Potentials of Microorganisms for Functional Food Production and Probiotics

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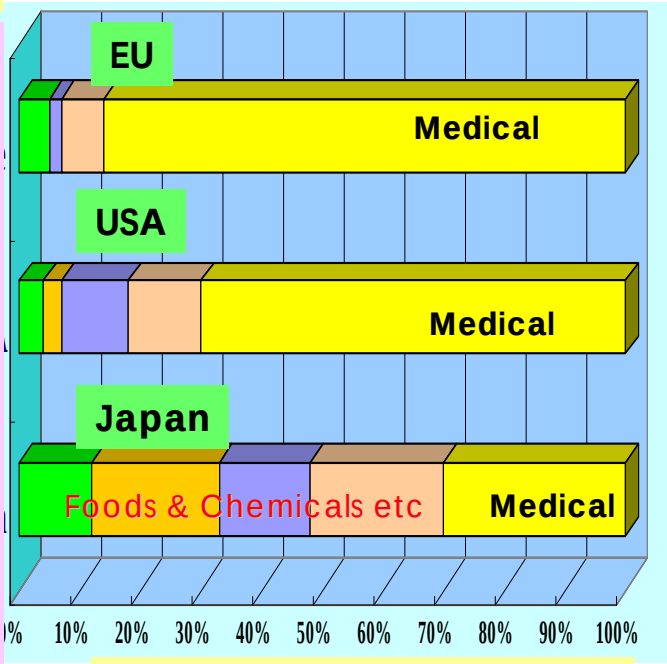
Base of Japan's Microbial Biotechnology

- Japan is a country rich in microbial resources.
- We have high affinity to microorganisms, that has been obtained traditionally and environmentally.
- There are many active industries using microorganisms.

豊かな微生物資源があります

微生物との共存ができます

Percentage of patent applicants in industry sector



バイオに強い化学工業があります

Potentials of Microorganisms for Functional Food Production and Probiotics

Creation of new food function based on unique microbial function:

Functional food materials produced by microbial transformation

- Production of 4-hydroxyisoleucine
- Production of polyunsaturated fatty acids
- Production of conjugated fatty acids

Food functions based on catalytic activity of microbial enzymes

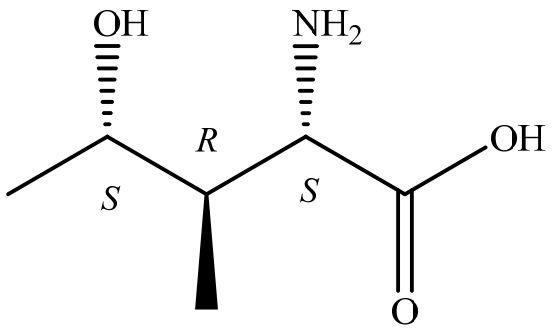
- Deodorizing activity derived from laccase

Probiotic use of lactic acid bacteria and their metabolisms

- Probiotics for hyperuricemia prevention

Searching unique microbial functions in Japanese microbial diversity and using them for food and chemical industries

4-Hydroxyisoleucine (HIL)

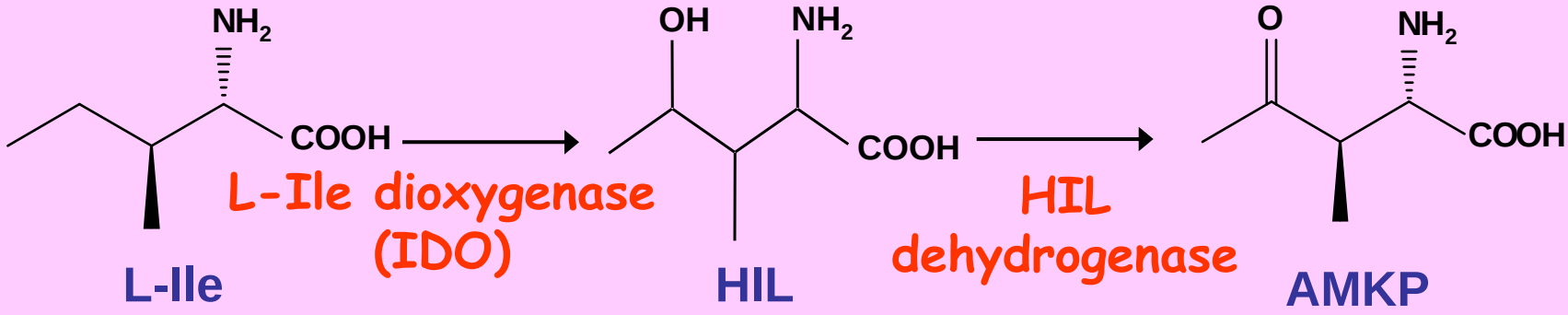


Fenugreek

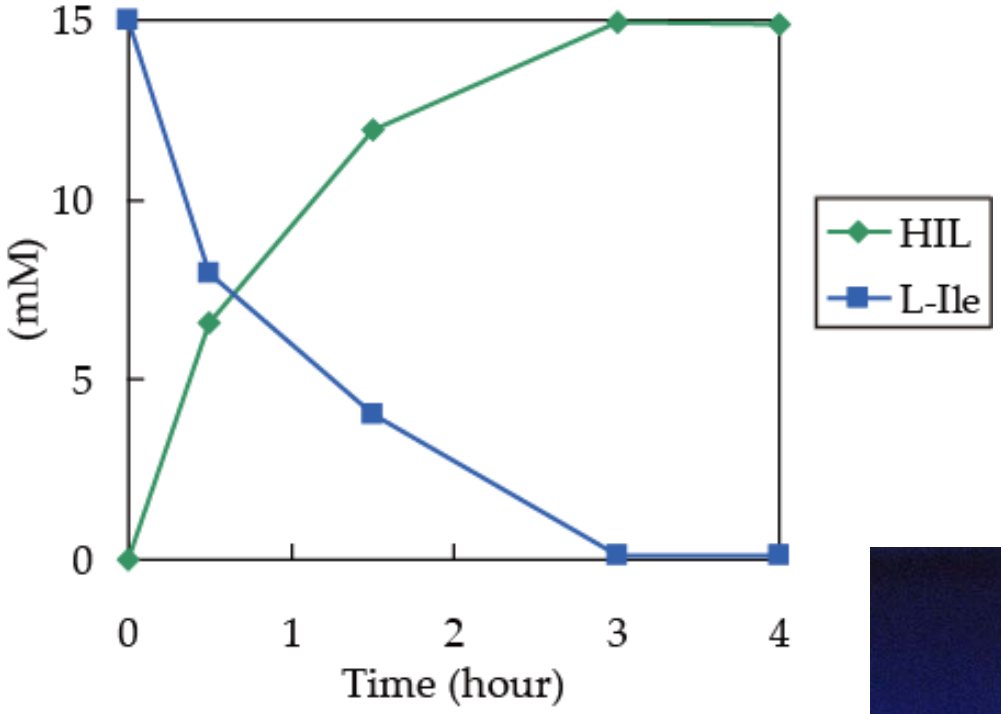
Target decision: by collaboration and discussion with industries.

