5st Annual Craft Sake Festival
@ Hot Springs, Arkansas, USA

30 Years of Learning from Lovely Yeasts: Discovery of Cellular Functions and their Application to Sake Brewing

Deer park, Nara

Historical Nara

April 12, 2025

Hiroshi Takagi, Ph.D.
Lab. of Fermentation Science
Institute for Research Initiatives
Nara Institute of Science and Technology, Japan

My Special Thanks to

Kengo Matsumura


Weston Konishi


Nick Lowry

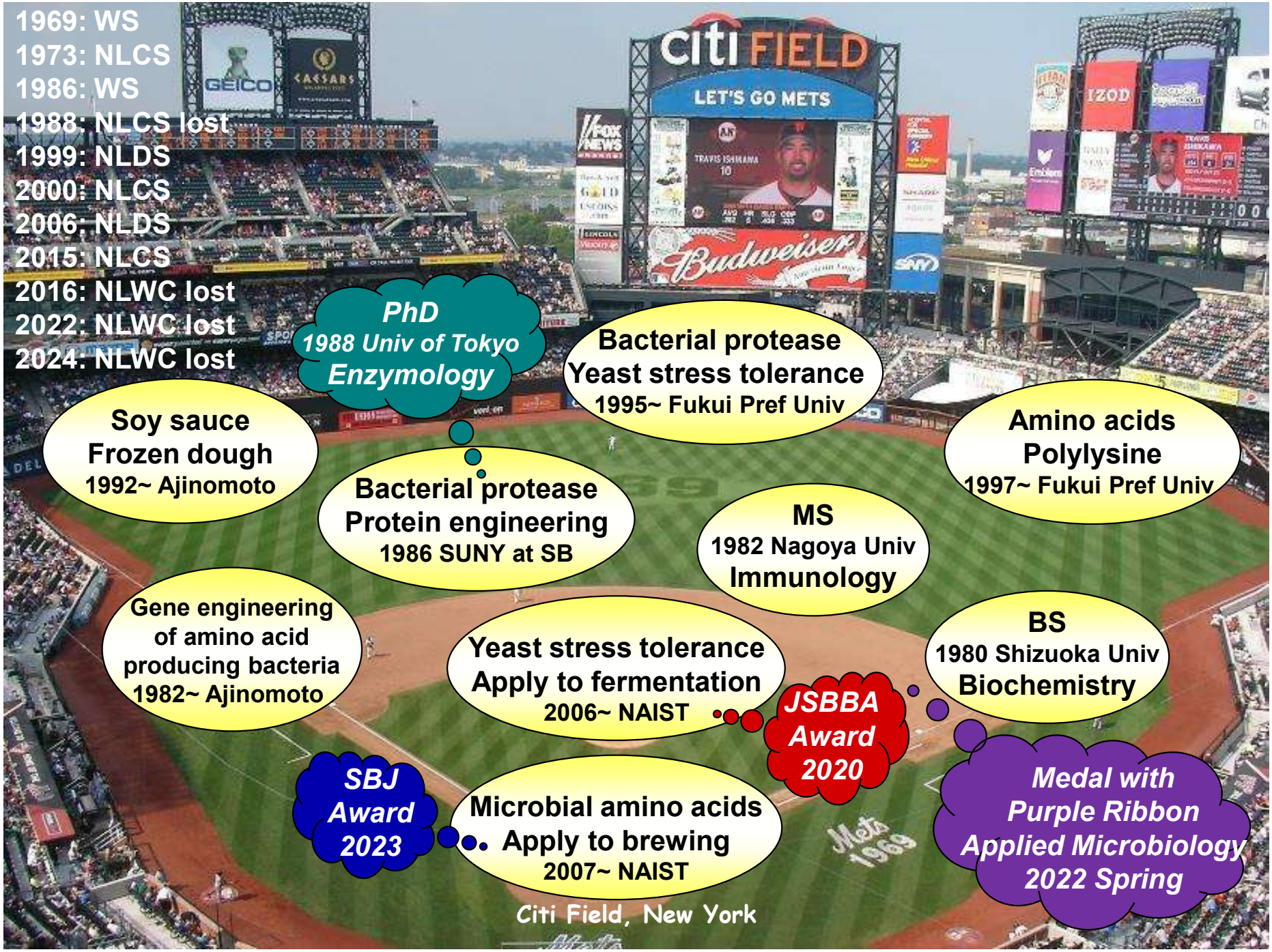

Chiaki & Tama


Byron Stithem


Brandon Doughan

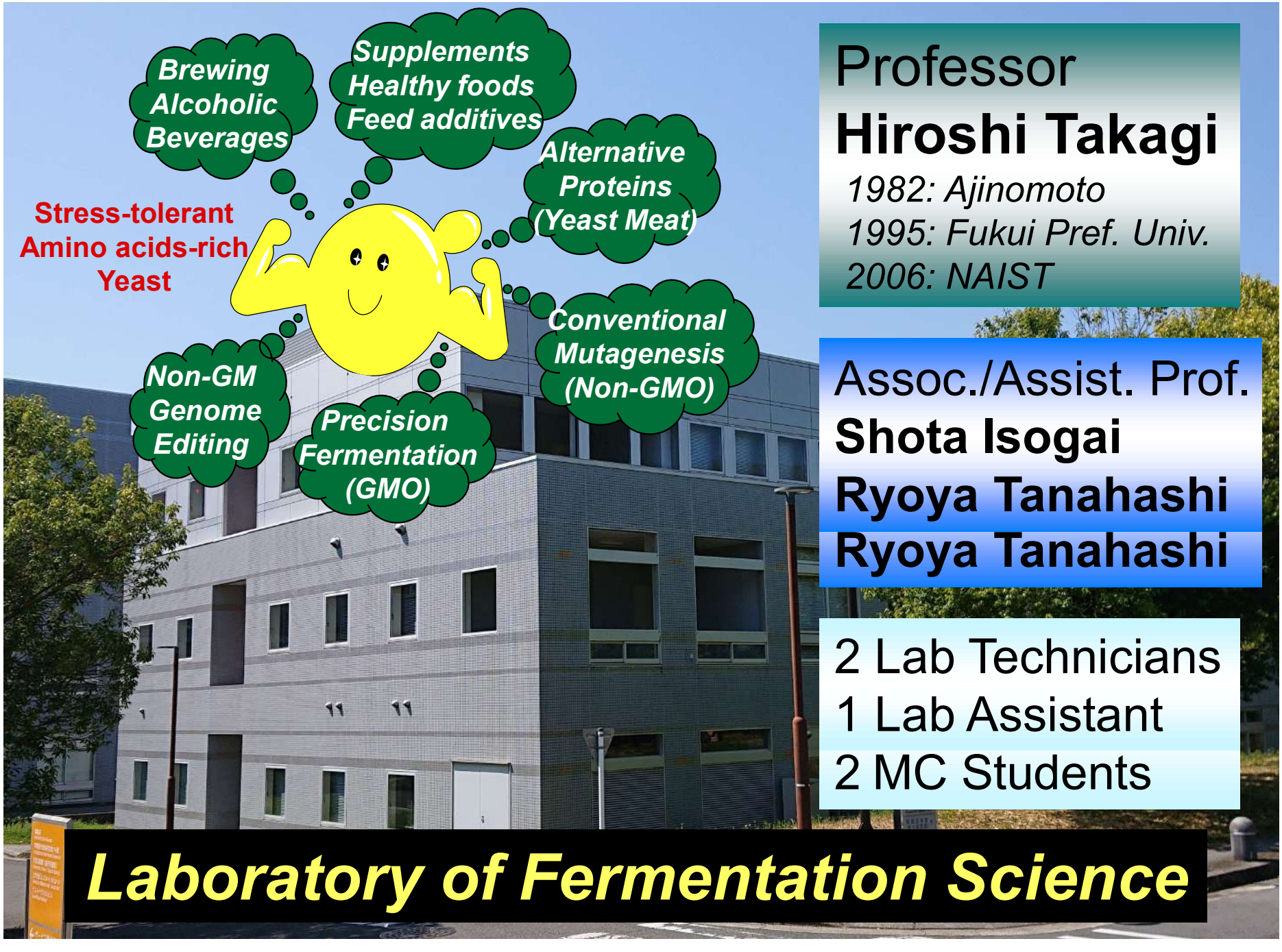
History of My Career and Research

Date of birth: July 15, 1957
Place of birth: Osaka, Japan

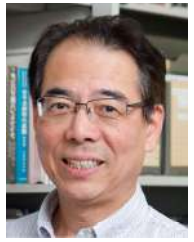


The New Takagi Lab

April, 2023 ~



Professor
Hiroshi Takagi
1982: Ajinomoto
1995: Fukui Pref. Univ.
2006: NAIST



Assoc./Assist. Prof.
Shota Isogai
Ryoya Tanahashi
Ryoya Tanahashi

2 Lab Technicians
1 Lab Assistant
2 MC Students

Laboratory of Fermentation Science

*The yeast **Saccharomyces cerevisiae** is …..*

Model for "Higher Eukaryotes"

