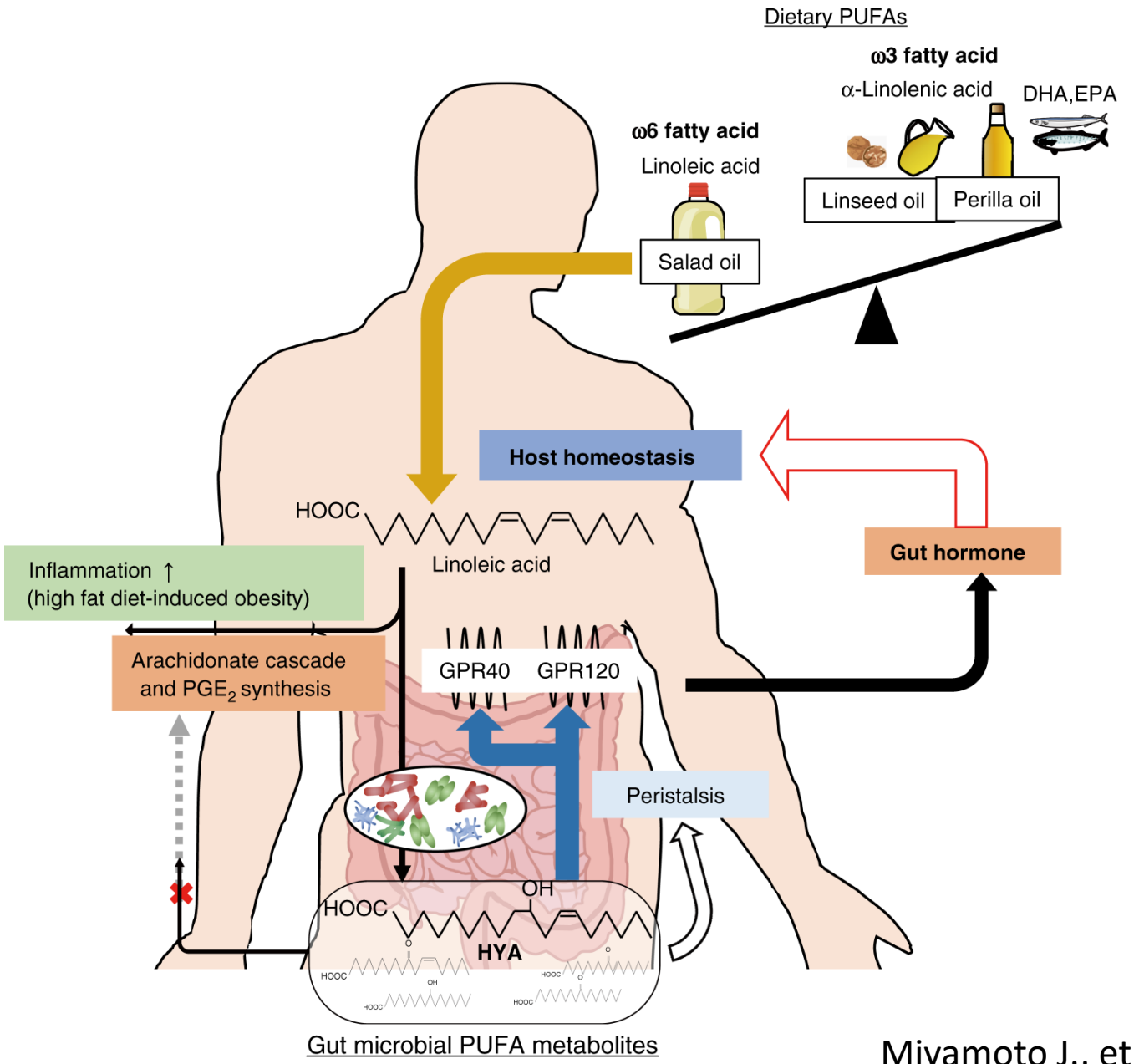




Miyamoto, J. et al, *Nat. Commun.* (2019)

HYA increase the abundance of the *Lactobacillaceae* family and specific metabolites in ileum

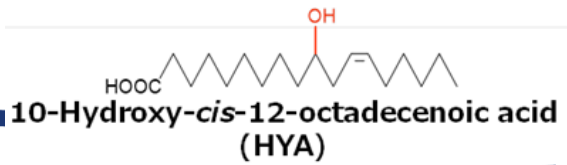
Miyamoto J., et al, NATURE COMMUNICATIONS 10:4007, 2019