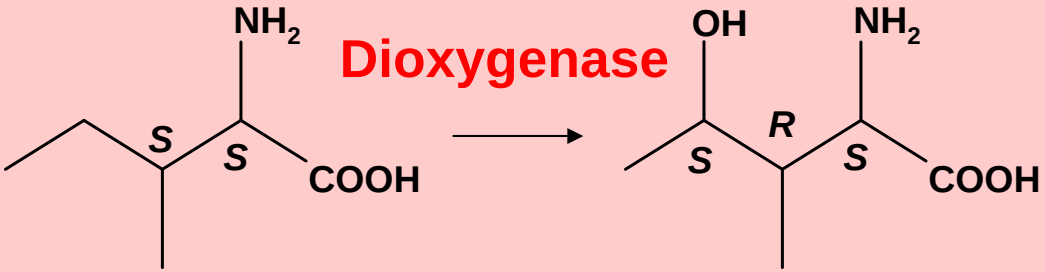
- 4-Hydroxyisoleucine (HIL) is a potential drug candidate for the treatment of diabetes and obesity.
- HIL is contained in fenugreek seeds, but the amount is low.
- Enzymatic processes are promissing for HIL synthesis that needs high stereo- and functional-group selectivity.

L-Ile transformation pathway found in *Bacillus thuringiensis* strain 2e2



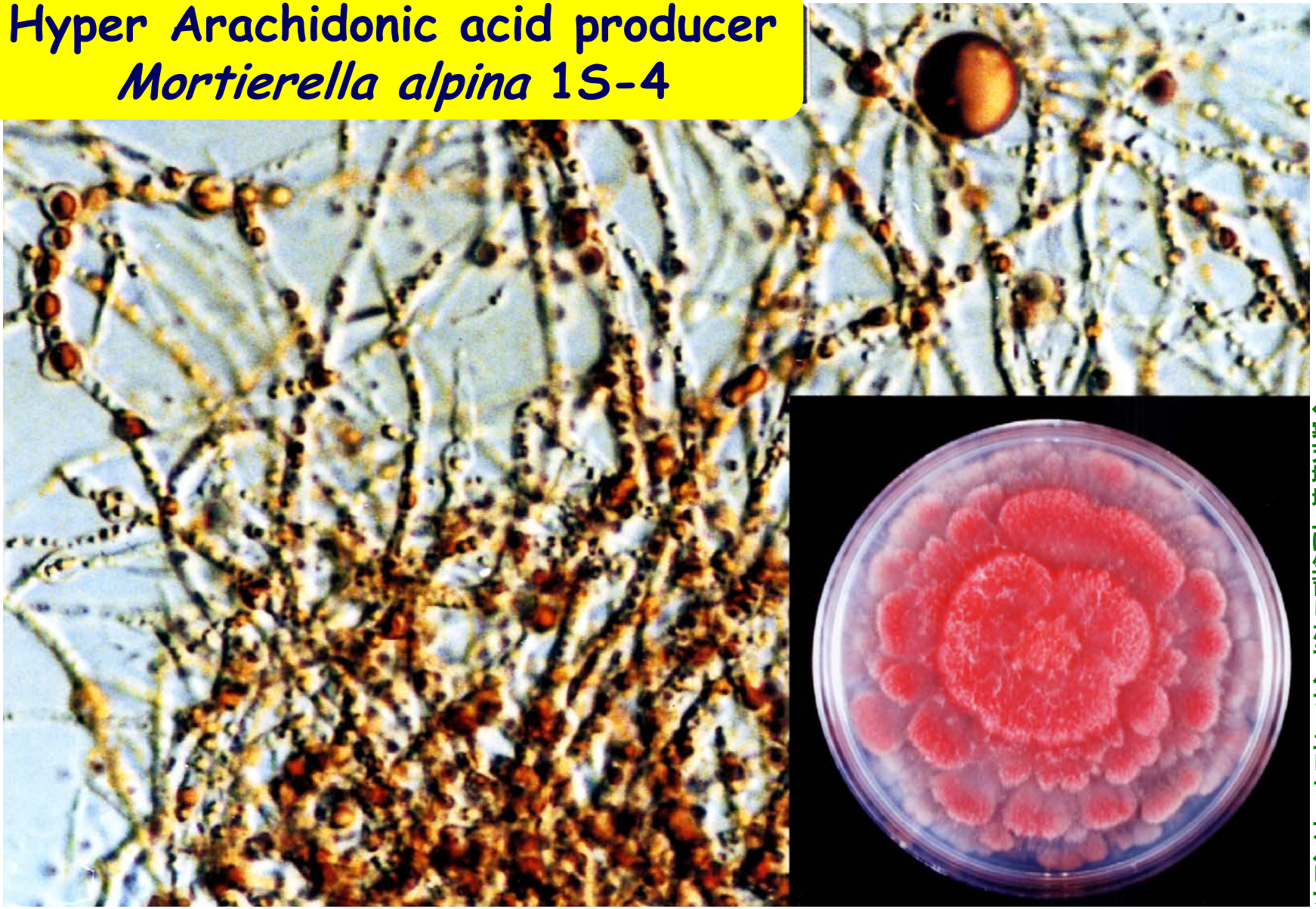
HIL production by cell-free extracts of *E. coli* expressing IDO from *B. thuringiensis* strain 2e2