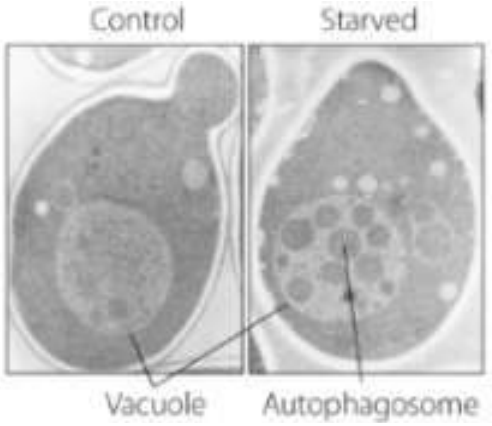
Autophagy

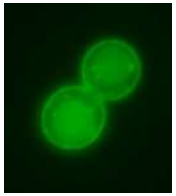
Nobel Prize winner
Dr. Yoshinori Ohsumi

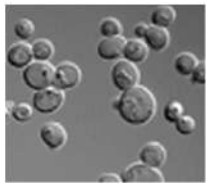
Awamori

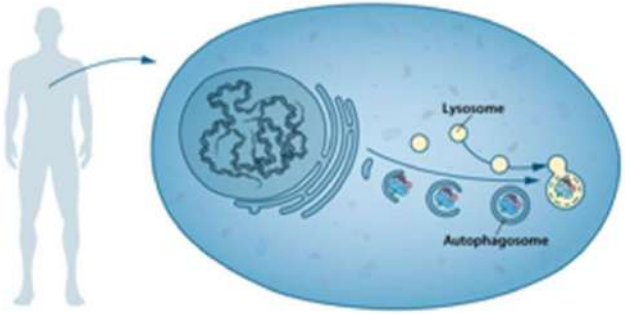
Alcohol beverage brewed by my Yeast

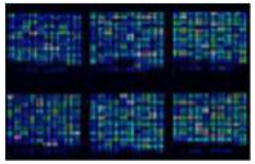
Great "Ethanol Producer"

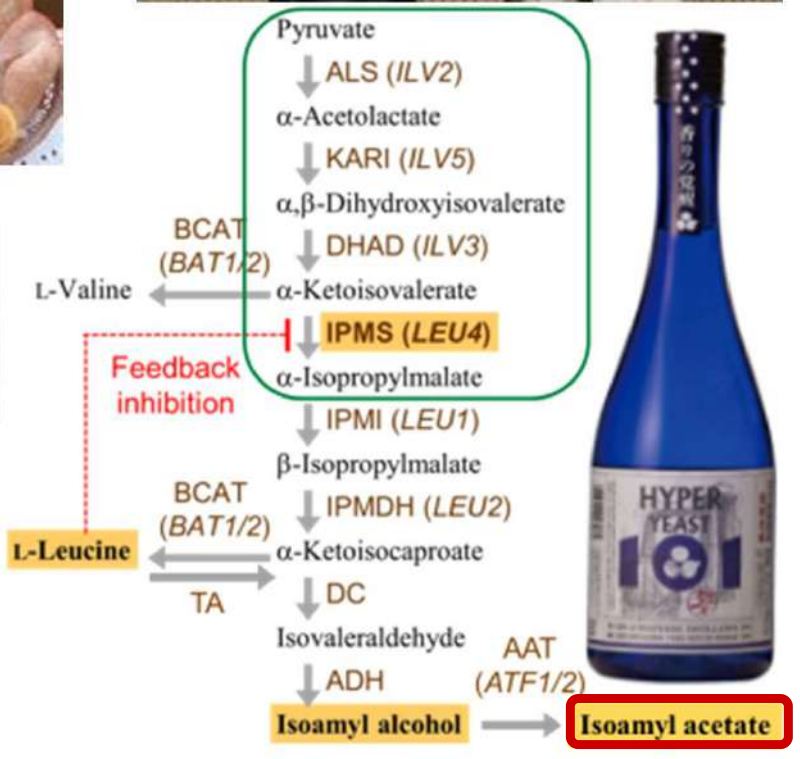




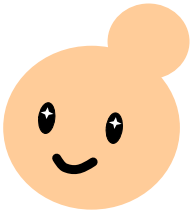






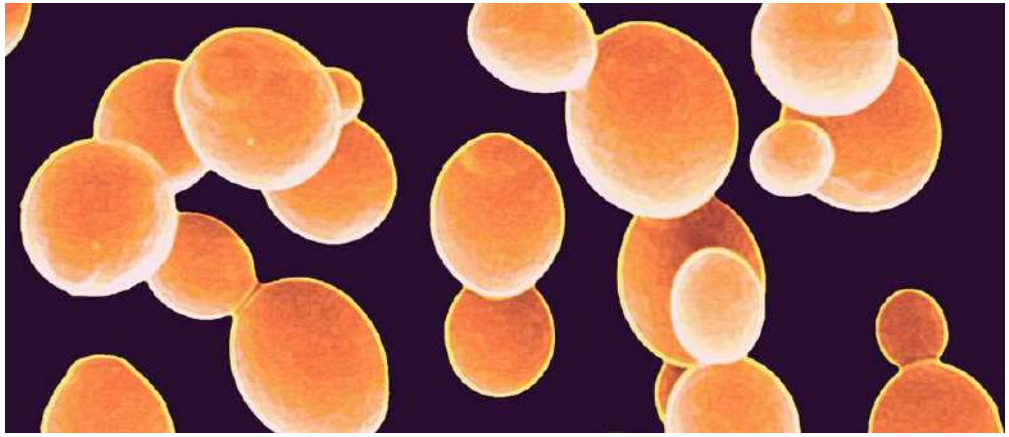


Ethanol *fermentation by* ***yeast***



$$C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2 \uparrow$$

GLUCOSE ETHANOL



Bread

Beer

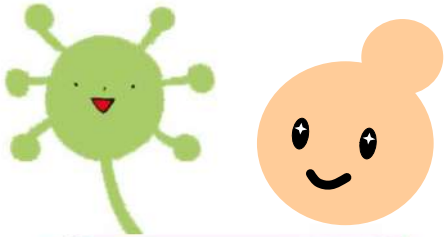
Wine

Sake

Bioethanol

Brewing

*We love **alcoholic beverages** !!*



Non-distilled beverages

Beer

Ethanol 4 – 7%

Wine

10 – 15%

Sake

15 – 20%

Distilled spirits

Whisky

Ethanol 40 – 45%

Brandy

40 – 50%

Shochu

25 – 45%

Congratulations !!

*Japan's "**Traditional Sake Brewing**" of **Sake**, **Shochu** and **Awamori** has been officially registered as an Intangible Cultural Heritage by UNESCO.*

unesco
Intangible Cultural Heritage

December 4, 2024

Paddling work for managing sake mash 'moromi'

Courtesy of Gekkeikan Sake Co., Ltd.