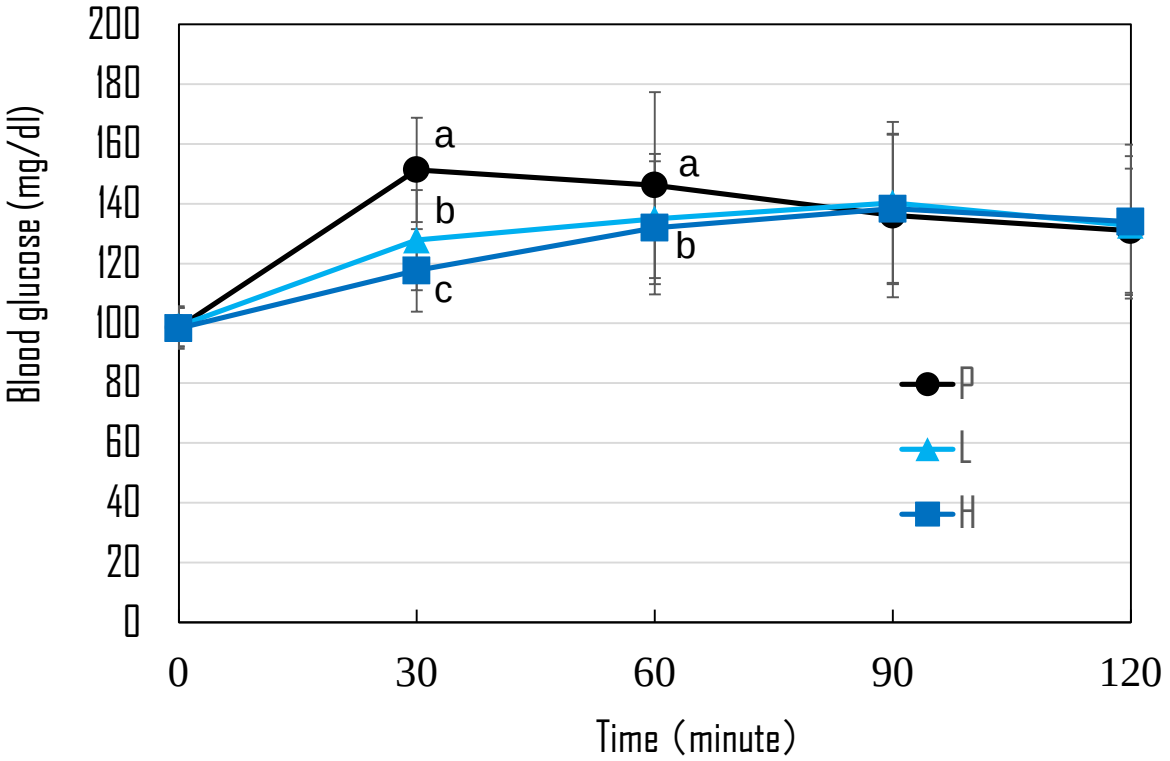
Gut microbiota confers host resistance to obesity by metabolizing dietary polyunsaturated fatty acids