Dioxygenase process

Hyper Arachidonic acid producer *Mortierella alpina* 1S-4

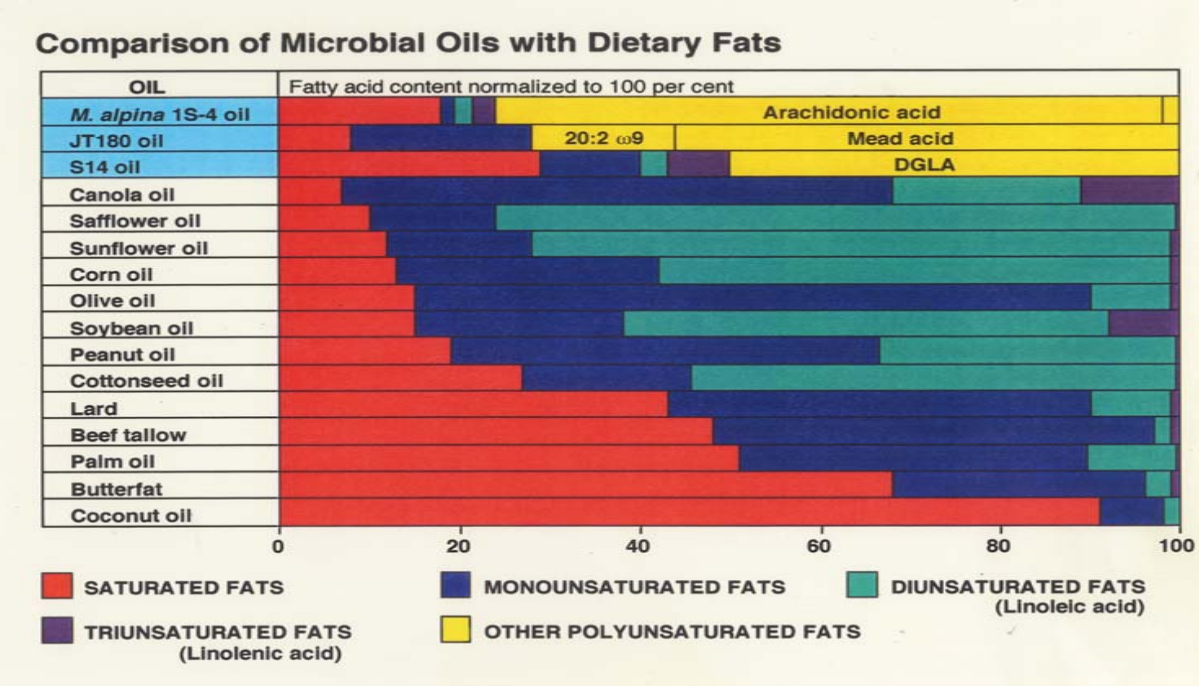


→高度不飽和脂肪酸の油滴が見られる顕微鏡写真

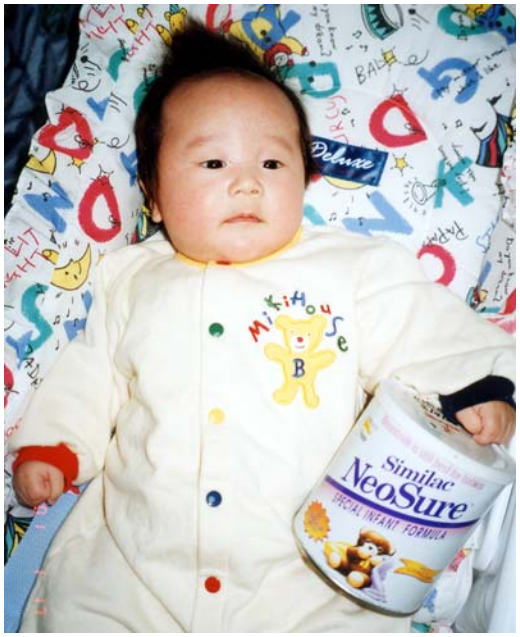
←寒天培地上に生育したモルティエレラ・アルピナ

Searching unique microbial functions in Japanese microbial diversity and using them for food and chemical industries

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.