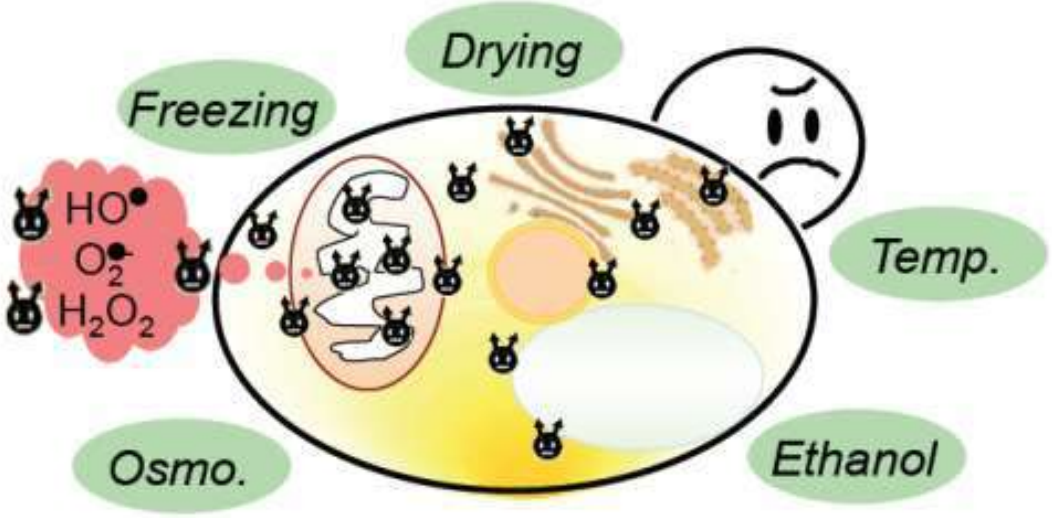
*Industrial **yeast breeding***

1) Improvement of cellular physiology
2) Improvement of yield or productivity
3) Extension of substrate range
4) Reduction of by-product formation
5) Pathways leading to new products

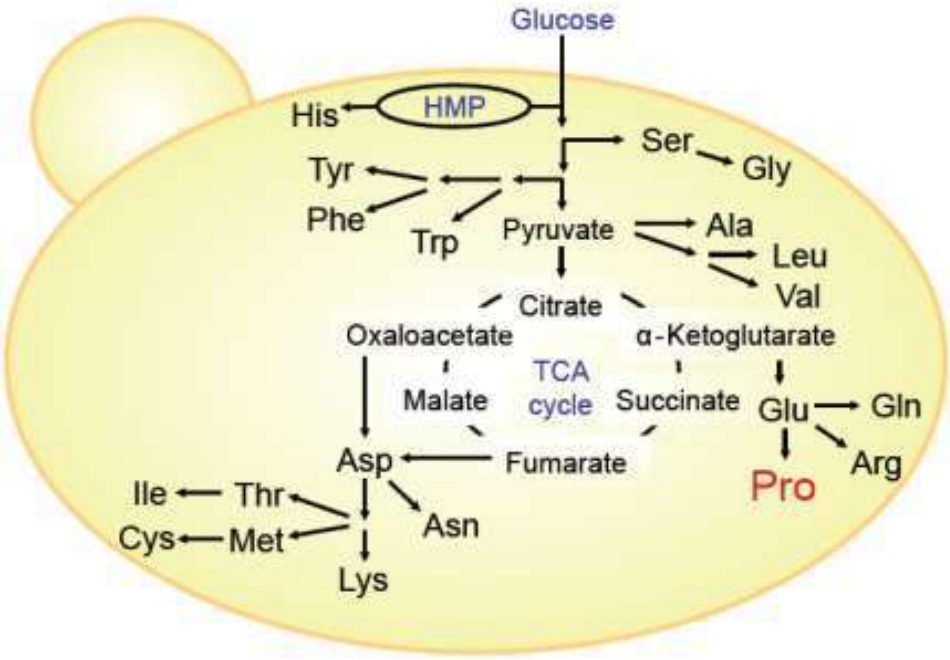
Improvement of fermentation ability

Diversity of product taste and flavor

Stress tolerance



Metabolism modification



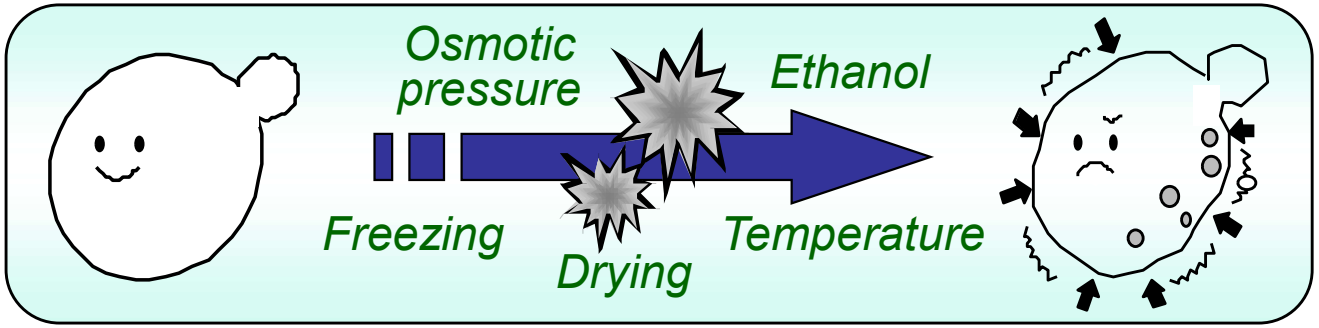
*Stress tolerance is the key for industrial **yeast**.*

Breads

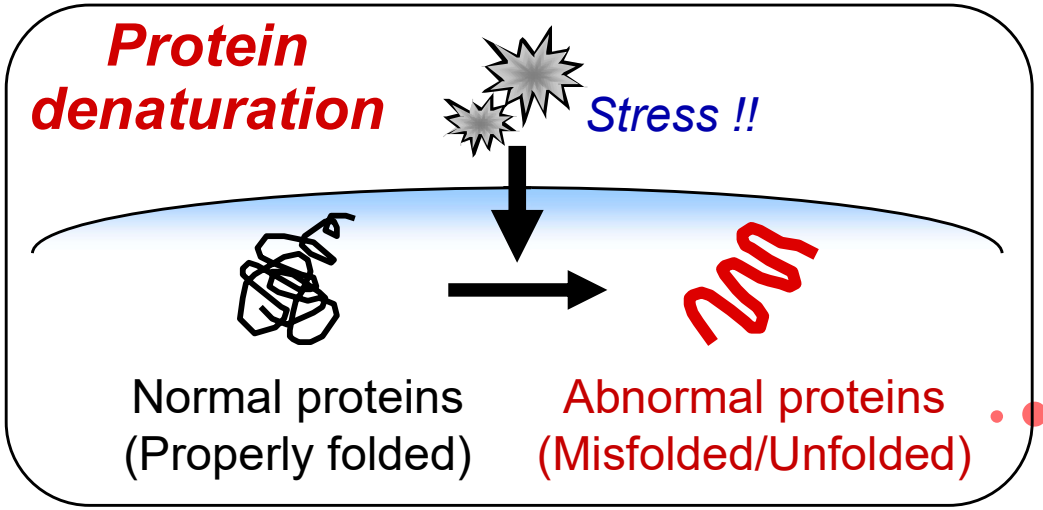
Alcoholic beverages

Bioethanol

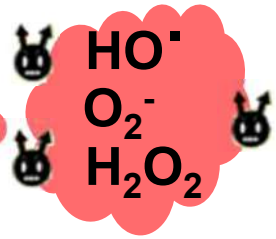
Multiple stresses



Protein denaturation



Reactive oxygen species (ROS) generation



- ***Growth inhibition***
- ***Cell death***

Fermentation ability is limited.

Yeast *cells are exposed to ethanol stress.*

Sake: high concentrations (~20%)

toxicity *Damage to membrane and proteins* *stress* *Cell death*

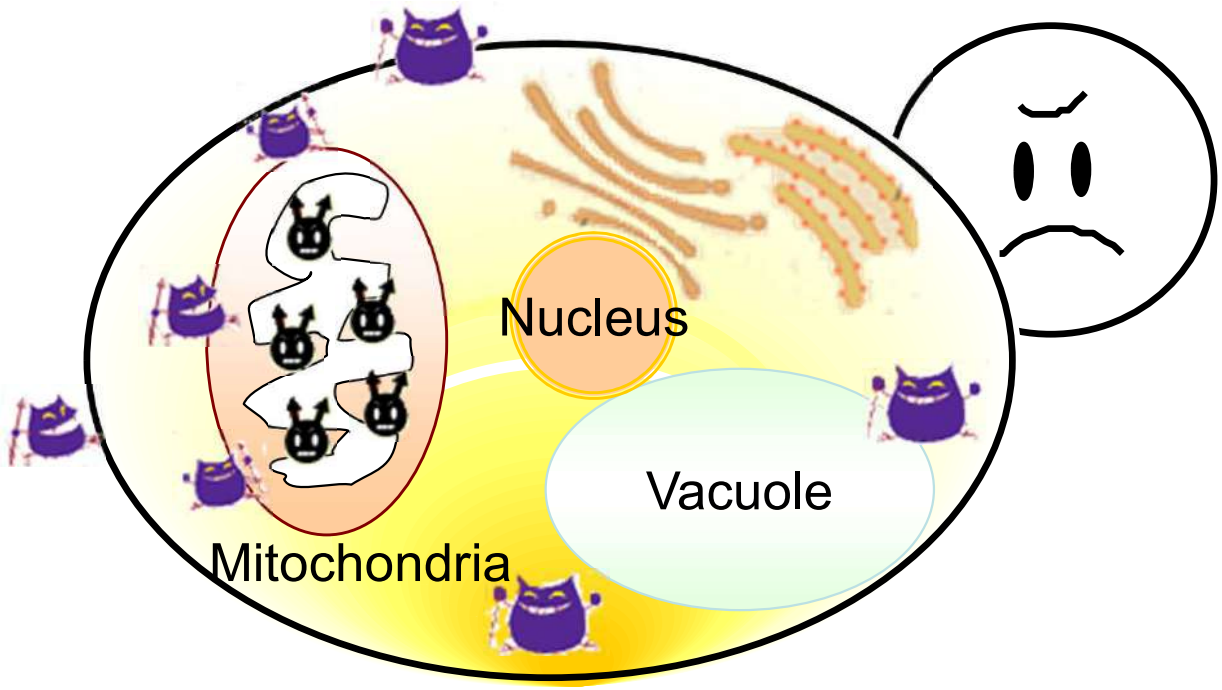
-denatures cellular proteins (enzymes)
-changes the membrane fluidity and permeability