Miyamoto J., et al,
NATURE COMMUNICATIONS
10:4007, 2019

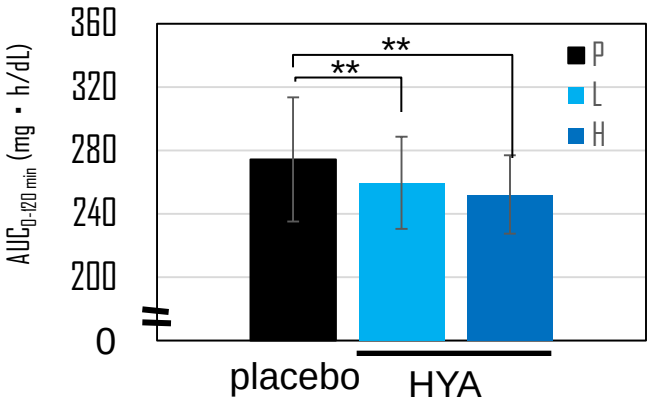
Clinical trial results



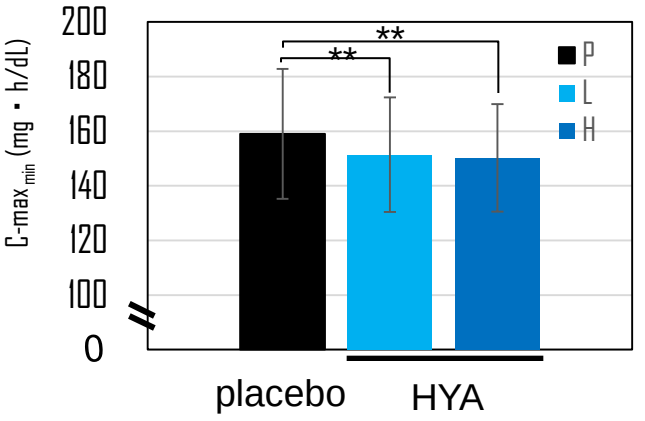
Changes in blood glucose after ingestion



$AUC_{0\text{-}120\ min}$



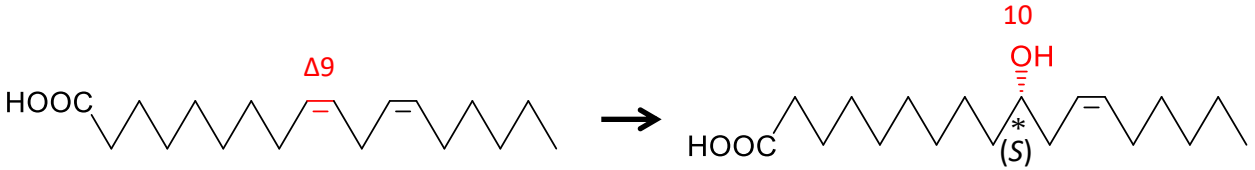
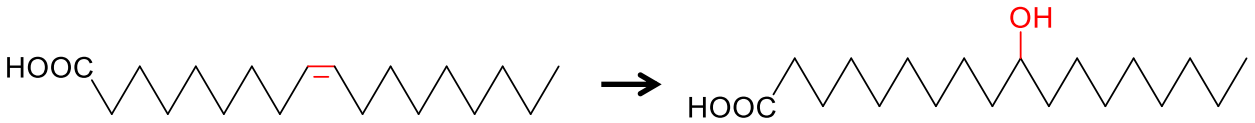
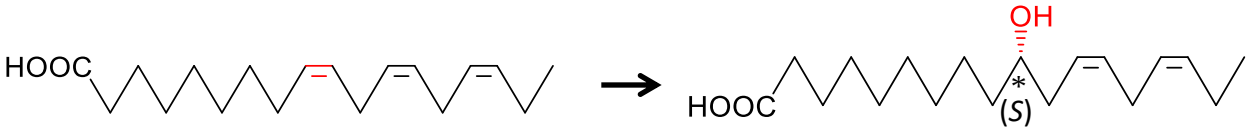
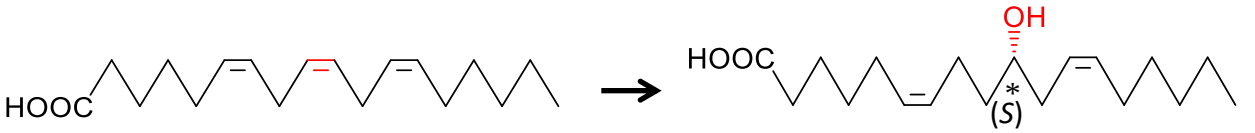
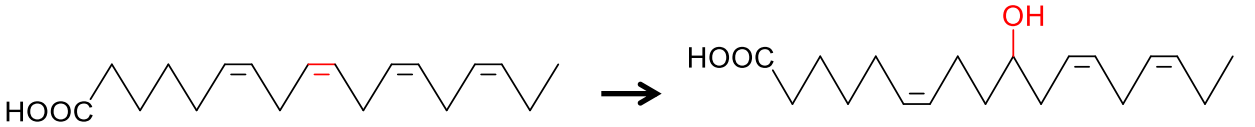
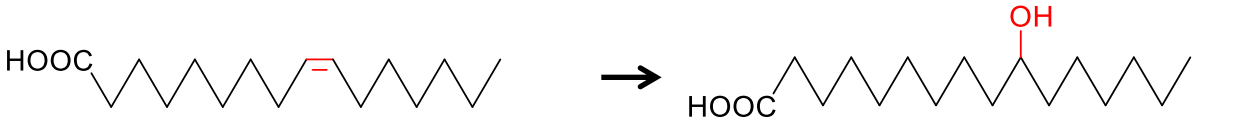
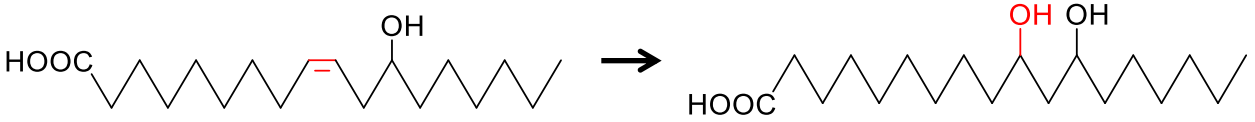
$C\text{-}max_{\ min}$



