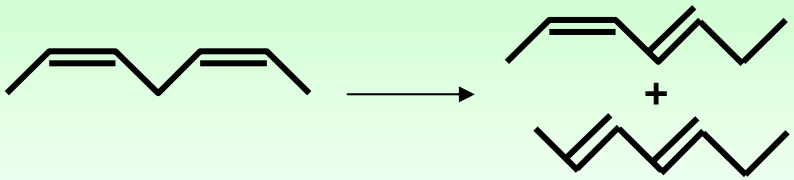
微生物による共役脂肪酸生産

小川　順[1]、岸野重信[1,2]、横関健三[2]、清水　昌[1]

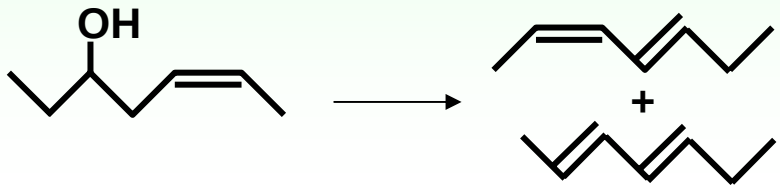
（[1]京大院農・応用生命、[2]京大院農・産業微生物）

Unique fatty acid transformation catalyzed by anaerobic bacteria

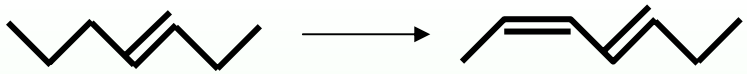
Isomerization



Dehydrating isomerization

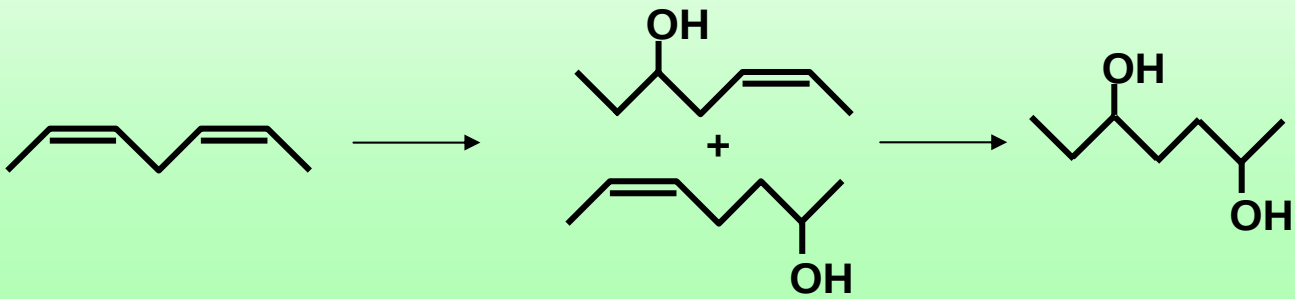


Desaturation



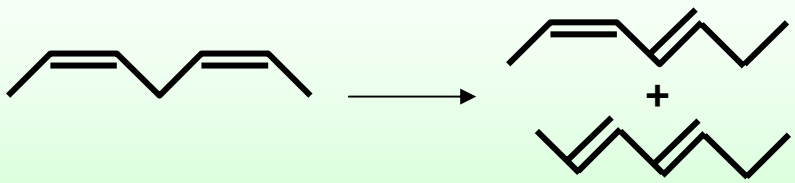
Saturation

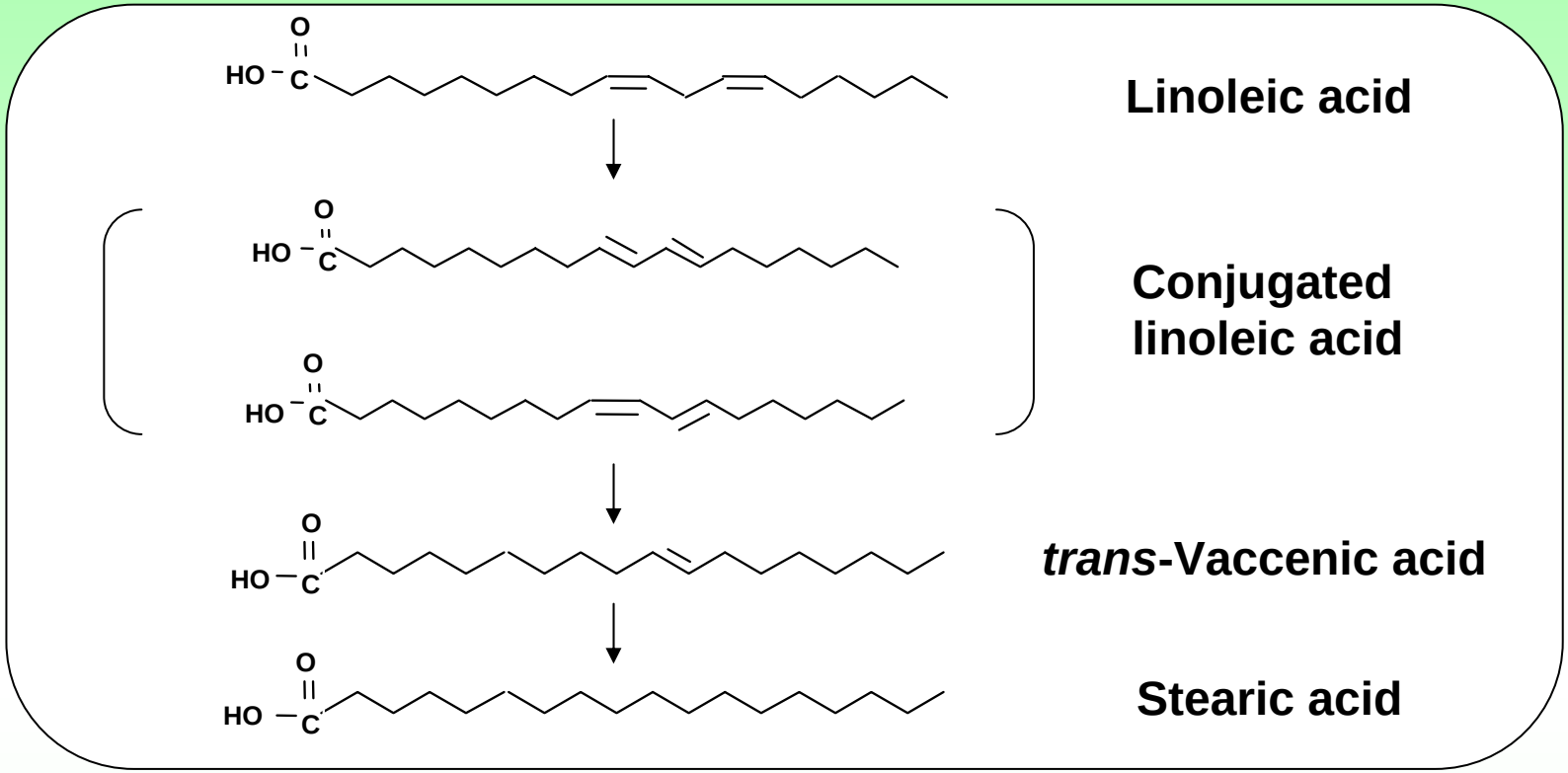
Hydration



Isomerization of non-conjugated diene to conjugated diene

Linoleic acid transformation to CLA by lactic acid bacteria





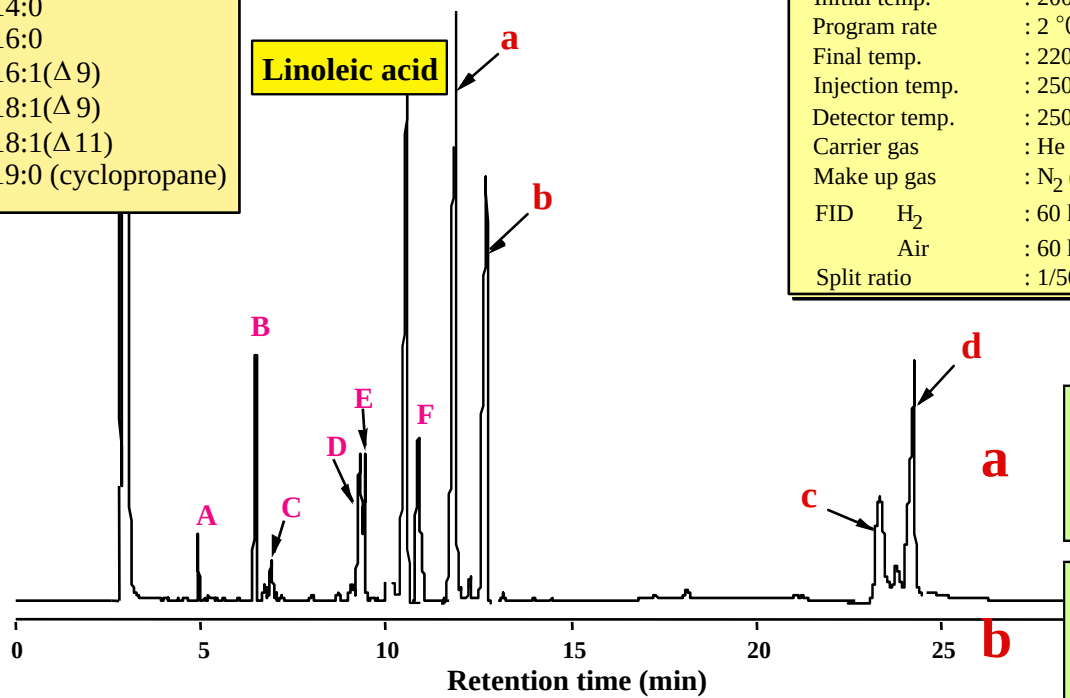
Analytical level (less than 0.5 mg/ml)