-inhibits cell growth
- cell viability
- fermentation

Mitochondria damage

ROS generation

DNAs, proteins and lipids injury

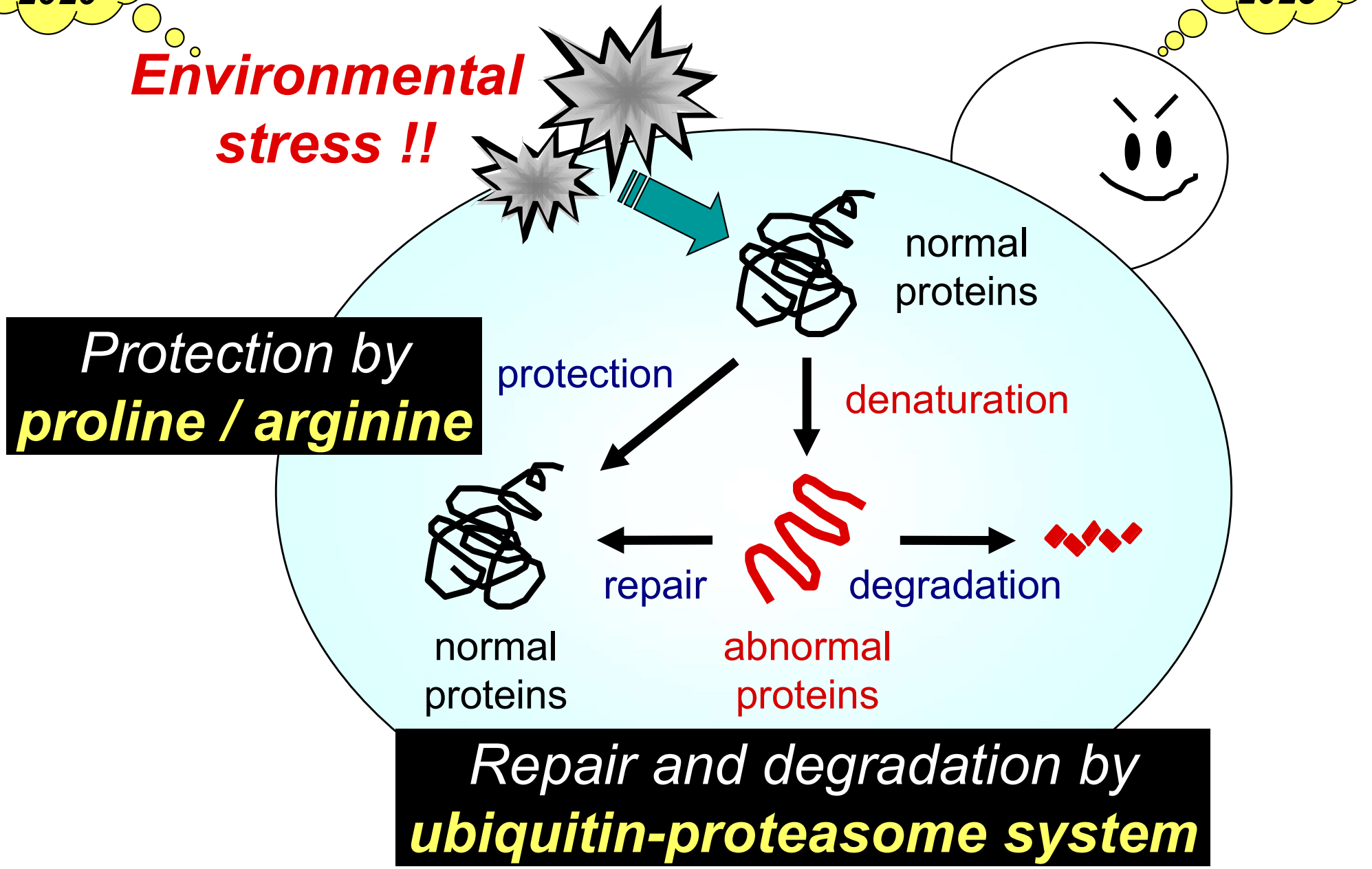


Tolerance to ethanol and oxidative stress is required !!

JSBBA Award 2020

SBJ Award 2023

Stress-tolerant mechanisms of yeast



Takagi, ***Biosci. Biotech. Biochem.***, **85**, 1017, 2021.






*I am **sensitive** to a high concentration of **ethanol** at night…*

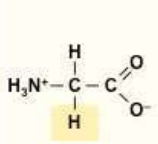



Amino Acids

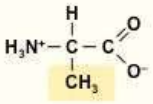
Fermented foods, health foods, beverages, infusions/medicine, cosmetics, feeds...

Most microorganisms synthesize all amino acids in the cell.

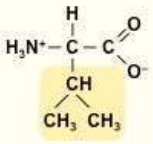
NON-POLAR



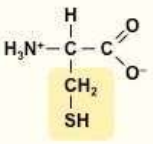
Glycine (Gly / G)



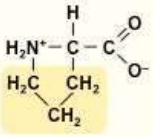
Alanine (Ala / A)



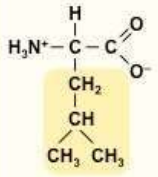
Valine (Val / V)



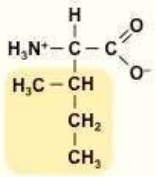
Cysteine (Cys / C)



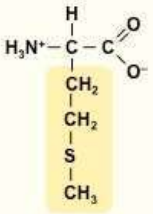
Proline (Pro / P)



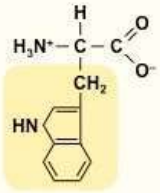
Leucine (Leu / L)



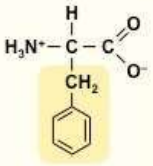
Isoleucine (Ile / I)



Methionine (Met / M)

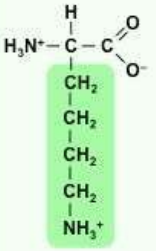


Tryptophan (Trp / W)

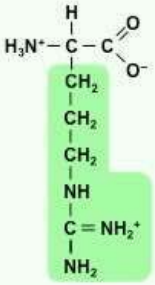


Phenylalanine (Phe / F)

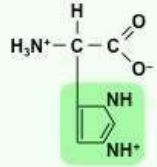
+ CHARGE



Lysine (Lys / K)

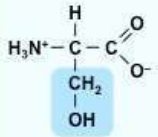


Arginine (Arg / R)

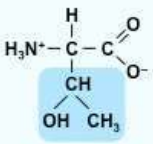


Histidine (His / H)

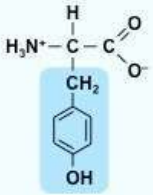
POLAR



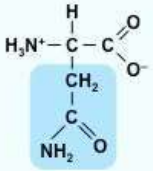
Serine (Ser / S)



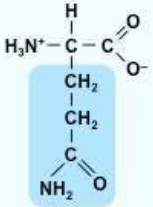
Threonine (Thr / T)



Tyrosine (Tyr / Y)

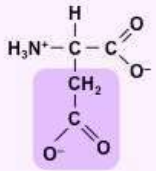


Asparagine (Asn / N)

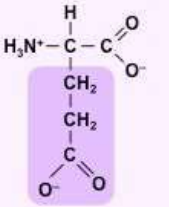


Glutamine (Gln / Q)

– CHARGE



Aspartic Acid (Asp / D)



Glutamic Acid (Glu / E)

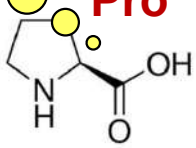
Amino acids in cells exert various physiological functions.