P: ingesting a placebo
L : ingesting low dose HYA (1000mg)
H : ingesting high dose HYA (2000mg)

Yonejima Y., et al, *Prog. Med.*, 37, 1105-1111 (2017)

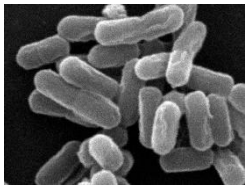
Hydroxy fatty acid production using CLA-HY expressed in *E. coli*

Substrates (280 g/L)		Yield [%]
Linoleic acid (18:2)		98% (292 g/L)
Oleic acid (18:1)		96% (286 g/L)
α-Linolenic acid (18:3)		96% (286 g/L)
γ-Linolenic acid (18:3)		95% (283 g/L)
Stearidonic acid (18:4)		96% (286 g/L)
Palmitoleic acid (16:1)		98% (294 g/L)
Ricinoleic acid (12-OH 18:1)		99% (294 g/L)

HYA production from edible plant oils using lactic acid bacteria

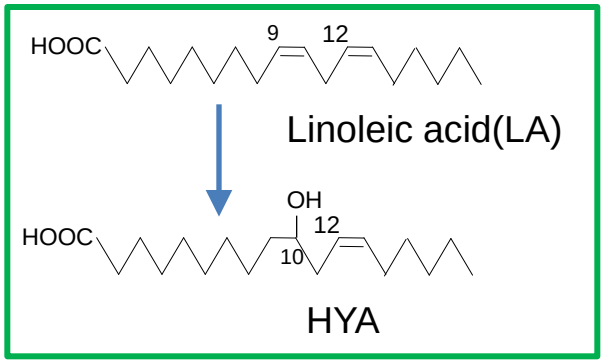
Oil

Lipase

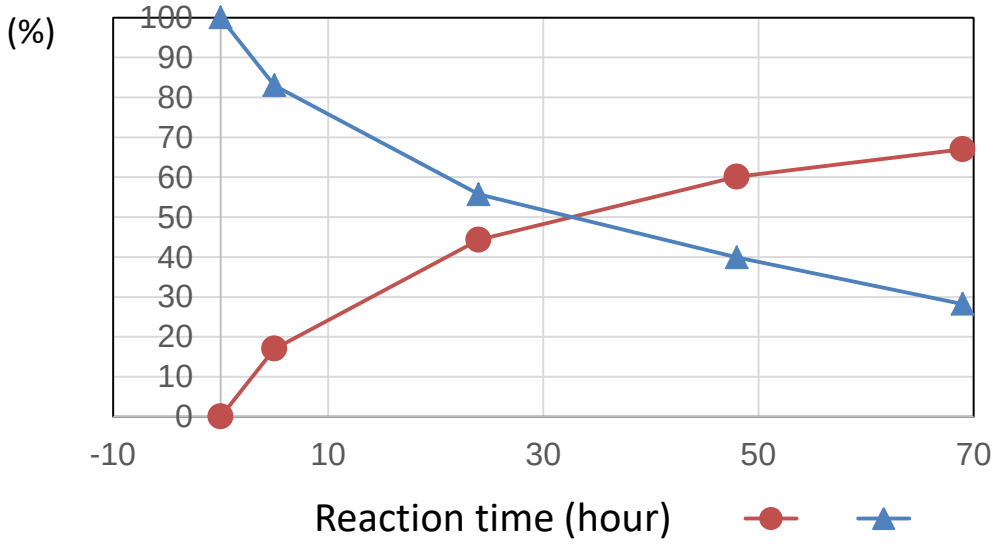


Lactic acid bacteria (LAB)