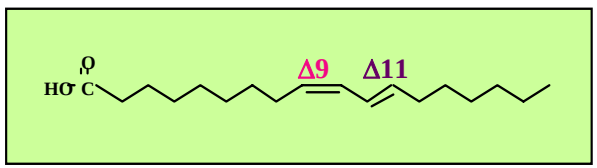
Bifidobacterium breve	***cis*-9,*trans*-11**
Bifidobacterium bifidum	***cis*-9,*trans*-11**
Bifidobacterium dentium	***cis*-9,*trans*-11(78%) , *trans*-9,*trans*-11(21%) , *trans*-10,*cis*-12(1%)**
Bifidobacterium lactis	***cis*-9,*trans*-11(90%), *trans*-9,*trans*-11(8%) , *trans*-10,*cis*-12(2%)**
Megasphaera elsdenii	***trans*-10,*cis*-12**
Propionibacterium freudenreichii	***cis*-9,*trans*-11 (>80%)**
***Butyrivibrio fibrisolvens* A38**	***cis*-9,*trans*-11 (95%)**
Lactobacillus reuteri	***cis*-9,*trans*-11(59%), *trans*-10,*cis*-12(41%)**
Lactobacillus acidophilus	***cis*-9,*trans*-11(86%), *trans*-9,*trans*-11(6%), *trans*-10,*cis*-12(8%)**
Lactobacillus acidophilus	***cis*-9,*trans*-11(85%), *trans*-9,*trans*-11(3%), *trans*-10,*cis*-12(12%)**

GLC chromatogram of methyl esters of fatty acids produced from LA by *Lactobacillus acidophilus* AKU1137

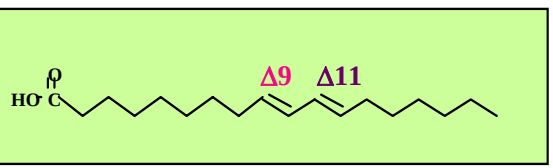
Cellular fatty acids
A, 14:0
B, 16:0
C, 16:1(Δ9)
D, 18:1(Δ9)
E, 18:1(Δ11)
F, 19:0 (cyclopropane)

Column : HR-SS-10, 0.25 mm I.D. x 50m
Initial temp. : 200℃
Program rate : 2 ℃ /min
Final temp. : 220 ℃
Injection temp. : 250 ℃
Detector temp. : 250 ℃
Carrier gas : He (225 kPa)
Make up gas : N_2 (55 mL/min)
FID H_2 : 60 kPa
Air : 60 kPa
Split ratio : 1/50