SBJ Award 2017

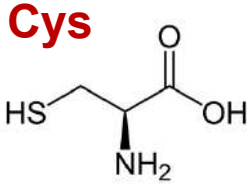
Functional **Amino Acids** Engineering

SBJ Award 2023

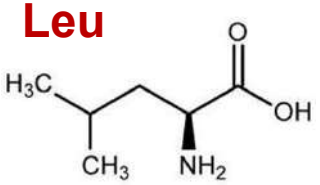
Pro



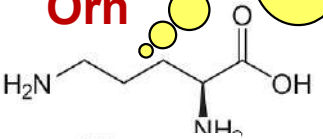
Cys



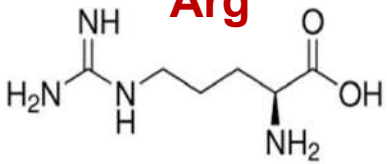
Leu



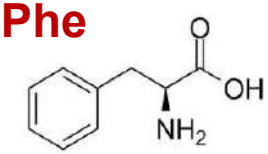
Orn



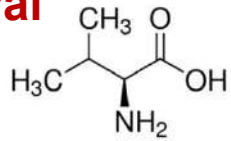
Arg



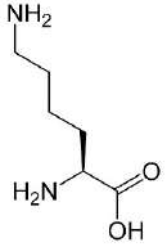
Phe



Val



Lys

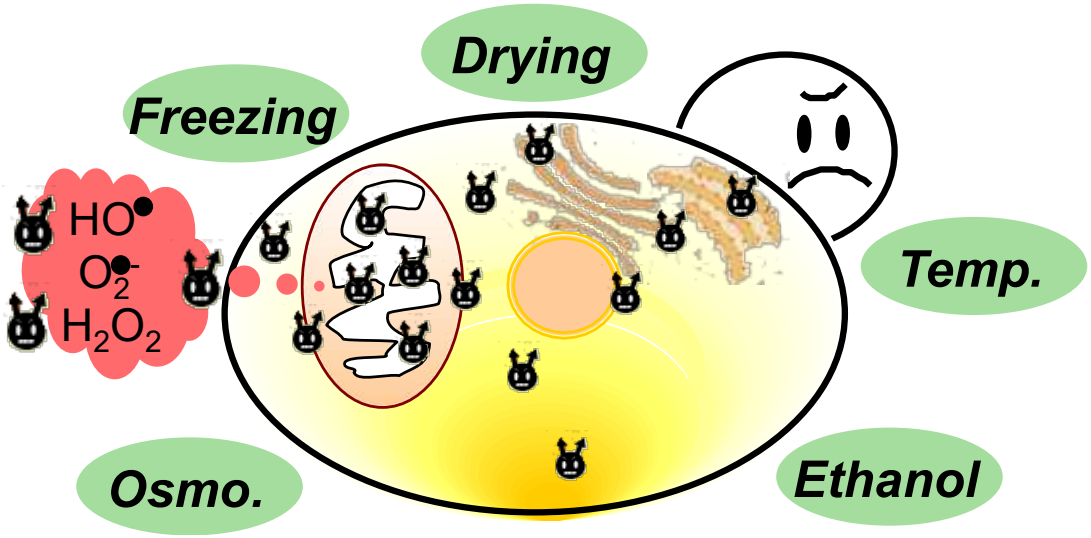


Improvement of fermentation ability

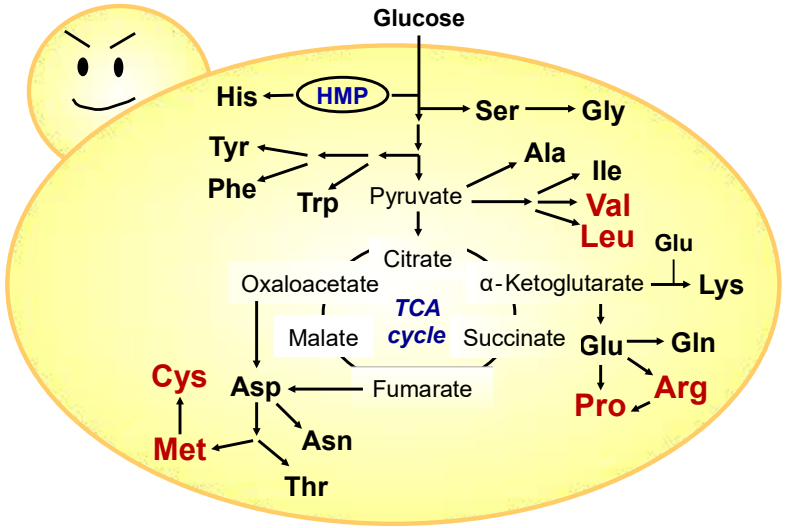
Diversity of taste and flavor

Enhancement of nutritional value

Stress tolerance

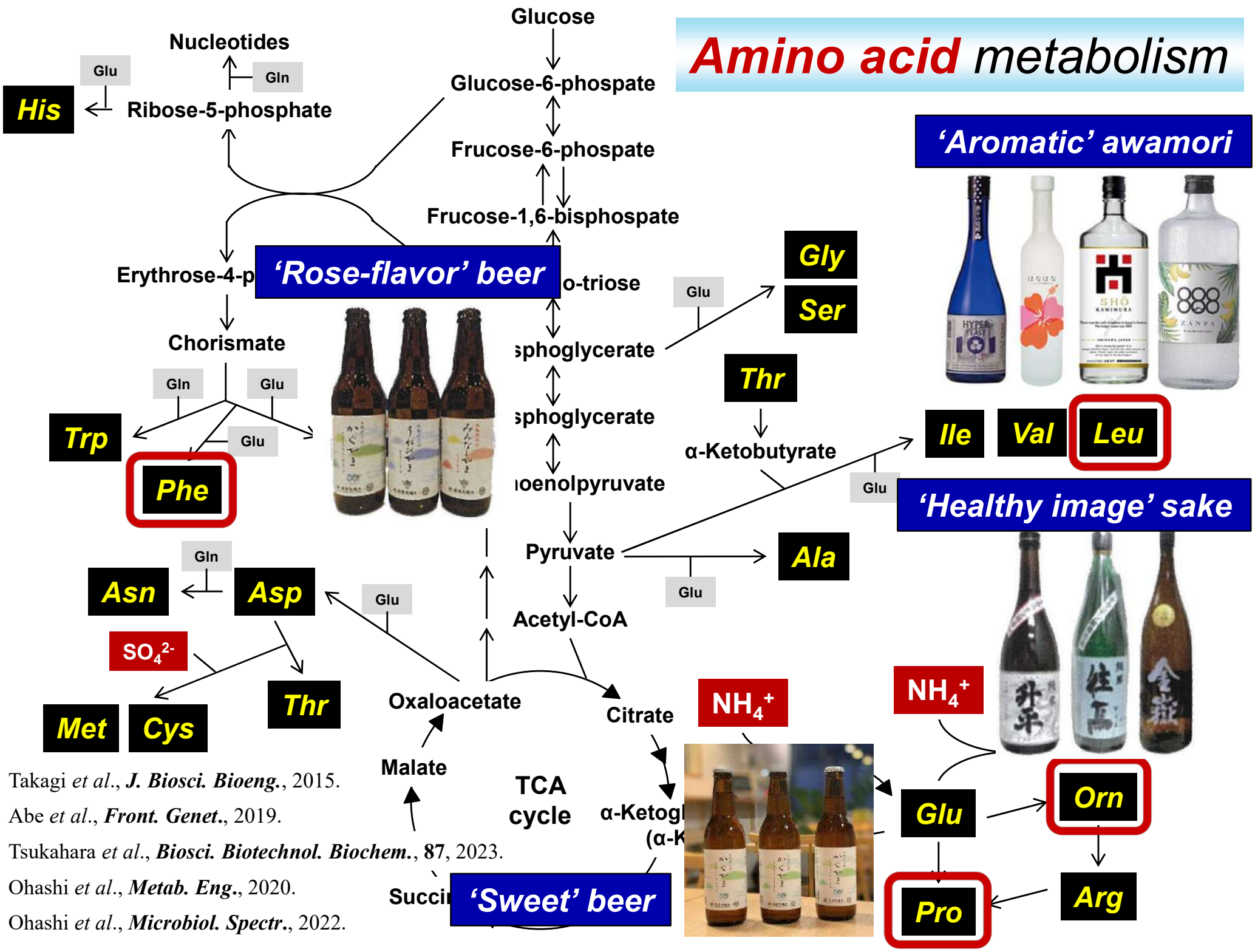


Metabolism modification



Takagi, ***Biosci. Biotechnol. Biochem.***, **83**, 1449, 2019; ***SIMB News***, **71**, 8, 2021.