"発酵油脂" は
乳幼児用ミルクの
栄養素として
世界中で
使われている。



見ためは同じでも
"発酵油脂" の脂肪酸組成は
植物・動物油脂とは全く違う。

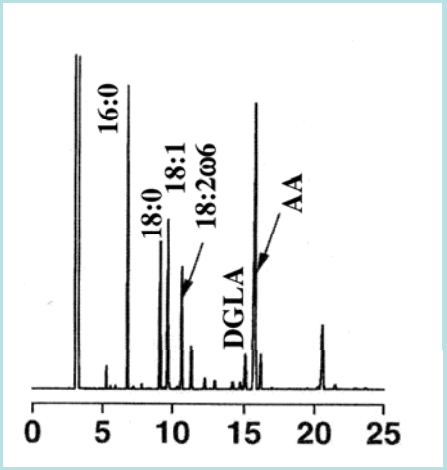
Mutant Screening

spore

NTG mutation

Mutant isolation, cultivation

Fatty acid analysis By GLC



Glucose

EL1 Δ9

18:0 18:1n-9

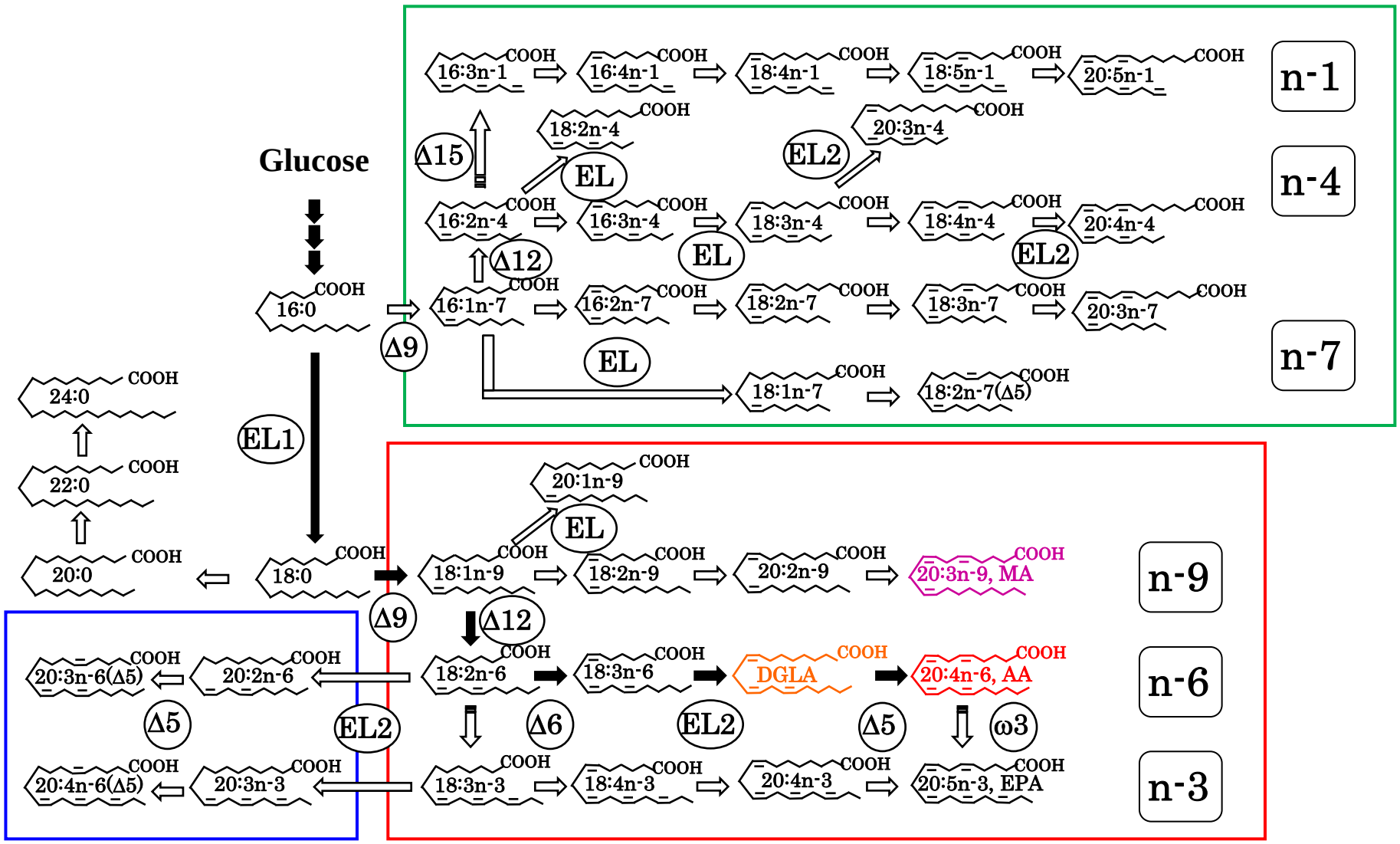
Δ12 Δ6 EL2 Δ5

18:2n-6 18:3n-6 20:3n-6, DGLA 20:4n-6, AA

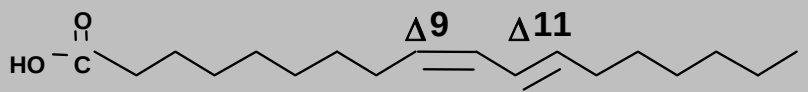
ω3 ω3

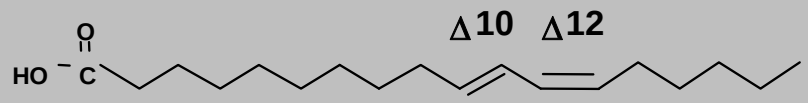
18:3n-3 Δ6 18:4n-3 EL2 20:4n-3 Δ5 20:5n-3, EPA

Various PUFAs produced by *M. alpina* 1S-4



Conjugated linoleic acid (CLA)





- inhibits initiation of skin carcinogenesis, and forestomach and mammary tumorigenesis.
- prevents the catabolic effects of immune stimulation.
- alters LDL / HDL cholesterol ratio.
- exhibits anti-arteriosclerosis activity.
- reduces body fat content and affects body weight gain.

Safe and selective CLA production process using lactic acid bacteria!!

Potential strains for CLA production from linoleic acid

Strain	Origin	Fatty acid (mg/ml reaction mixture)					
		Cellular FA	LA	Total CLA	(CAL1:CLA2)	HY1	HY2
Enterococcus faecium	AKU 1021	0.09	0.72	0.10	(0.04: 0.06)	0.02	0.06
Pediococcus acidilactici	AKU 1059	0.14	1.29	1.40	(1.00: 0.40)	0.30	0.43
Propionibacterium shermnjii	AKU 1254	0.11	1.42	0.11	(0.09: 0.02)	-	0.07
Lactobacillus acidophilus	AKU 1137	0.14	0.24	1.50	(0.85: 0.65)	0.11	0.07
Lactobacillus acidophilus	IAM10074	0.25	0.22	0.60	(0.18: 0.42)	0.60	0.18
Lactobacillus acidophilus	AKU 1122	0.09	0.91	0.12	(0.02: 0.10)	-	0.02
Lactobacillus brevis	IAM 1082	0.10	0.16	0.55	(0.23: 0.32)	0.79	-
Lactobacillus paracasei	IFO12004	0.18	0.83	0.20	(0.05: 0.15)	0.22	0.45
Lactobacillus paracasei	JCM 1109	0.17	0.76	0.07	(0.02: 0.05)	-	0.57
Lactobacillus paracasei	AKU 1142	1.08	0.90	0.07	(0.04: 0.03)	0.05	1.00
Lactobacillus paracasei	IFO 3533	0.32	0.93	0.09	(0.05: 0.04)	0.06	0.68
Lactobacillus pentosus	AKU 1148	0.10	1.24	0.08	(0.05: 0.03)	0.08	0.05
Lactobacillus pentosus	IFO12011	0.09	0.89	0.13	(0.10: 0.03)	0.13	0.74
Lactobacillus plantarum	AKU 1138	0.11	0.10	0.45	(0.10: 0.35)	1.21	-
Lactobacillus plantarum	AKU1009a	0.07	0.06	3.41	(0.25: 3.16)	0.11	0.16
Lactobacillus plantarum	JCM 8341	0.18	0.43	0.19	(0.04: 0.15)	0.27	0.40
Lactobacillus plantarum	JCM 1551	0.36	0.02	2.02	(0.10: 1.92)	0.02	0.46
Lactobacillus rhamnosus	AKU 1124	0.10	0.22	1.41	(0.69: 0.72)	0.13	0.15