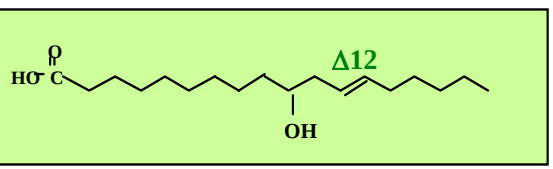


a


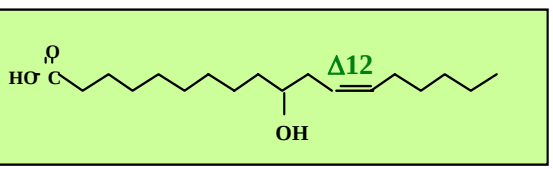
CLA1

b


CLA2

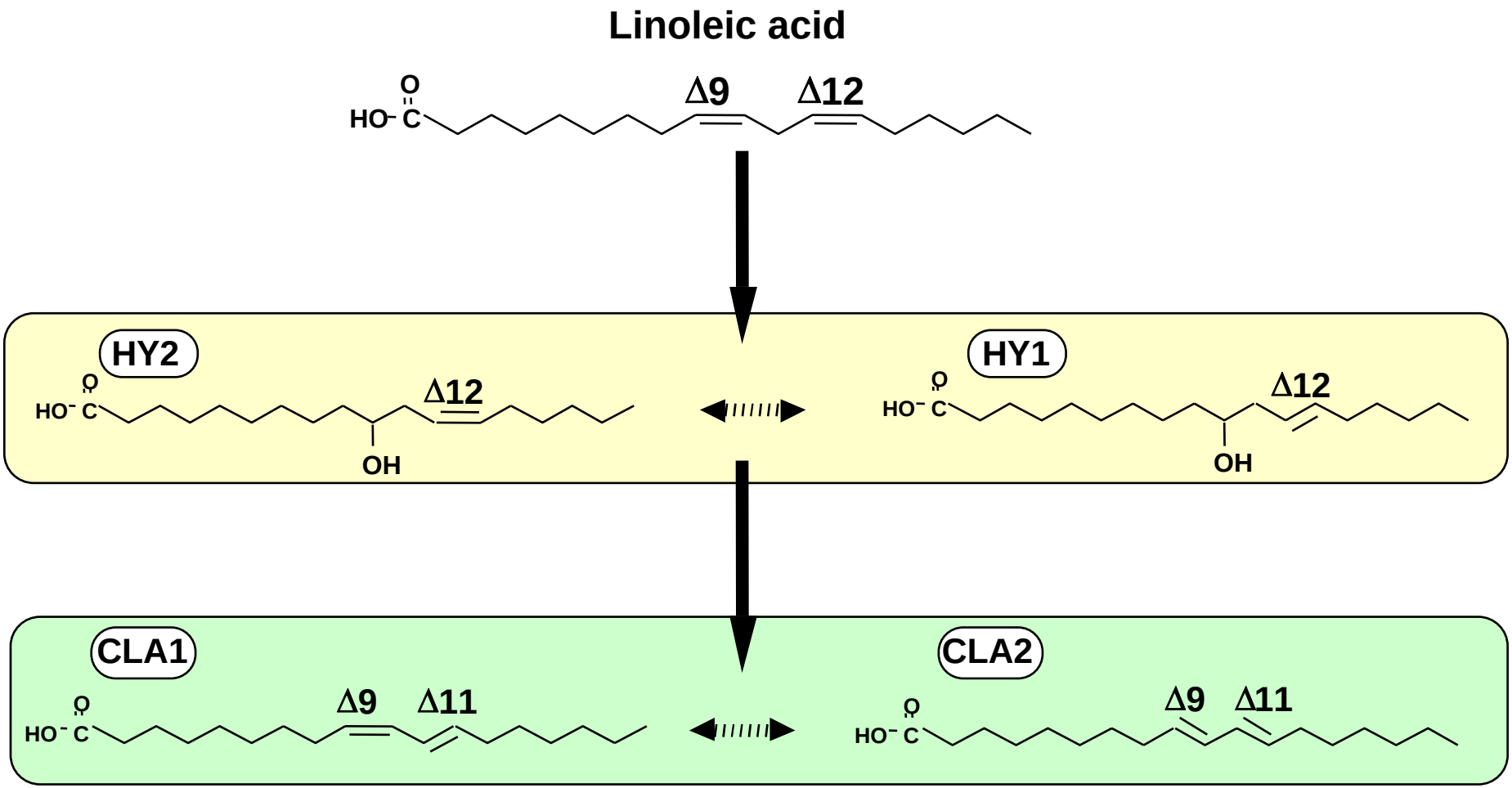
c


HY1

d


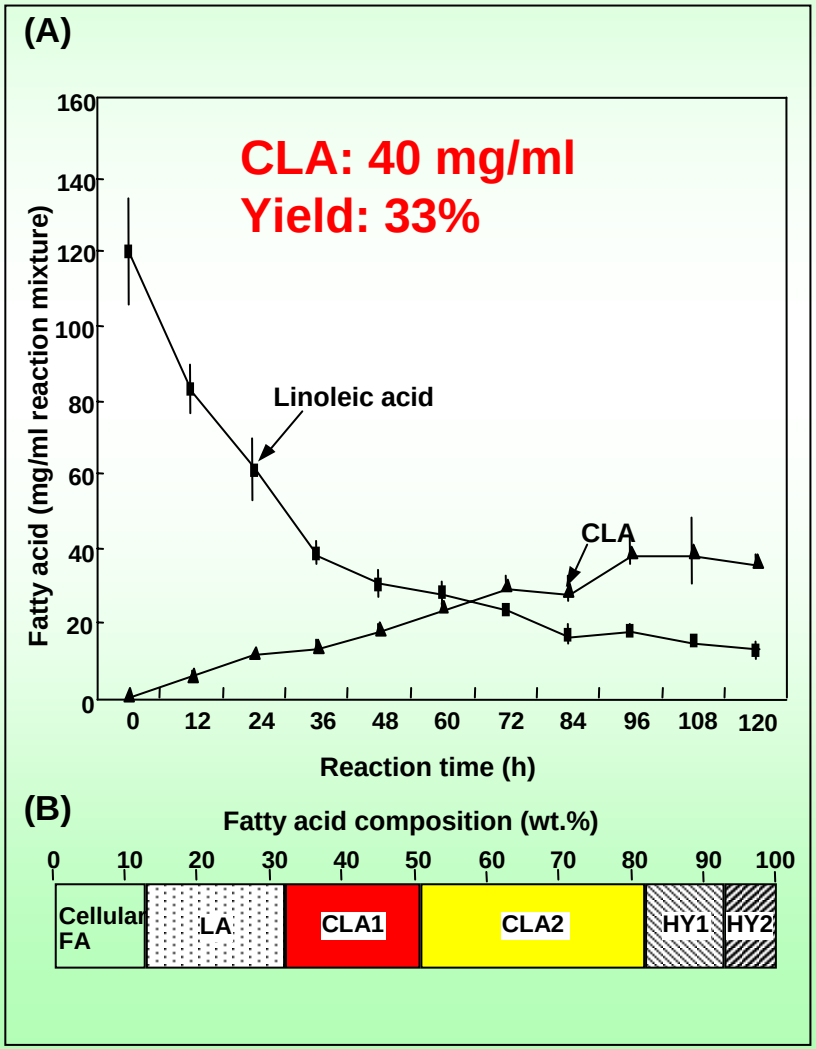
HY2

CLA production from linoleic acid by washed cells of lactic acid bacteria

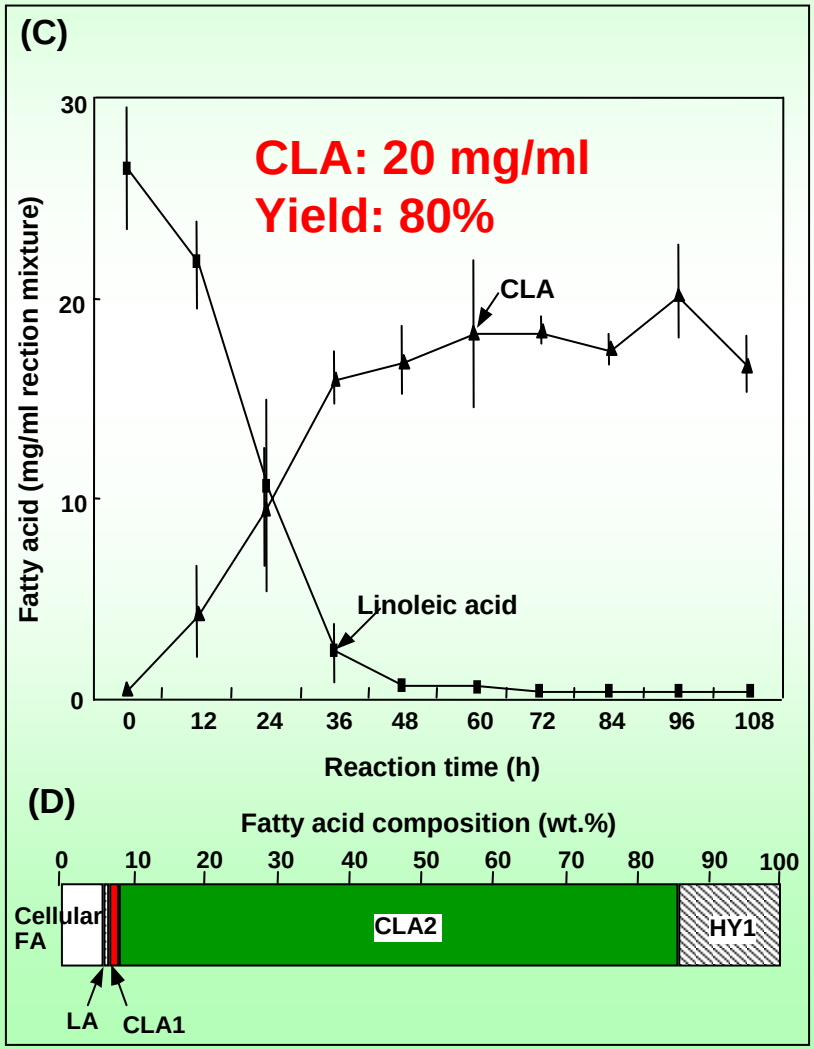


CLA production from linoleic acid by washed cells of *L. plantarum* AKU 1009a

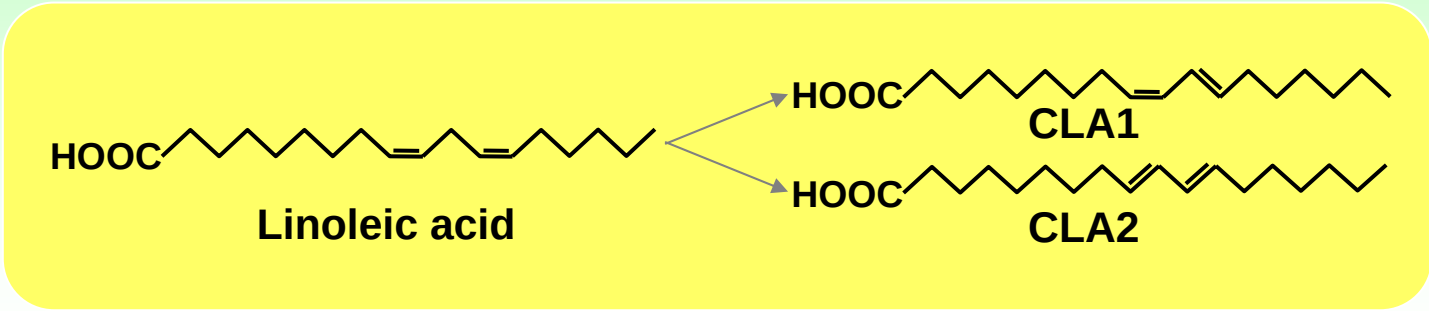
Linoleic acid : 12% (w/v)
Washed cells : 33% (w/v)
Aerobic



Linoleic acid : 2.6% (w/v)
Washed cells : 23% (w/v)
Aerobic



CLA production by *Lactobacillus plantarum* AKU 1009a

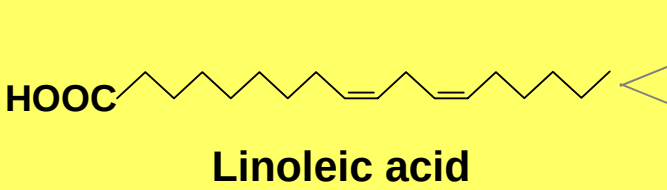


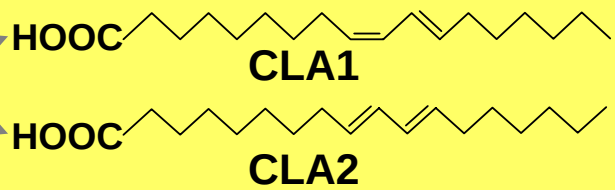
CLA production: ～40 mg/ml

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

CLA was obtained as free fatty acid form almost associated with the cells

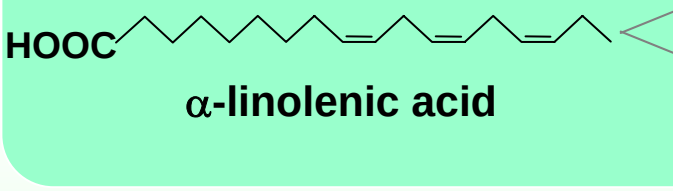
Conjugated fatty acids production by lactic acid bacteria

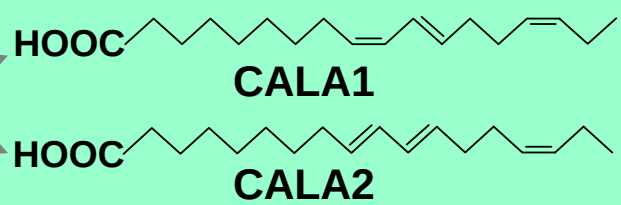




CLA production: ~40 mg/ml

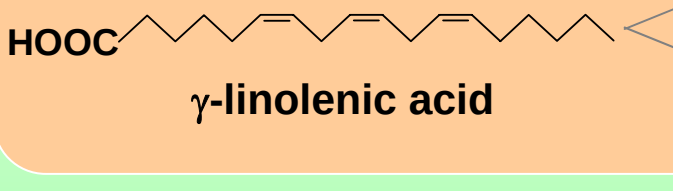
- CLA1 selective condition
  CLA1 purity: ~75%
- CLA2 selective condition
  CLA2 purity: ~97%