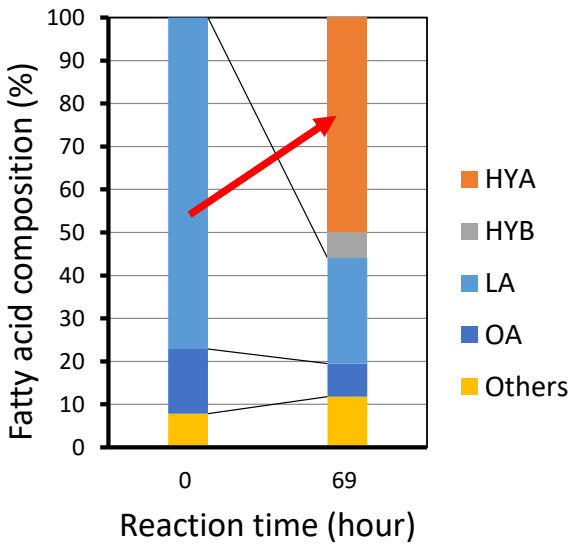
HYA



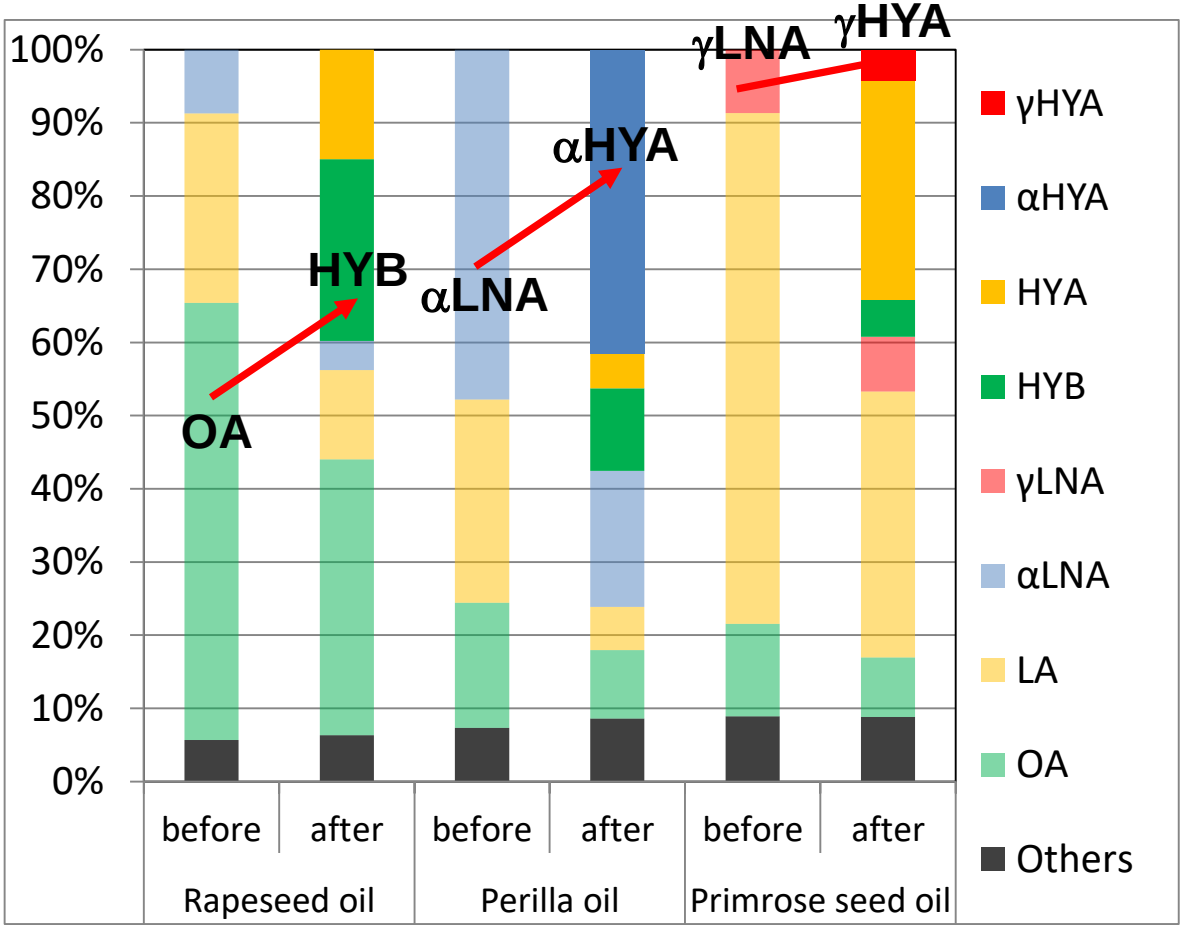
Time course of HYA production

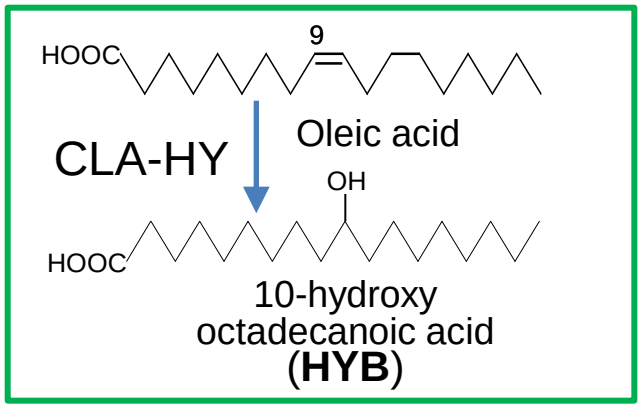


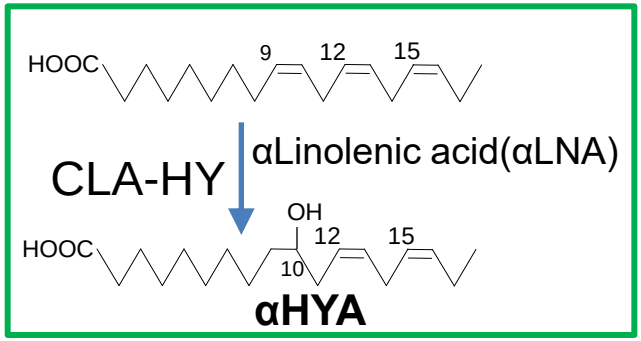