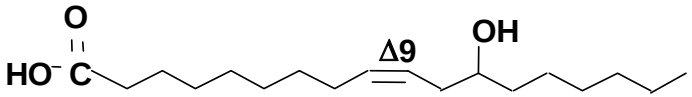
Searching unique microbial functions in Japanese microbial diversity and using them for food and chemical industries

CLA production by microorganisms

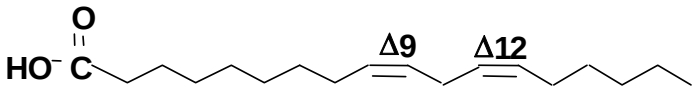
Castor oil

↓ Lipase

Ricinoleic acid
(12-hydroxy-*cis*-9-octadecaenoic acid)



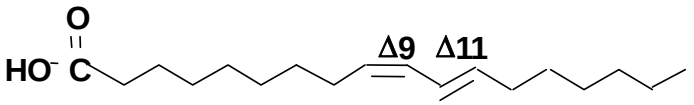
Linoleic acid
(*cis*-9,*cis*-12-octadecadienoic acid)



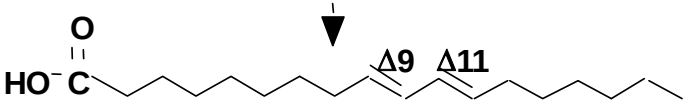
CLA production : 2.5 ~ 7.5 mg/ml
CLA1 : ~ 50%
CLA2 : ~ 82%
Free fatty acid

Lactic acid bacteria

CLA production : 20 ~ 40 mg/ml
CLA1 : ~ 75%
CLA2 : ~ 97%
Free fatty acid

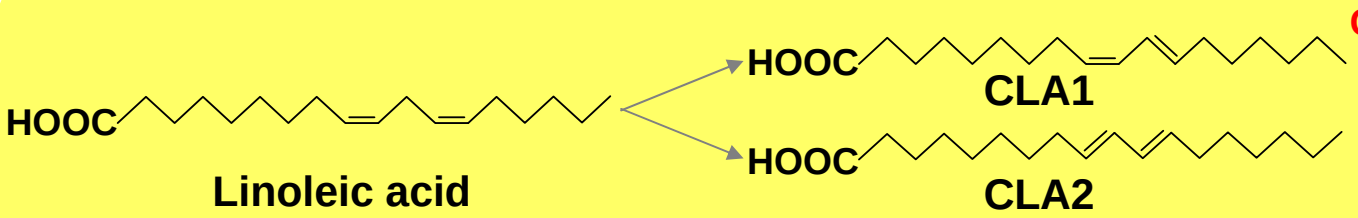


cis-9,*trans*-11-octadecadienoic acid



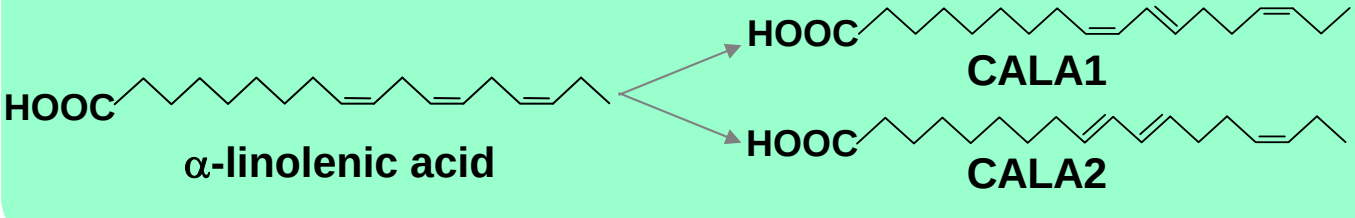
trans-9,*trans*-11-octadecadienoic acid

Conjugated fatty acids production by lactic acid bacteria



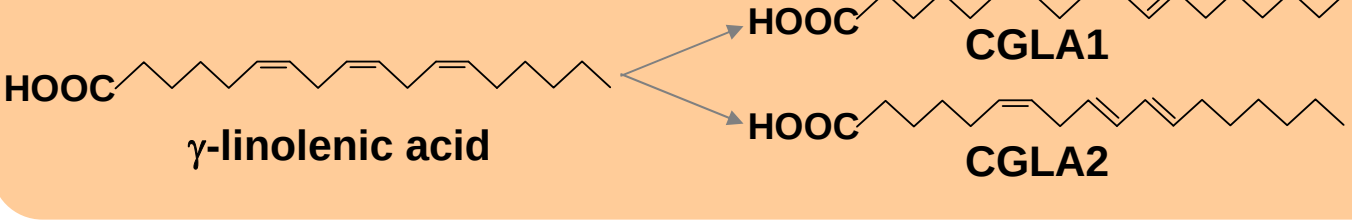
CLA production: ～40 mg/ml

- CLA1 selective condition
  CLA1 purity: ～75%
- CLA2 selective condition
  CLA2 purity: ～97%