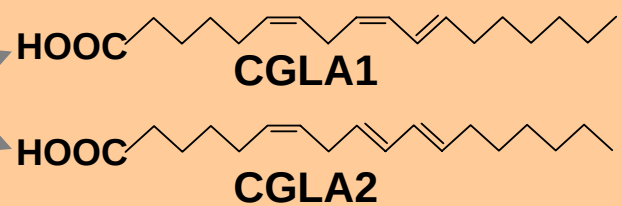




CALA production: ~25 mg/ml

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



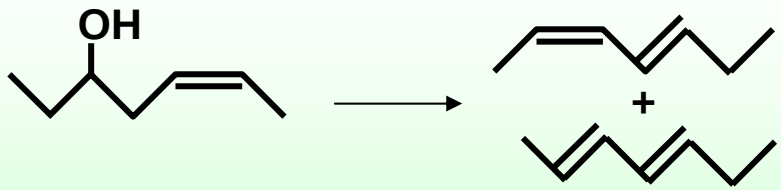


CGLA production: ~9 mg/ml

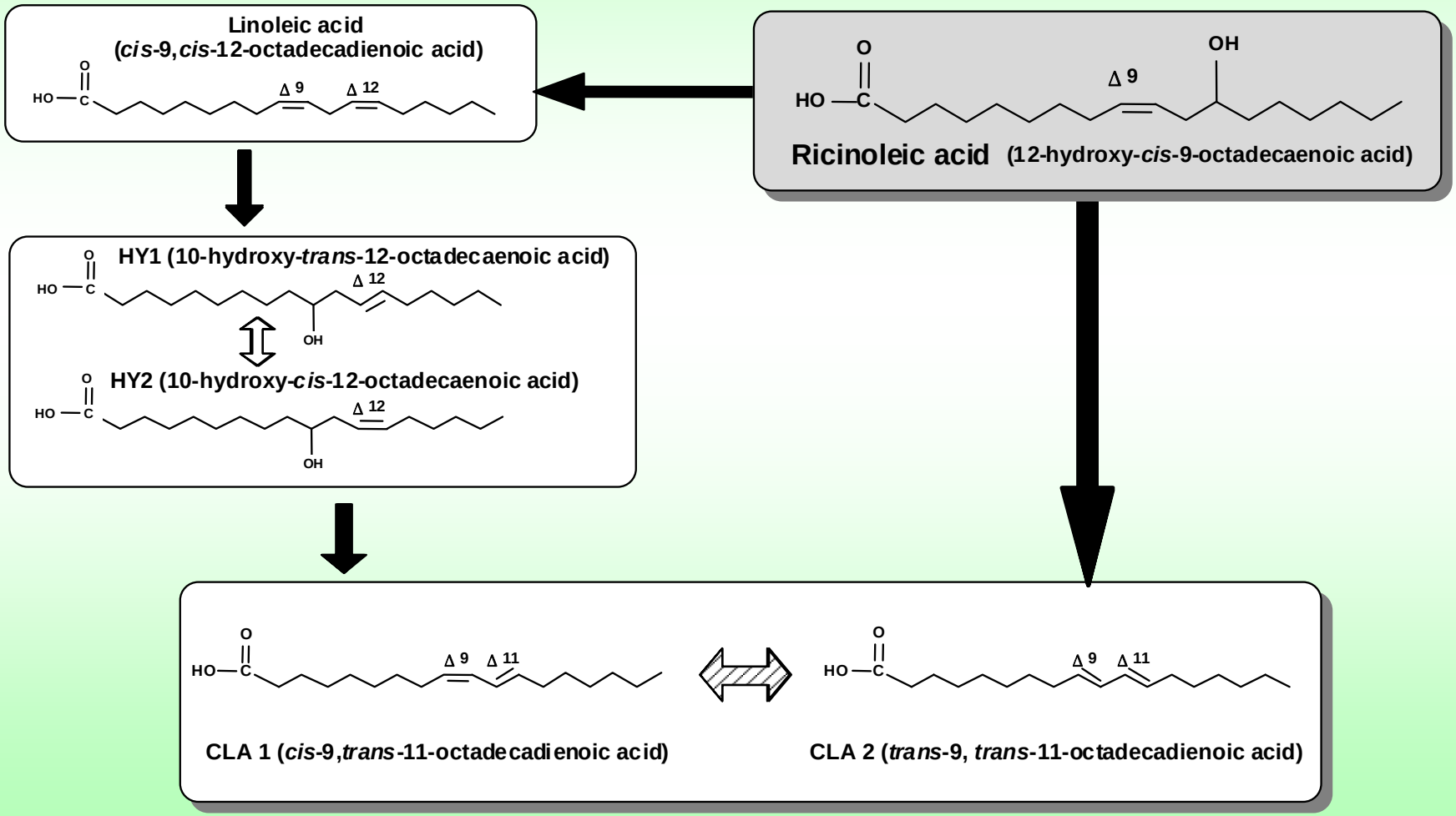
- CGLA1 selective condition
  CGLA1 purity: ~80%
- CGLA2 selective condition
  CGLA2 purity: ~87%

Dehydration of hydroxy fatty acid to conjugated fatty acid

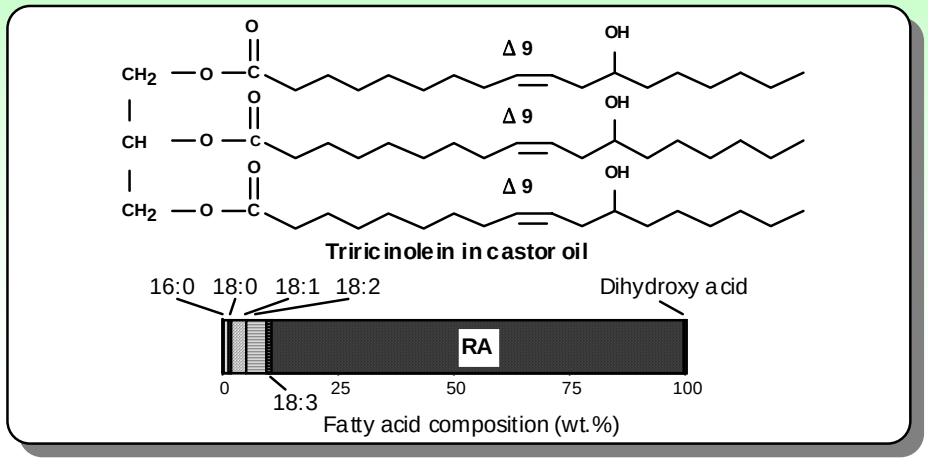
Ricinoleic acid transformation to CLA by lactic acid bacteria



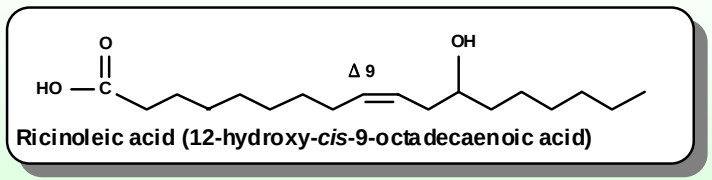
CLA production from ricinoleic acid by washed cells of lactic acid bacteria

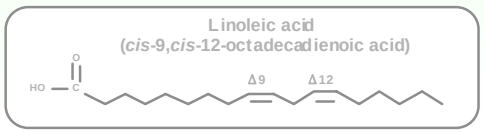


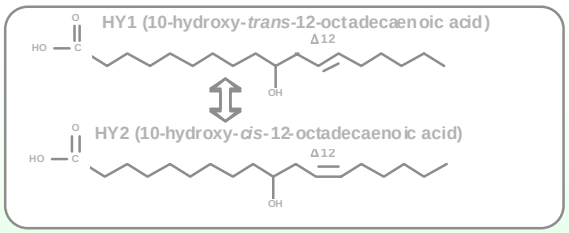
CLA production from castor oil by washed cells of lactic acid bacteria