Amino acid metabolism



Takagi *et al.*, ***J. Biosci. Bioeng.***, 2015.

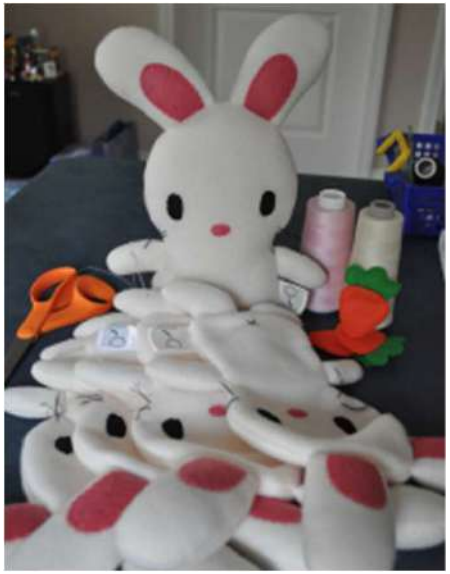
Abe *et al.*, ***Front. Genet.***, 2019.

Tsukahara *et al.*, ***Biosci. Biotechnol. Biochem.***, **87**, 2023.

Ohashi *et al.*, ***Metab. Eng.***, 2020.

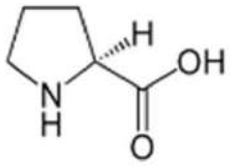
Ohashi *et al.*, ***Microbiol. Spectr.***, 2022.

*Construction of commercial **brewing yeasts***

Genetic engineering	***Self-cloning***	***Traditional breeding***
GM yeast	Non-recombinant yeast	
		
	Homologous recombination	Isolation, mutation mating, fusion etc.

Traditional breeding is only acceptable for food industry.

Improved fermentation performance of industrial yeasts by proline



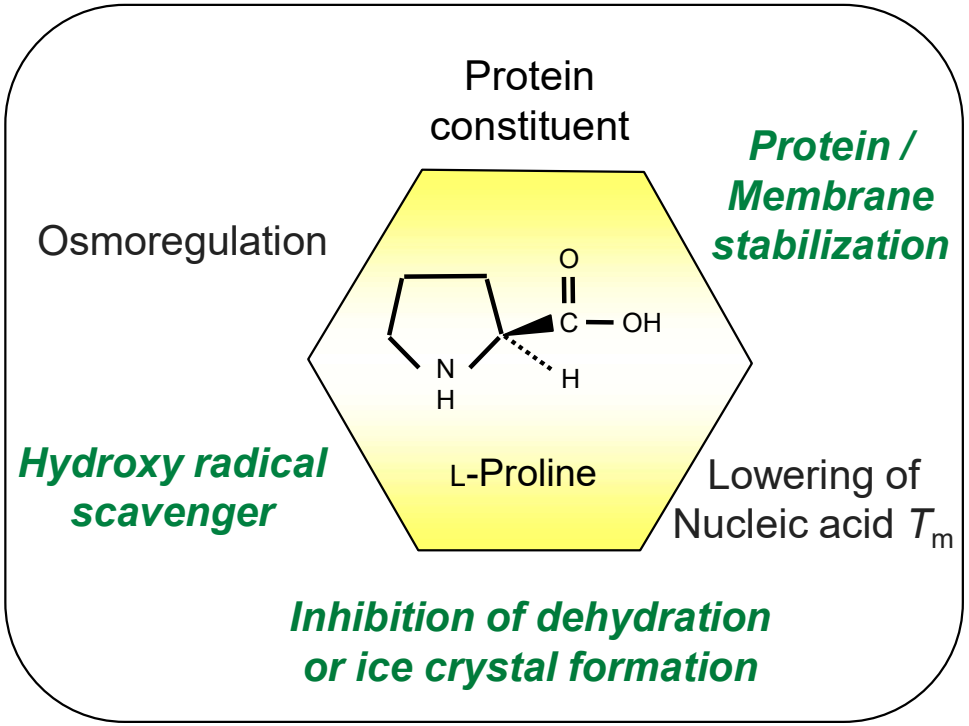
L-Proline

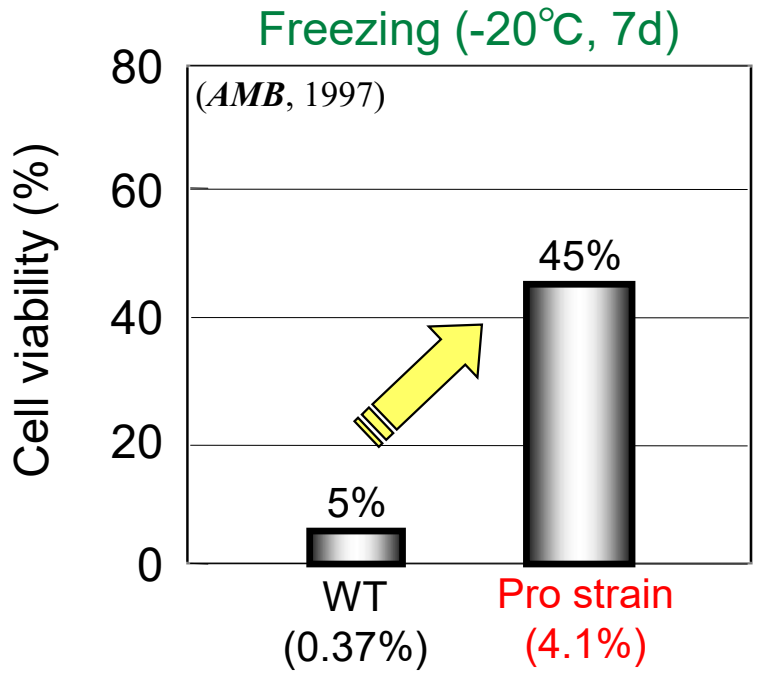
What is **proline** ?

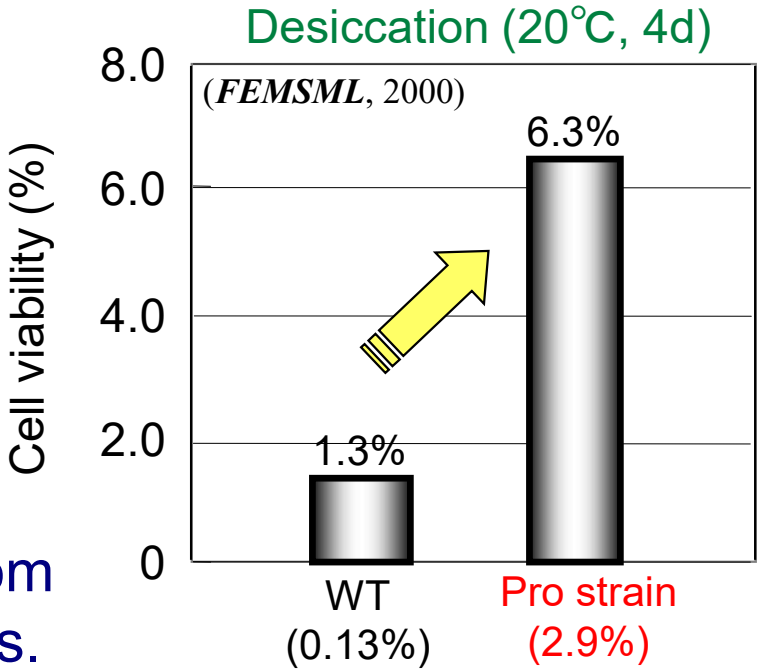
Protects yeast cells from various stresses

- In response to osmotic stresses, many bacterial and plant cells accumulate proline.
- Yeast cells induce glycerol or trehalose synthesis, but do NOT increase proline level.