CALA production: ～25 mg/ml

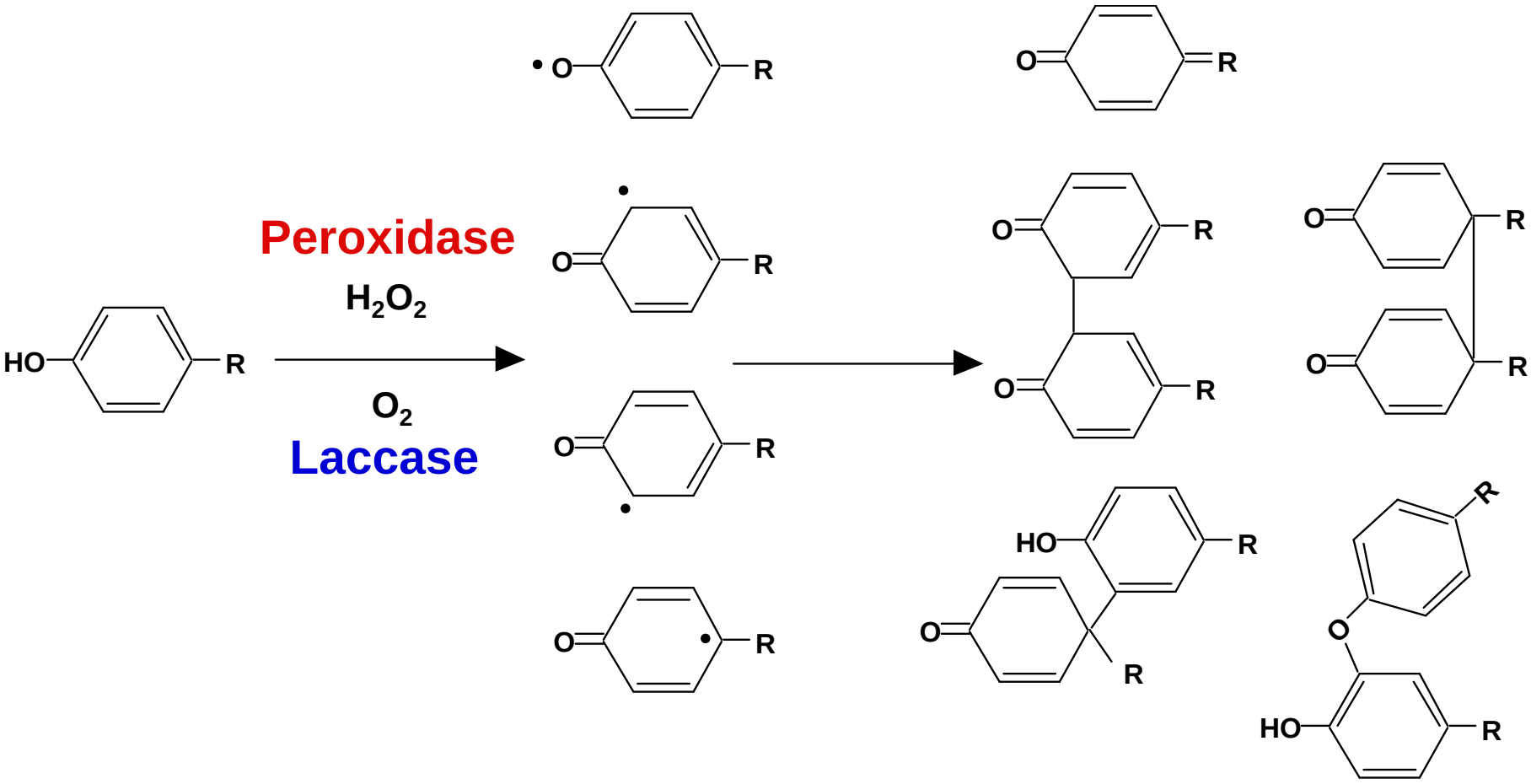
- CALA1 selective condition
  CALA1 purity: ～85%
- CALA2 selective condition
  CALA2 purity: ～85%



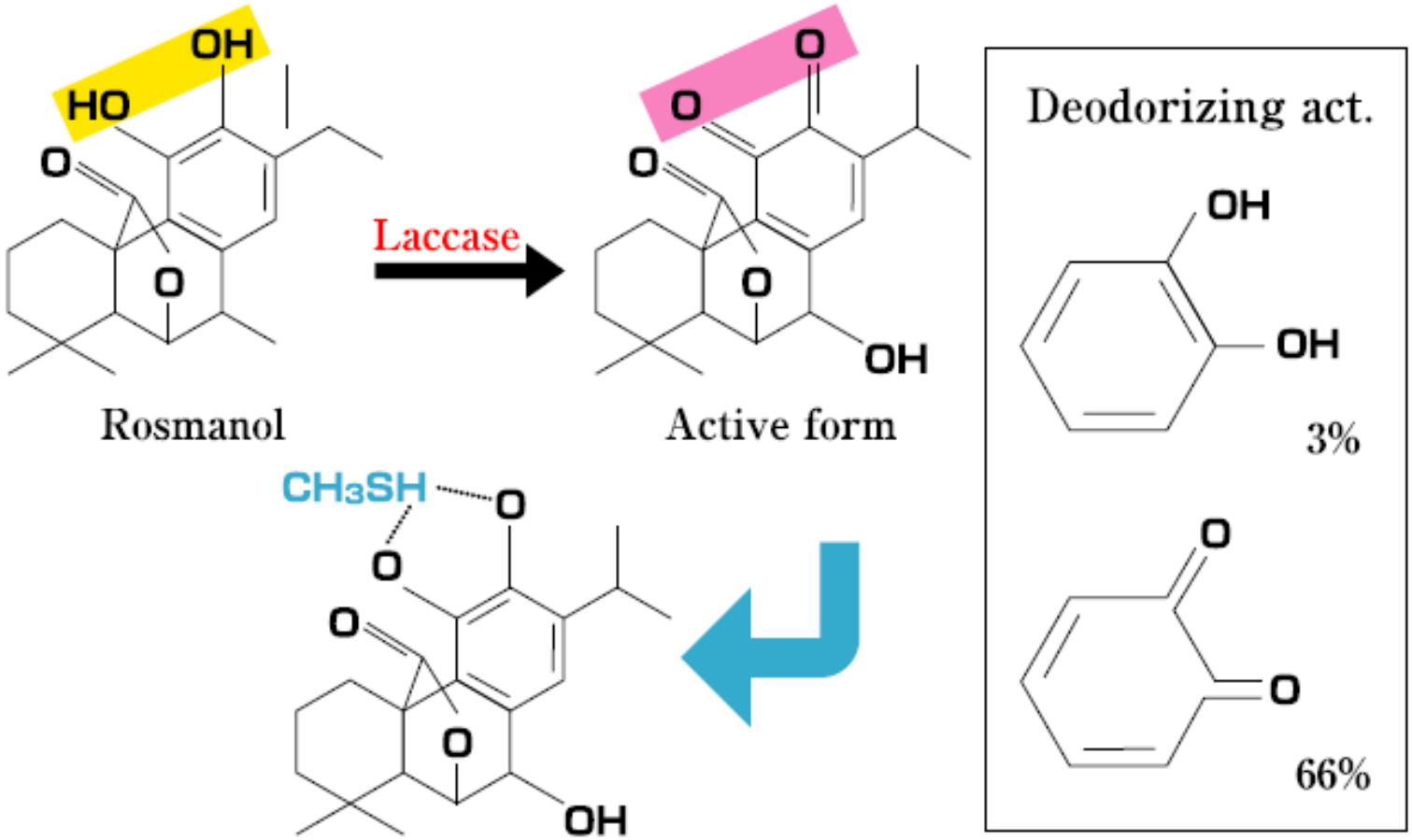
CGLA production: ～9 mg/ml

- CGLA1 selective condition
  CGLA1 purity: ～80%
- CGLA2 selective condition
  CGLA2 purity: ～87%