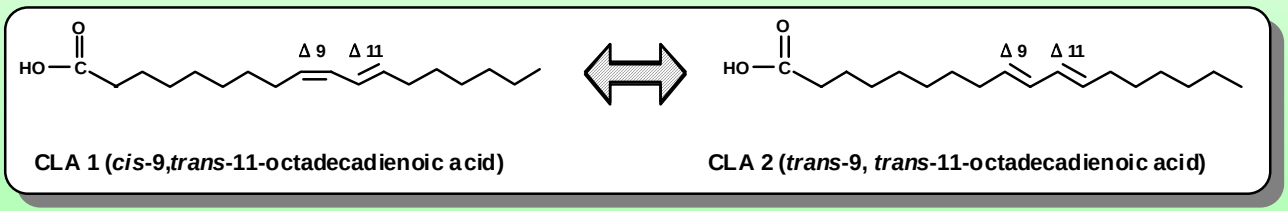


Lipase (Lipase M"Amano"10)
Detergent (Lubrol PX)







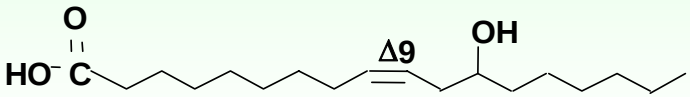


CLA production by microorganisms

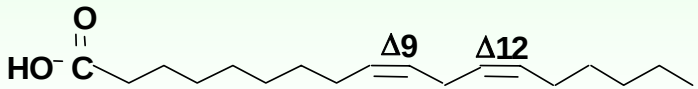
Castor oil

Lipase

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



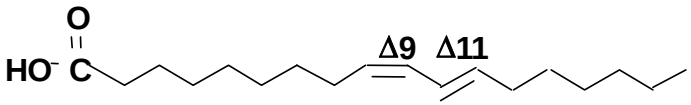
Linoleic acid
(*cis*-9,*cis*-12-octadecadienoic acid)



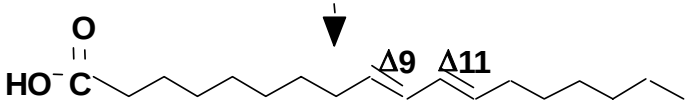
CLA production : 2.5 ~ 7.5 mg/ml
CLA1 : ~ 50%
CLA2 : ~ 82%
Free fatty acid

Lactic acid bacteria

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

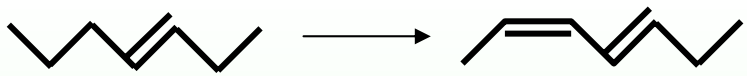


cis-9,*trans*-11-octadecadienoic acid

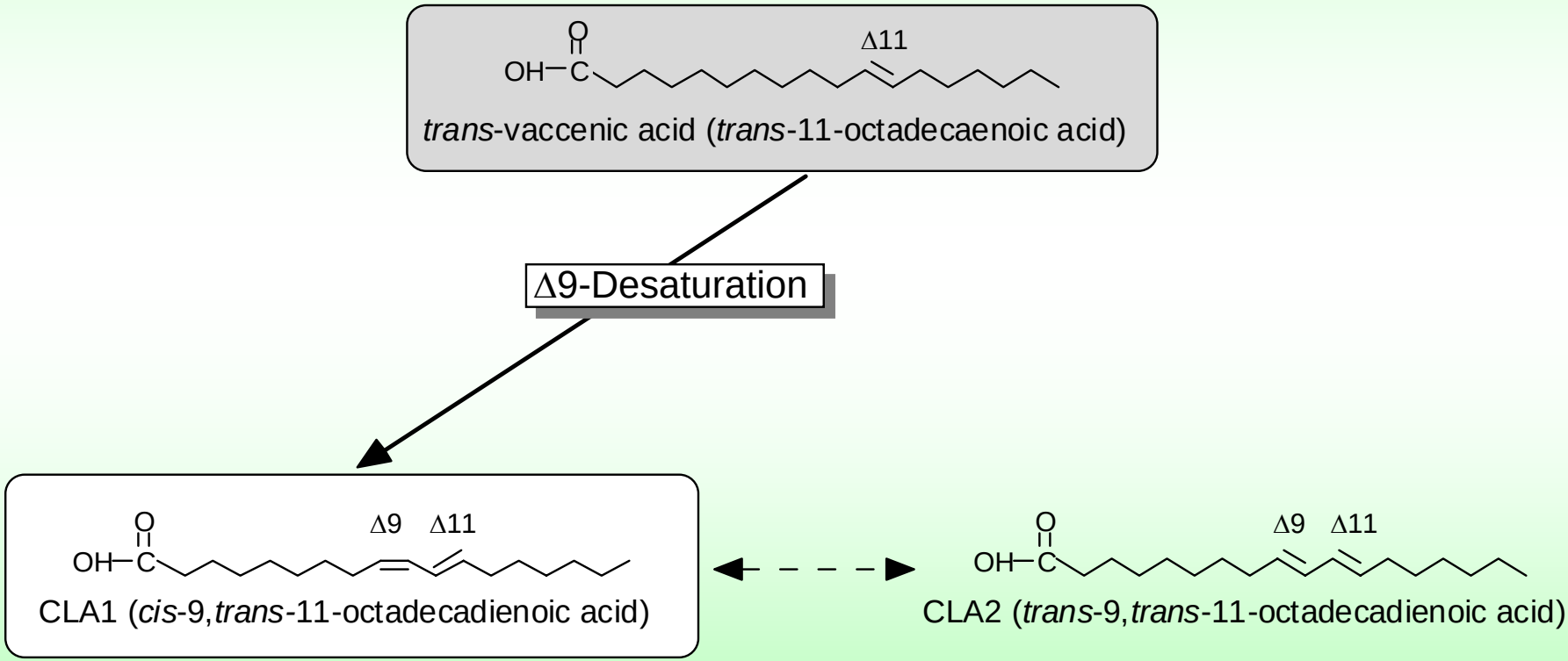


trans-9,*trans*-11-octadecadienoic acid

trans-Vaccenic acid desaturation to CLA by filamentous fungi



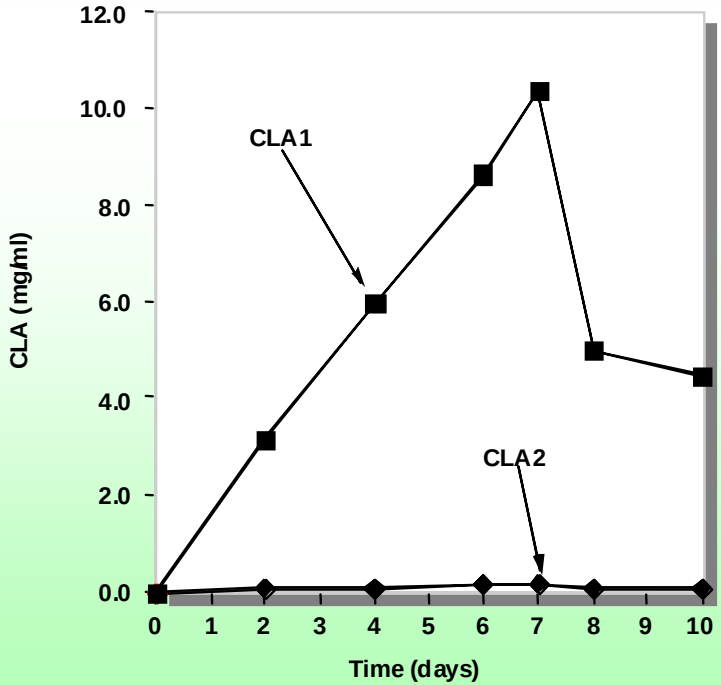
CLA production from *trans*-vaccenic acid through Δ9 desaturation



Culture conditions

Strain	:	*Delacroiaxia coronata* IFO 8586
Substrate conc.	:	33.3 mg/ml
Substrate addition	:	Inoculation
Medium	:	Dextrin 5.0%, Tryptone 2.0% Thiourea 0.83 mmol/ml
pH	:	9.0
Culture temp.	:	28 ℃

Time courses of CLA production from *t*-VAME



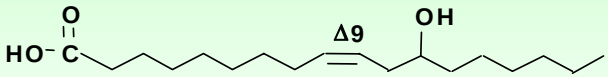
CLA production by microorganisms

Castor oil

Lipase

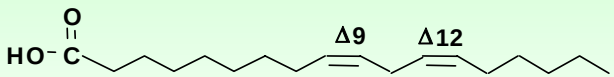
Ricinoleic acid

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



Linoleic acid

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



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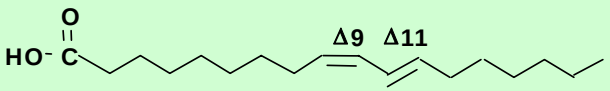
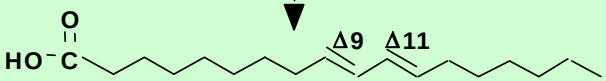
Free fatty acid