Fatty acid composition after reaction

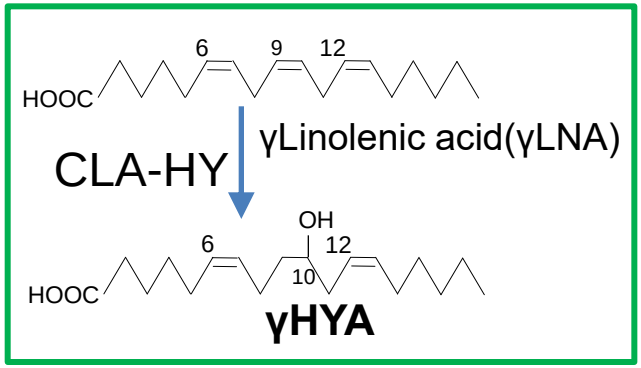


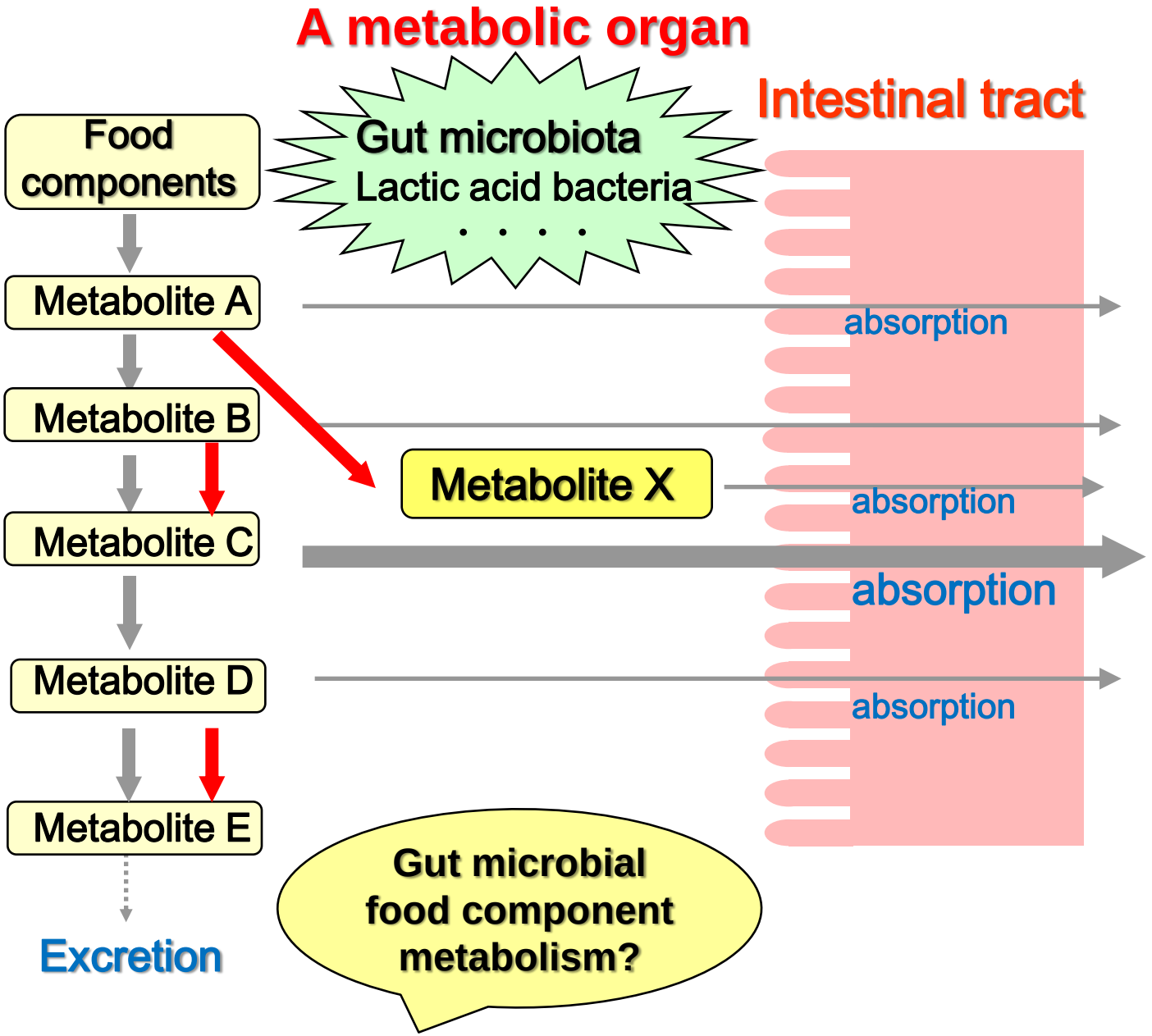
HYA production from edible plant oils using lactic acid bacteria











for Human Health and Good Life

Summary

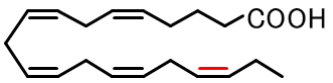
Functional lipid production by microbial metabolisms and enzymes: beyond common polyunsaturated fatty acids