Non-specific oxidases

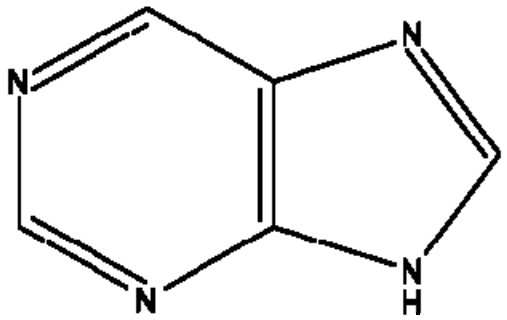


Deodorization of methylmercaptane by laccase with rosemary extract as a mediator

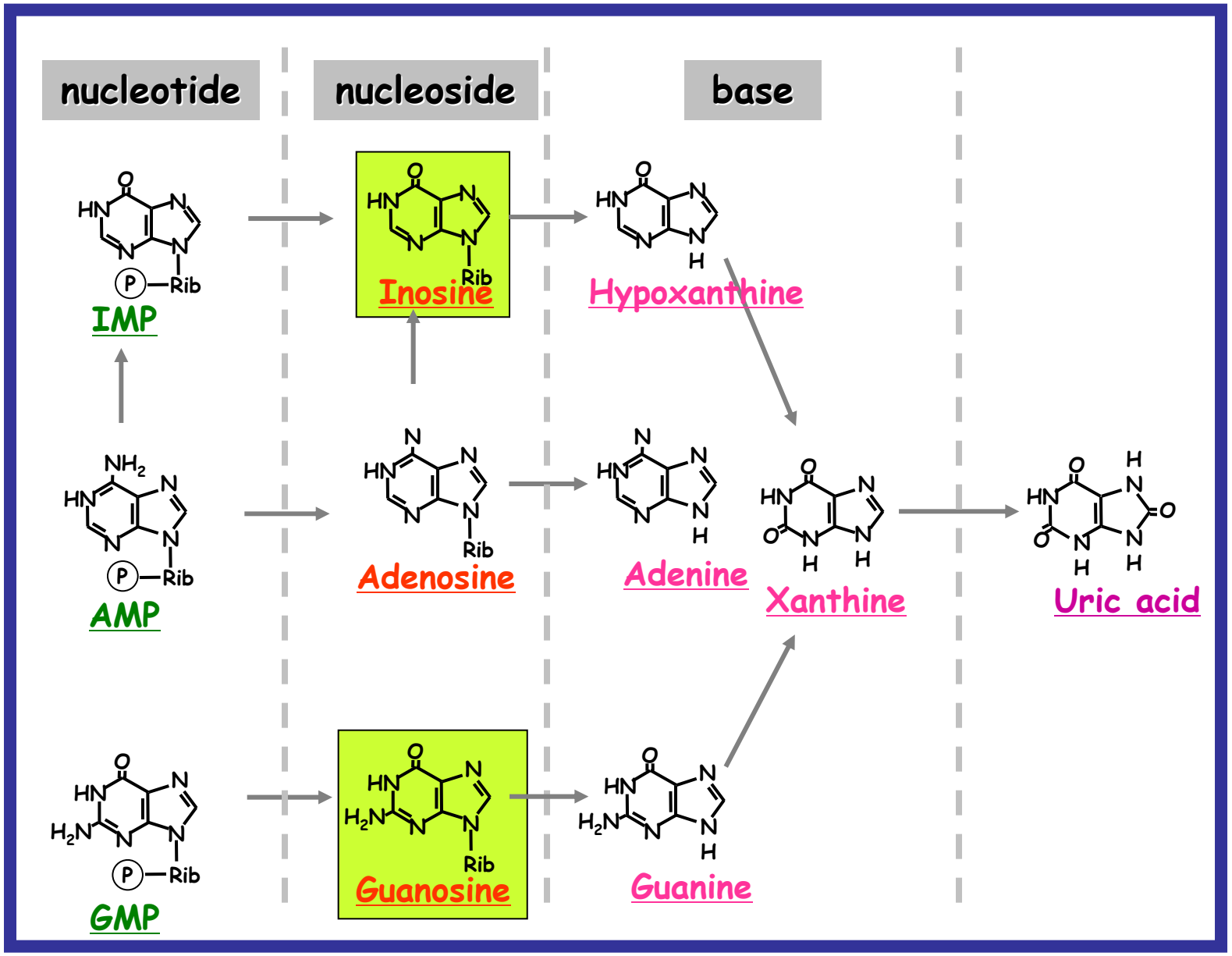


Background (Hyperuricemia & Purine)

- Hyperuricemia is a disease, which results from upper serum uric acid level. Some hyperuricemic individuals develop gout .
- Over 20% of male adults (in Japan) develop hyperuricemia.
- Hyperuricemia is influenced by a high dietary intake of purine (meat, seafood and alcoholic beverages).
- Low-purine diets are used for hyperuricemia clinic, but difficult for patients to adhere.