Lactic acid bacteria

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



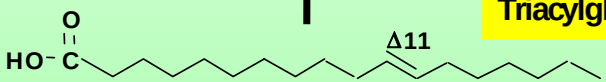
cis-9,*trans*-11-octadecadienoic acid



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

Molds

CLA production : ~ 13 mg/ml
CLA1 : ~ 98%
CLA2 : ~ 10%
Triacylglycerol



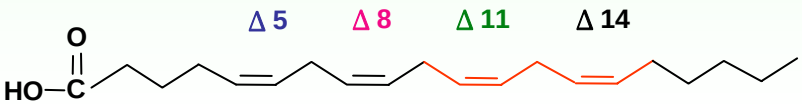
(*trans*-11-octadecaenoic acid)

***trans*-Vaccenic acid**

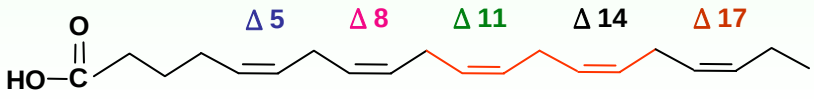
Saturation of polyunsaturated fatty acid by anaerobic microorganisms

Saturation of arachidonic acid and EPA by *Clostridium bifermentans* JCM 1386

Arachidonic acid (*cis*-5,*cis*-8,*cis*-11,*cis*-14-eicosatetraenoic acid)

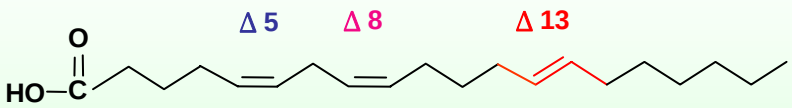


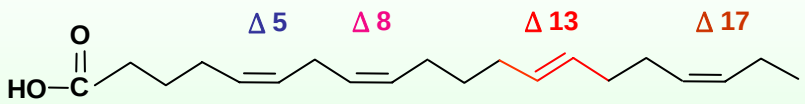
EPA (*cis*-5,*cis*-8,*cis*-11,*cis*-14,*cis*-17-eicosapentaenoic acid)



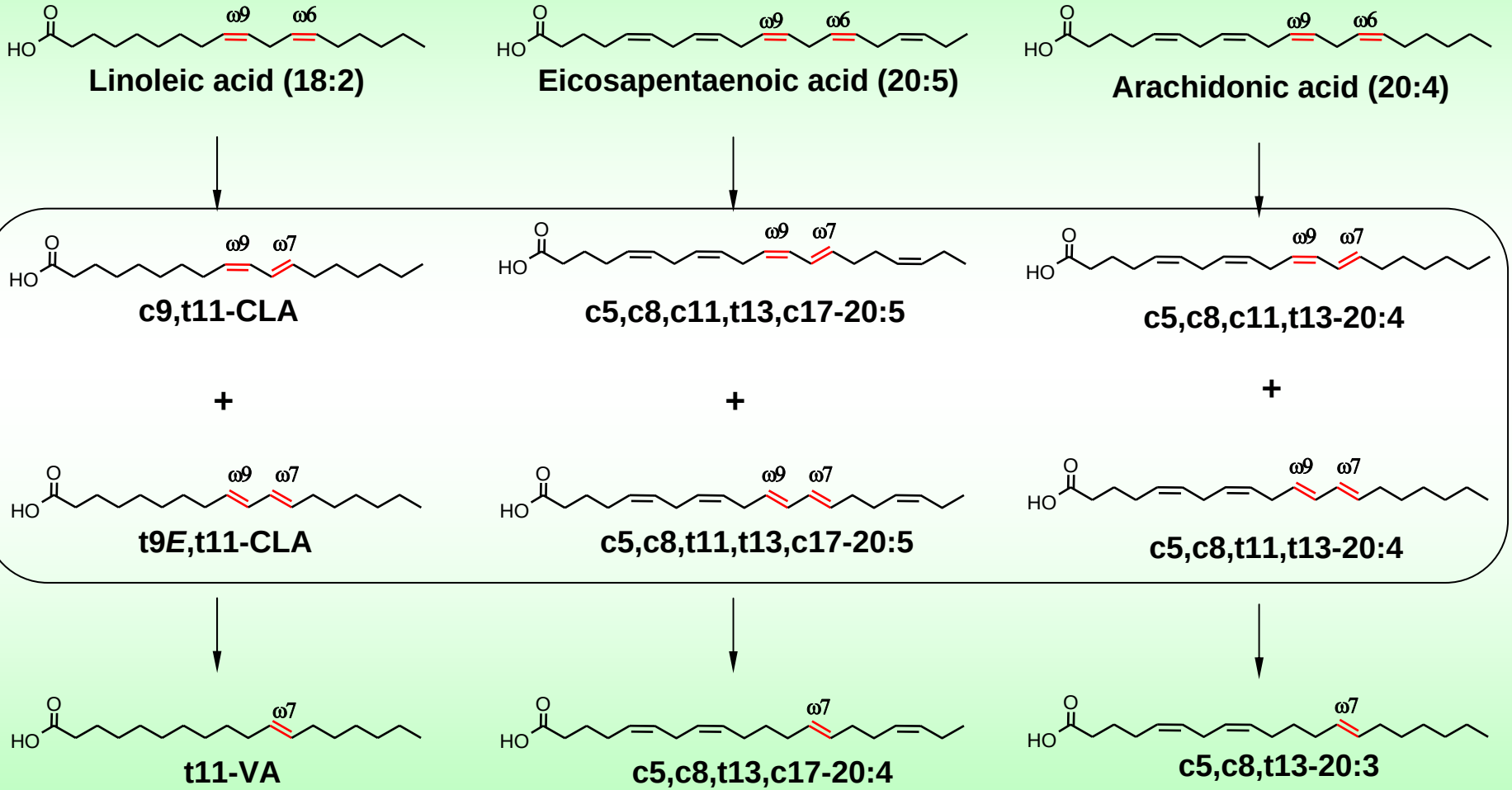
(UK1)

(UK2)

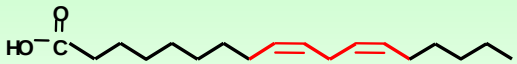
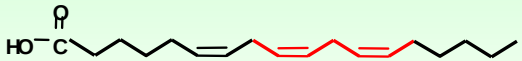
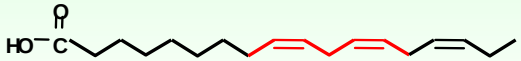
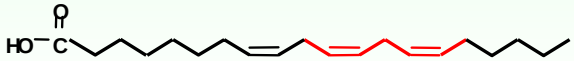
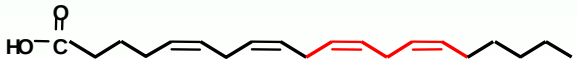
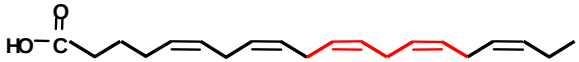
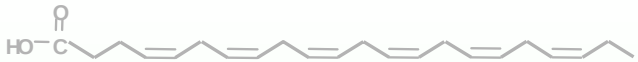