Physiological functions

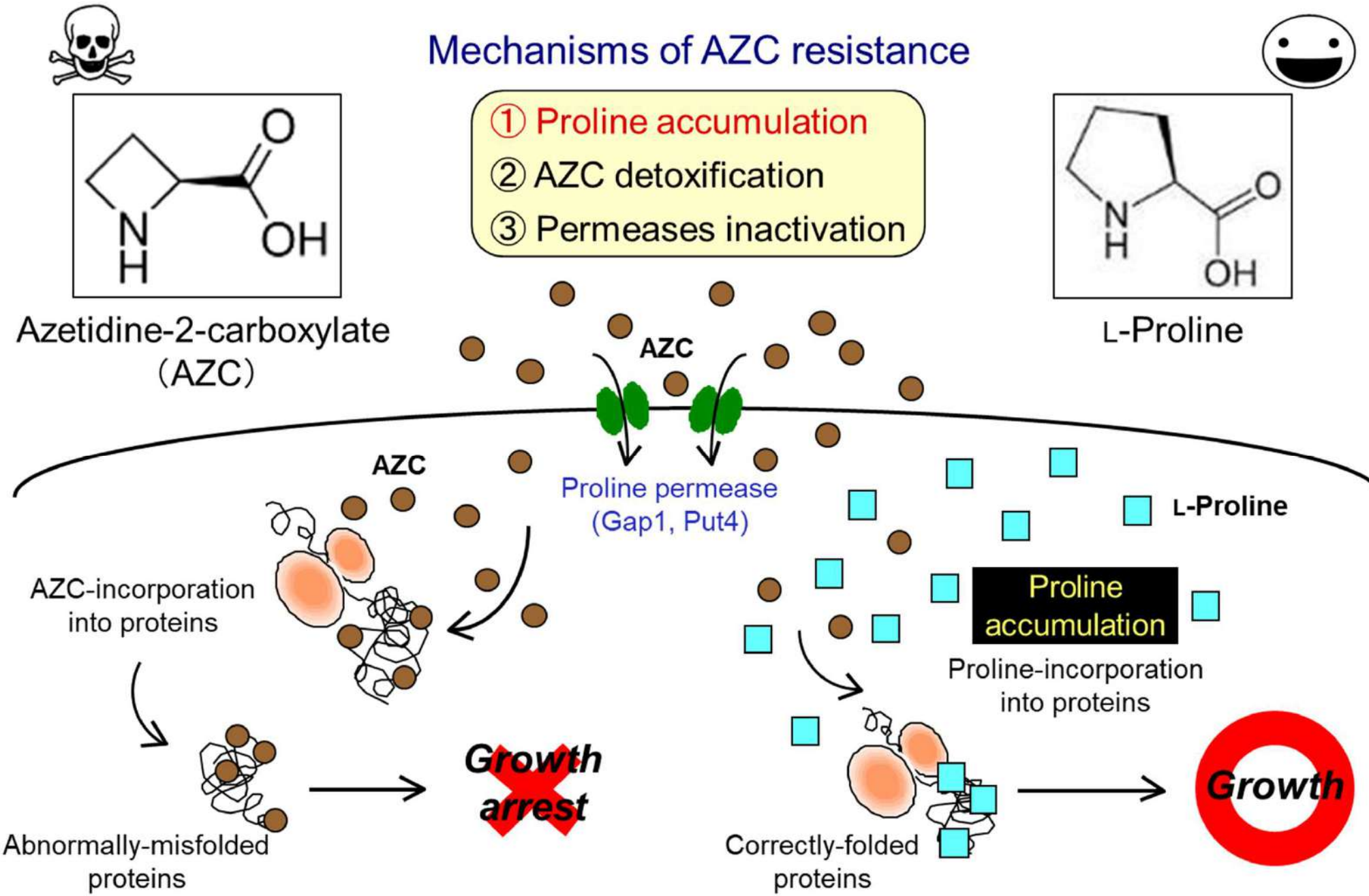






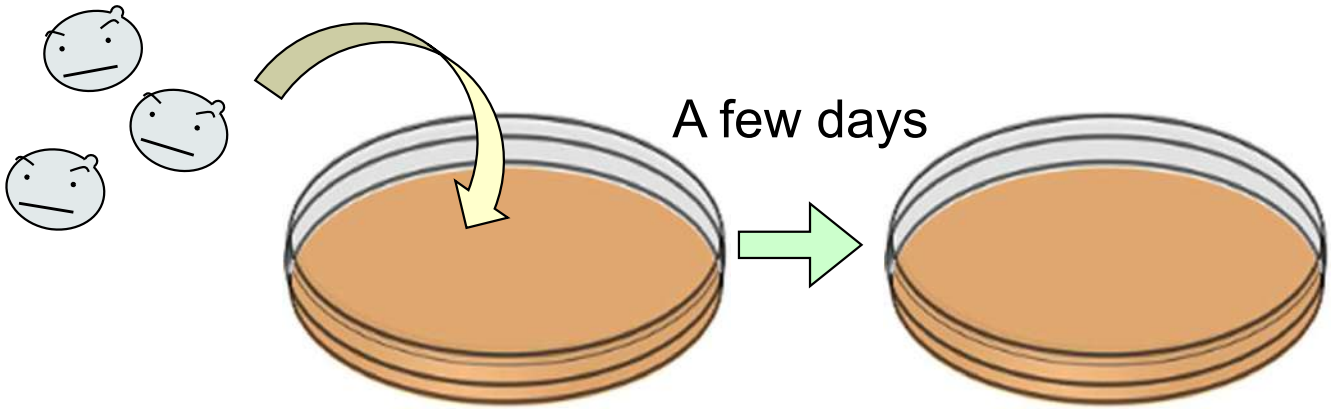
Proline-accumulating strains were isolated from **the proline analogue (AZC)-resistant** mutants.

*Isolation of yeast mutants with **proline** accumulation*

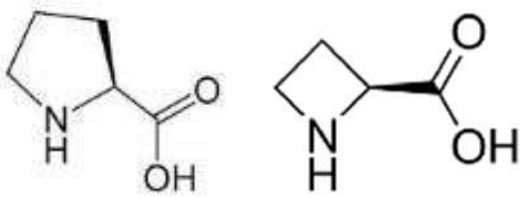


*Isolation of **proline**-acculumulating mutants*

Sake yeast



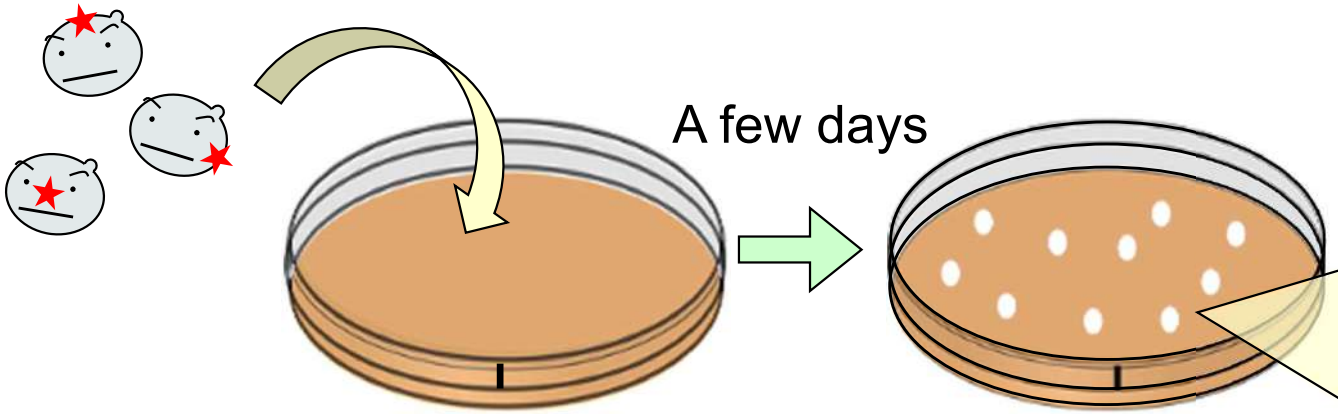
Proline



Azetidine 2-carboxylate (AZC)

Minimal medium agar plate containing **AZC** (5 mg/mL)