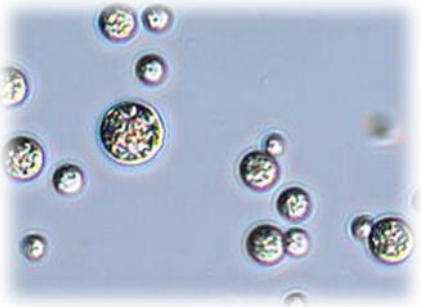
Fermentative production of PUFAs

EPA: 52 % 14.4g/L

M. alpina transformant expressing

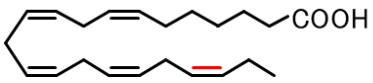
Δ12, Δ17 desaturases





n−3 DPA: 165 mg/L

Aurantiochytrium sp. T7

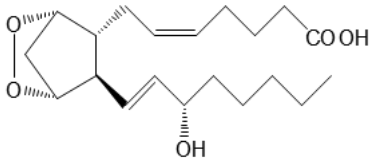


Biotransformation of PUFAs

$PGF_{2\alpha}$: 6 mg/L

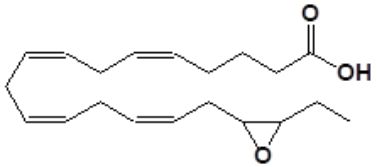
M. alpina transformant expressing

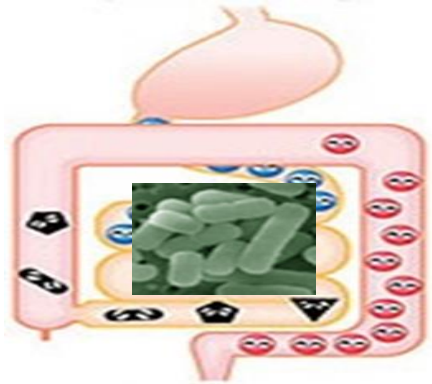
COX from alga



17,18−EpETe: 0.38 mg /mL

P450 MO from Bacillus species

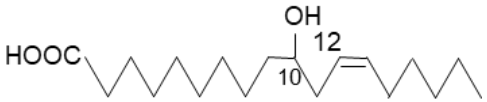




HYA: 292 g /L

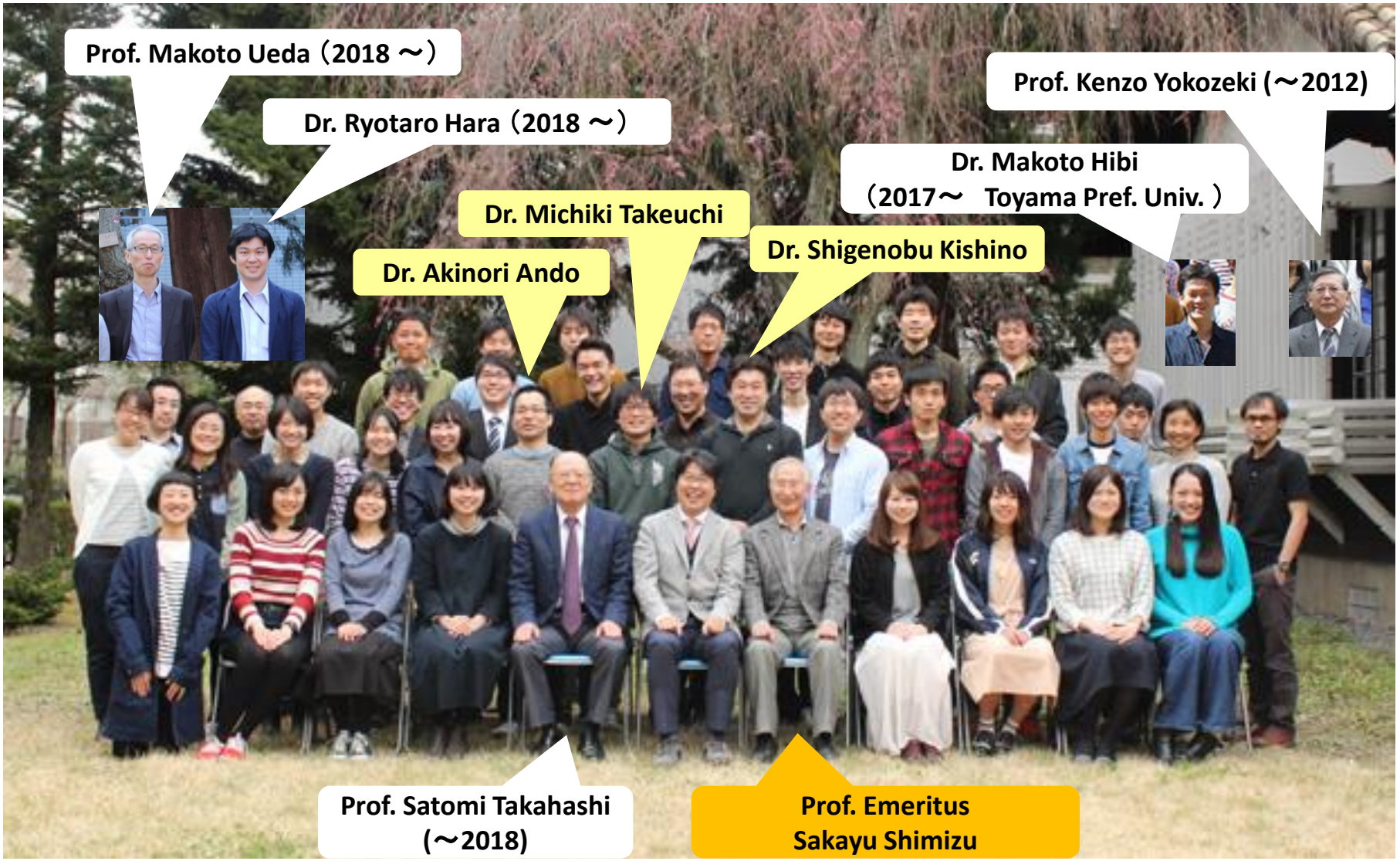
Other hydroxy FAs, Oxo−FAs, enone FAs

Lactobacillus species



Acknowledgments

Hakko Lab. (Div. Appl. Life Sci., Grad. Sch. Agric. Kyoto Univ.)



JSPS･NEDO･BRAIN･JST-MIRAI･AMED

Thank you for your kind attention !