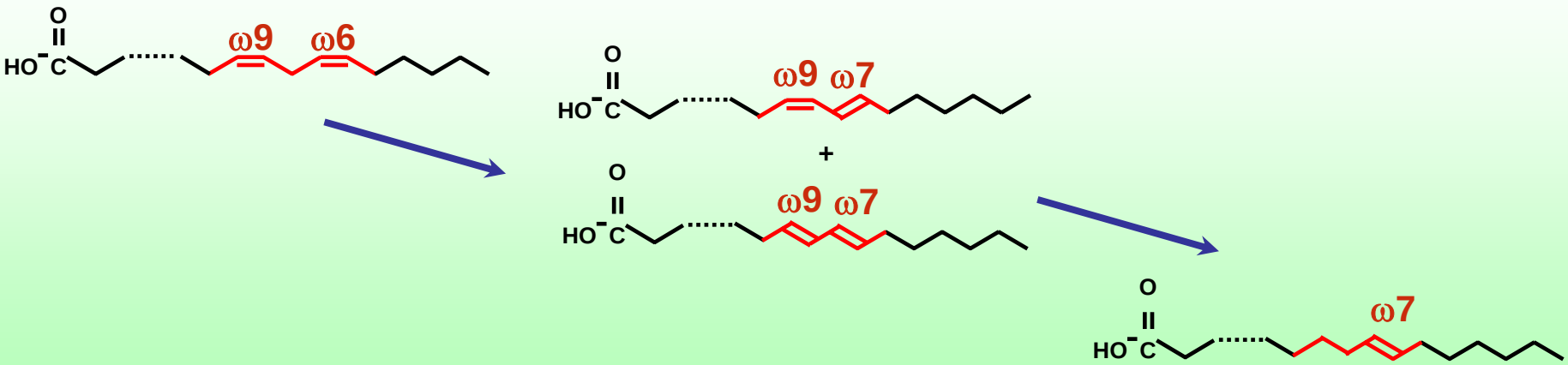


Putative pathway of unsaturated fatty acids by *C. bifermentans* JCM1386

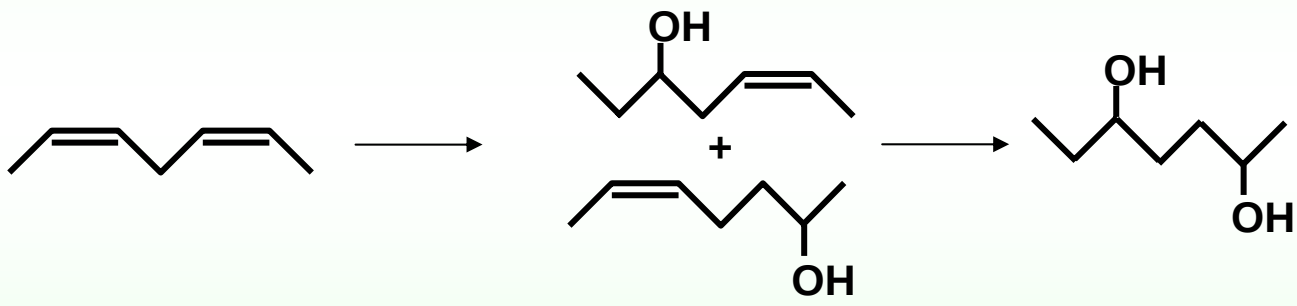


Transformation of polyunsaturated fatty acids by cell-free extracts of *C. Bifermentans* JCM 1386

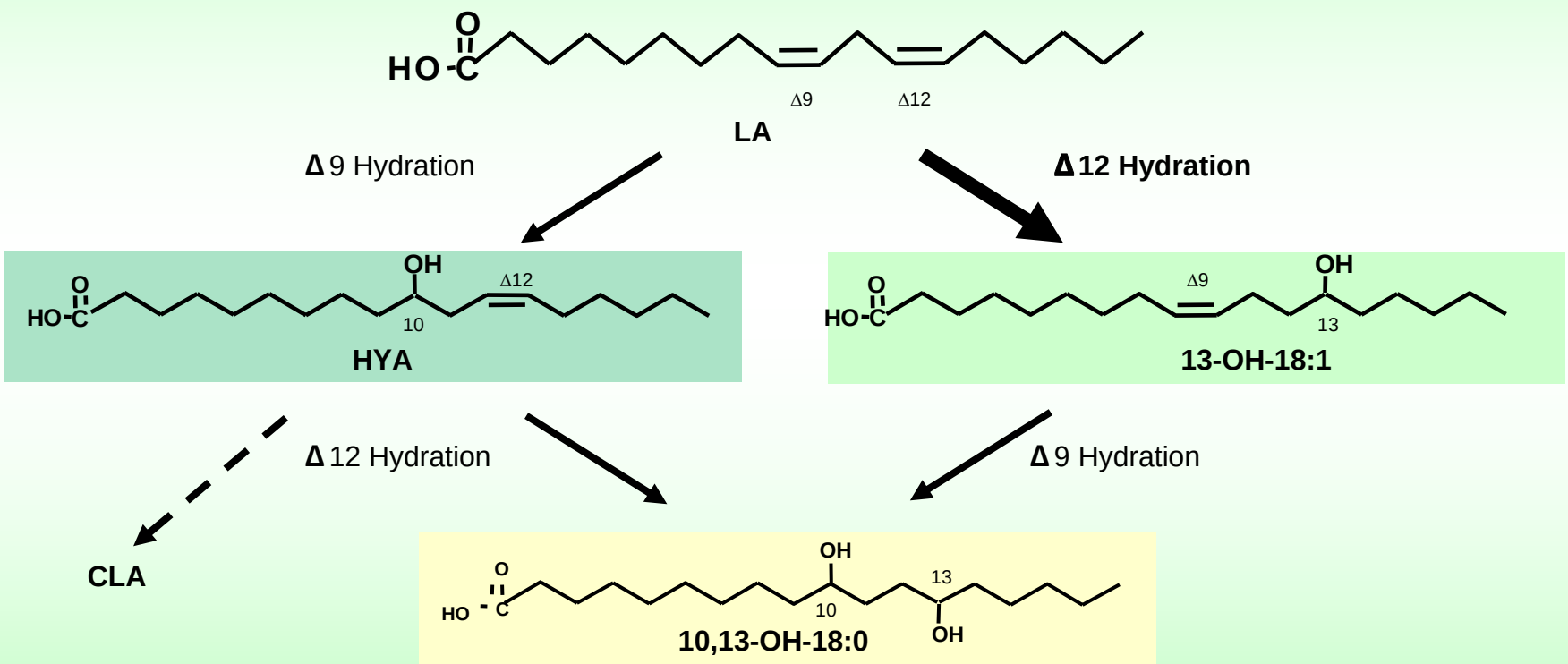
Substrate	Structure	Transformation
Linoleic acid (LA; 18:2 n-6)		+
gLinolenic acid (GLA; 18:3 n-6)		+
a-Linolenic acid (ALA; 18:3 n-3)		+
Dihomo-glinolenic acid (DGLA; 20:3 n-6)		+
Arachidonic acid (AA; 20:4 n-6)		+
Eicosapentaenoic acid (EPA; 20:5 n-3)		+
Docosahexaenoic acid (DHA; 22:6 n-3)		-



Hydration of unsaturated fatty acid to hydroxyl fatty acid



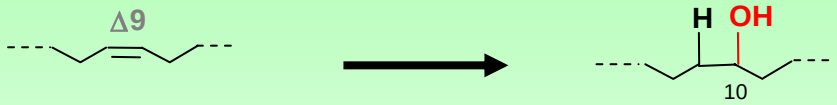
Putative pathway of LA transformation by *Pediococcus* sp. LBK 454



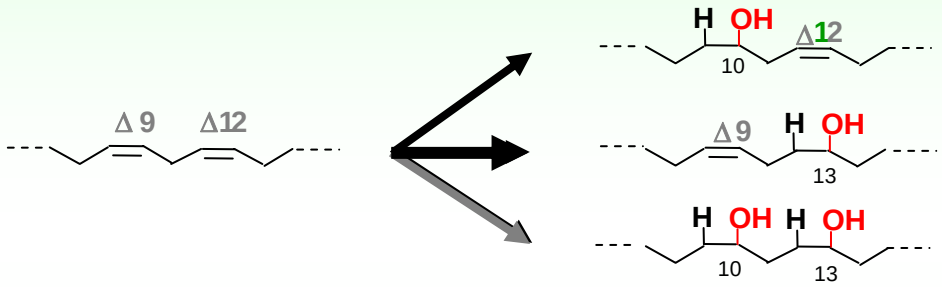
Transformation of unsaturated fatty acid

Substrate		Transformation
α-Linolenic acid	*cis*-9,*cis*-12,*cis*-15-18:3	+
γ-Linolenic acid	*cis*-6,*cis*-9,*cis*-12-18:3	+
Stearidonic acid	*cis*-6,*cis*-9,*cis*-12,*cis*-15-18:4	+
Oleic acid	*cis*-9-18:1	+
13-OH-18:1	13-hydroxy-*cis*-9-18:1	+
Ricinolecic acid	12-hydroxy-*cis*-9-18:1	+
HYA	10-hydroxy-*cis*-12-18:1	+
Dihomo-γ-linolenic acid	*cis*-8,*cis*-11,*cis*-14-20:3	-
Arachidonic acid	*cis*-5,*cis*-8,*cis*-11,*cis*-14-20:4	-

Lactic acid bacteria



Pediococcus sp. LBK 454



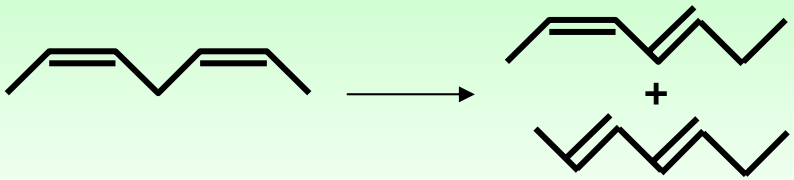
Stereoselective for *R*-configuration

Hydroxy fatty acids

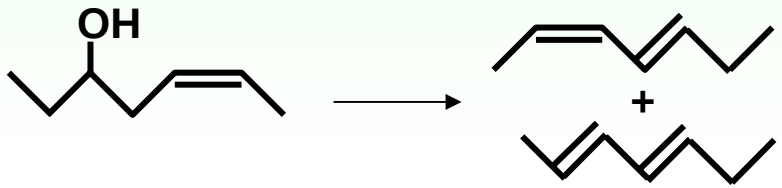
Resins, waxes, nylons, plastics, corrosion inhibitors, cosmetics, coatings, lubricants, etc…..