Mammalian purine metabolism



Result of screening

Searching unique microbial functions in Japanese microbial diversity and using them for food and chemical industries

267 strains	*Lactobacillus* (187), *Bifidobacterium* (29), *Leuconostoc* (15), *Enterococcus* (13), *Pediococcus* (9), *Clostridium* (1), *Bacillus* (12), *Saccharomyces* (1)

↓ First screening; reaction time 2 h

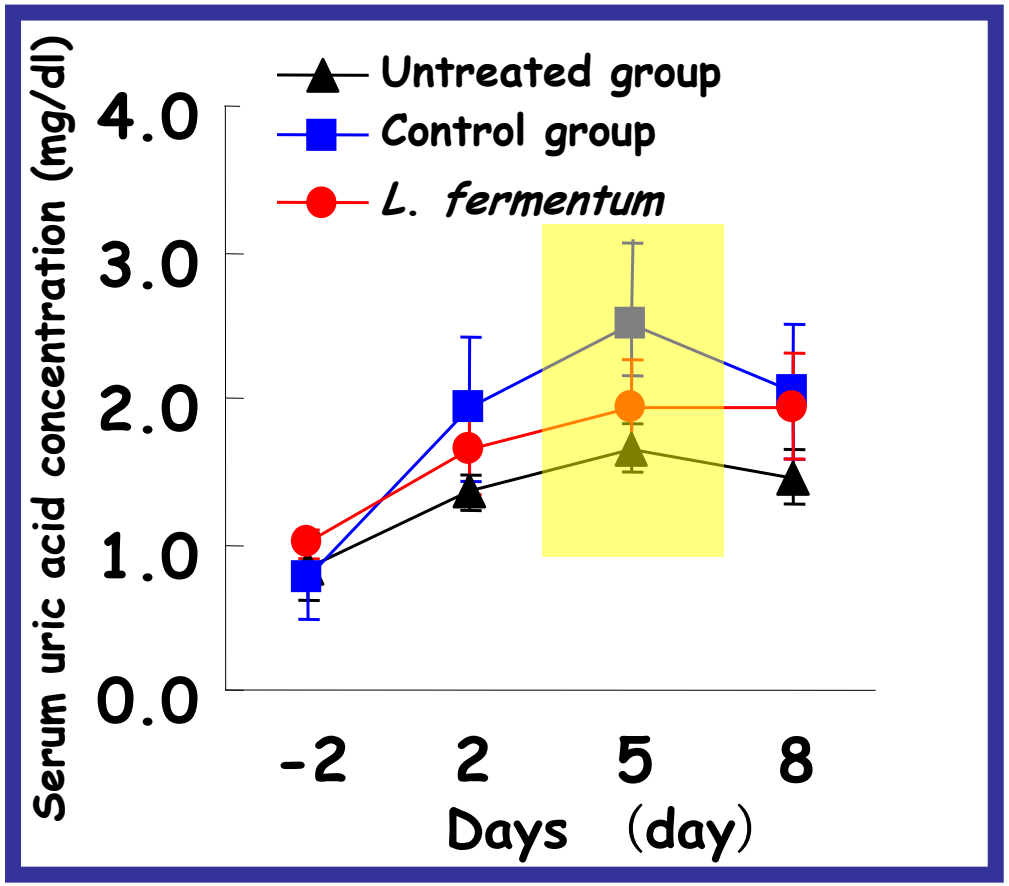
66 strains	*Lactobacillus* (58), *Leuconostoc* (4), *Pediococcus* (2), *Clostridium* (1), *Saccharomyces* (1)

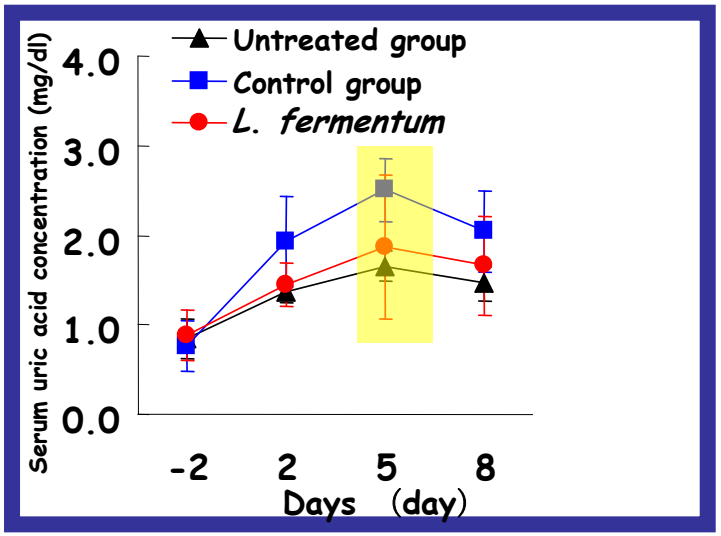
↓ Second screening; reaction time 30 min

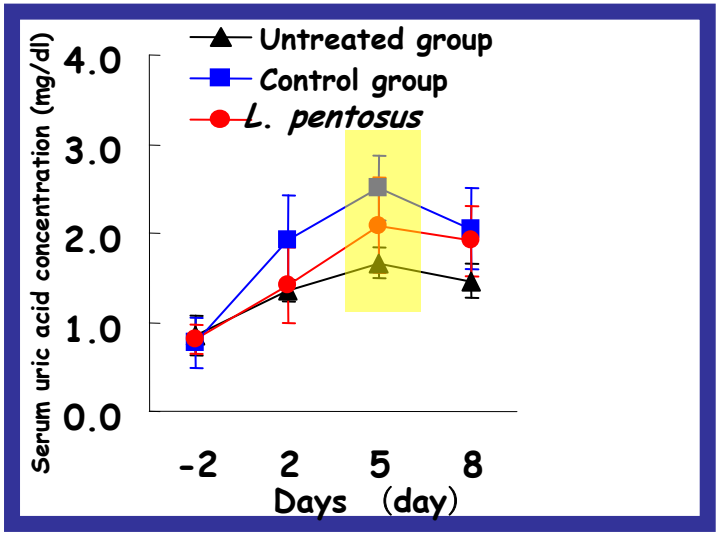
13 strains	*L. fermentum* (7) , *L. brevis* (2) , *L. mali* (1) *L. vaccinostercus* (1) , *L. homohiochi* (1) , *L. pentosus* (1)

↓ Third screening; Rat model experiment

Results of rat experiment







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Food functions based on catalytic activity of microbial enzymes

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Probiotic use of lactic acid bacteria and their metabolisms

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These microbial functions might be useful by themselves and to endow agricultural products with extra-qualities increasing added values of primary agro-products.