Unique fatty acid transformation catalyzed by anaerobic bacteria

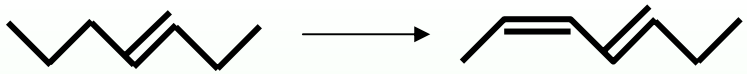
Isomerization
(乳酸菌)



Dehydrating isomerization
(乳酸菌)

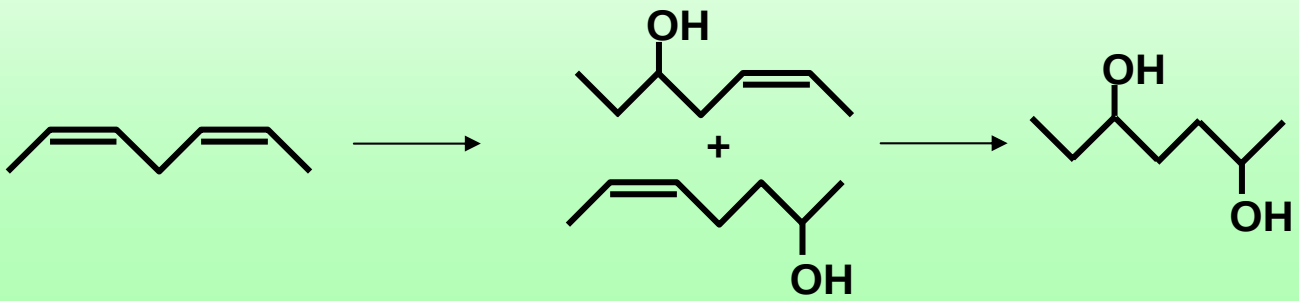


Desaturation
(糸状菌・酵母)



Saturation
(嫌気性細菌)

Hydration
(乳酸菌)



微生物機能の活用法

●機能性脂質生産のための酵素触媒として用いる

野生株を用いた生産　→　食品・サプリメント用途

形質転換株を用いた選択的・高効率生産　→　化学素材・医薬品

●機能性脂質増強食品・サプリメントとして、生産菌と共に用いる

ex) ＣＬＡ強化ヨーグルト植物性乳酸菌入り（*L. plantarum*）

生育条件下（発酵条件下）における阻害の解除、

代謝制御（飽和化反応の抑制）が課題

●脂質変換活性をもつ腸内細菌のプロバイオティクスとしての活用

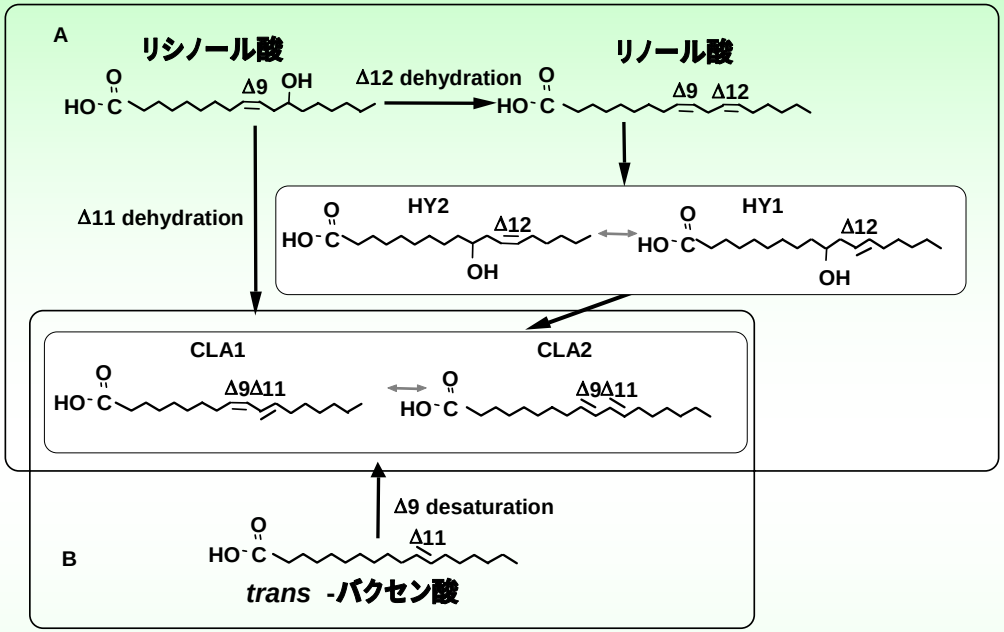
腸内細菌の腸内環境下における脂質代謝機能の解析

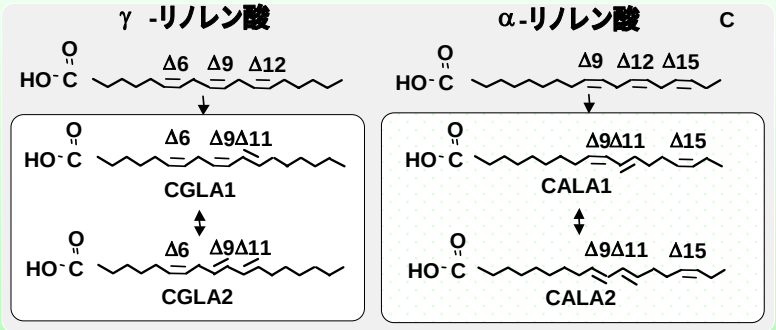
（腸管内の脂質の存在形態を考慮→シンバイオティクスとして基質を投与）

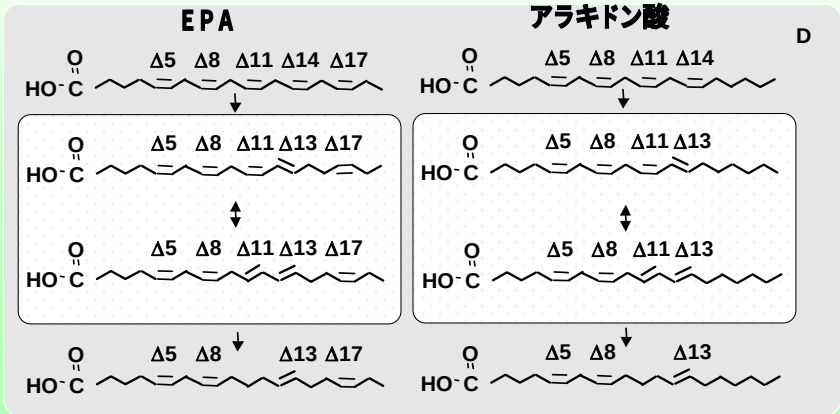
作れるようになった脂質の機能評価が必要（異性体別の機能も含め）

◎　微生物機能をより向上させるための工夫

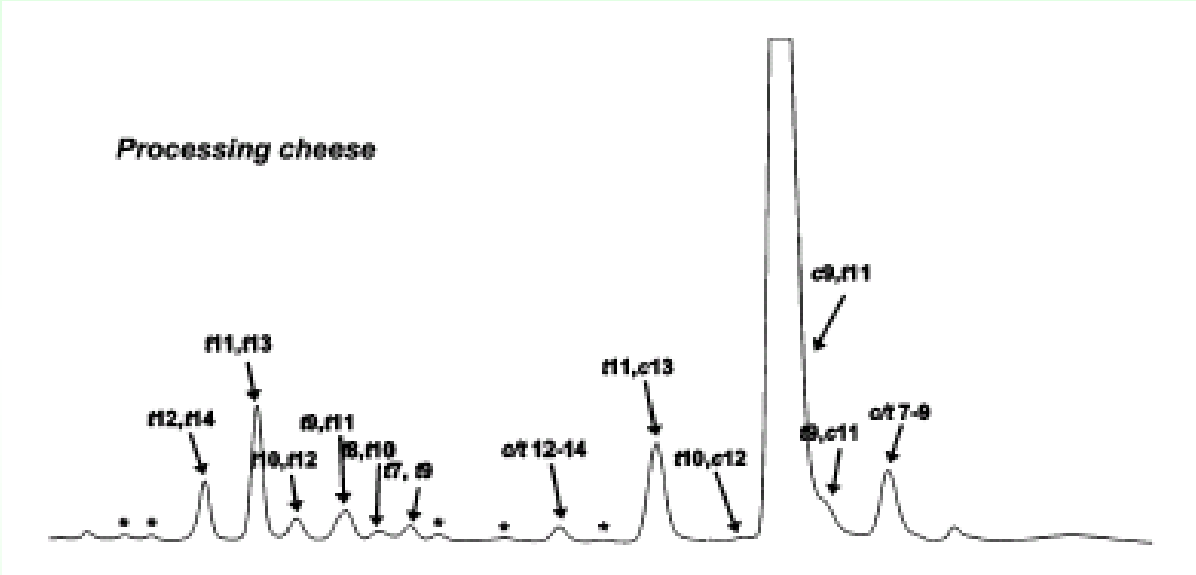
×　微生物機能を低下させるための工夫







反芻動物由来の食品には、
さまざまな脂肪酸が含まれている



ルーメン細菌に代表される嫌気性微生物における
脂質代謝機能のさらなる解明