Mutagen-treated Sake yeast



AZC-resistant colonies

Measure cellular **proline** level

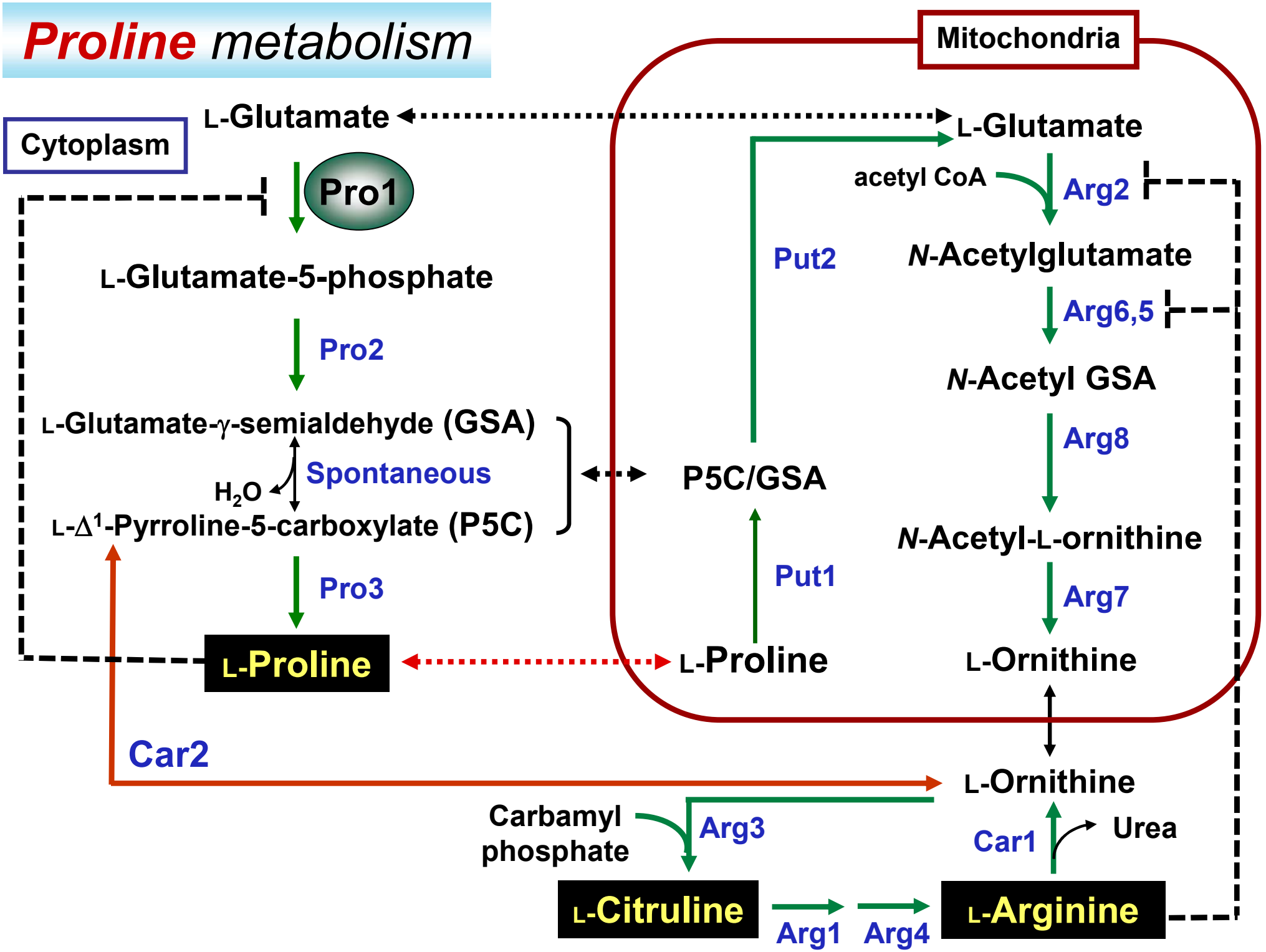
↓

Select **proline** overproducers

↓

Test stress tolerance

Proline metabolism

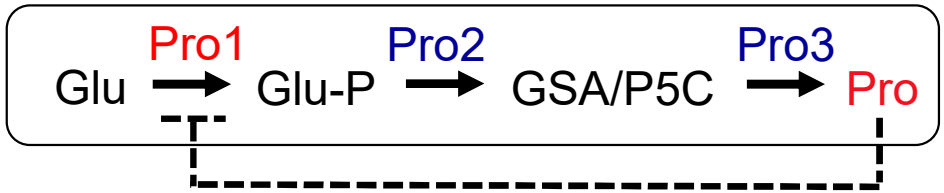


Pro1 variants with enhanced *proline*-synthetic activity

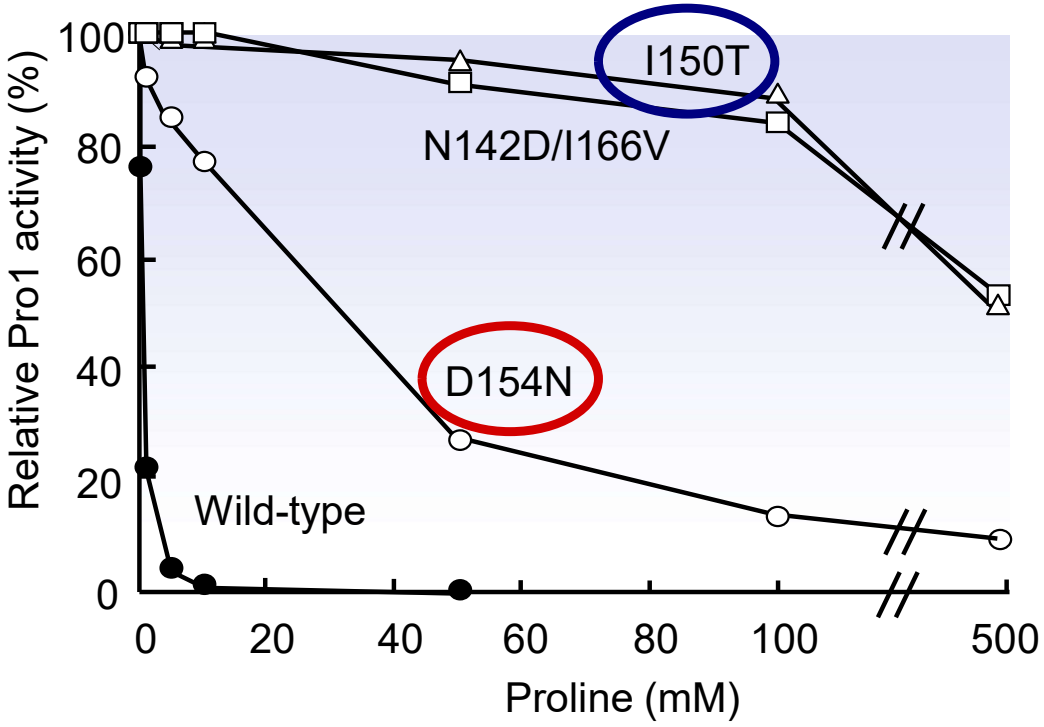
Sekine *et al.*, ***Appl. Environ. Microbiol.***, **73**, 4011, 2007.

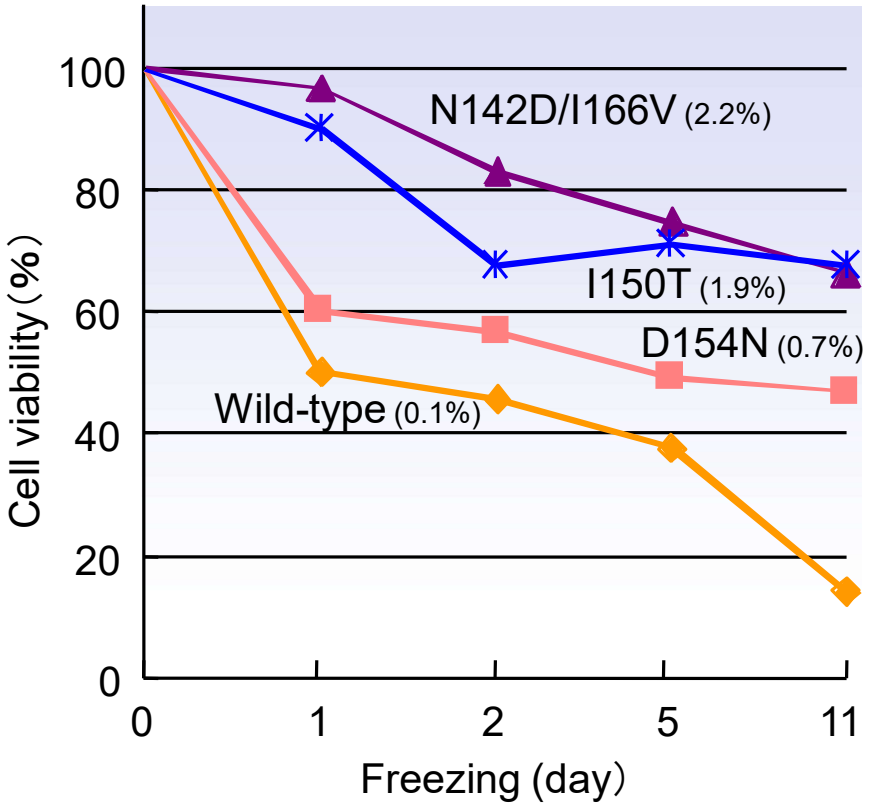
γ-Glutamyl kinase (Pro1)

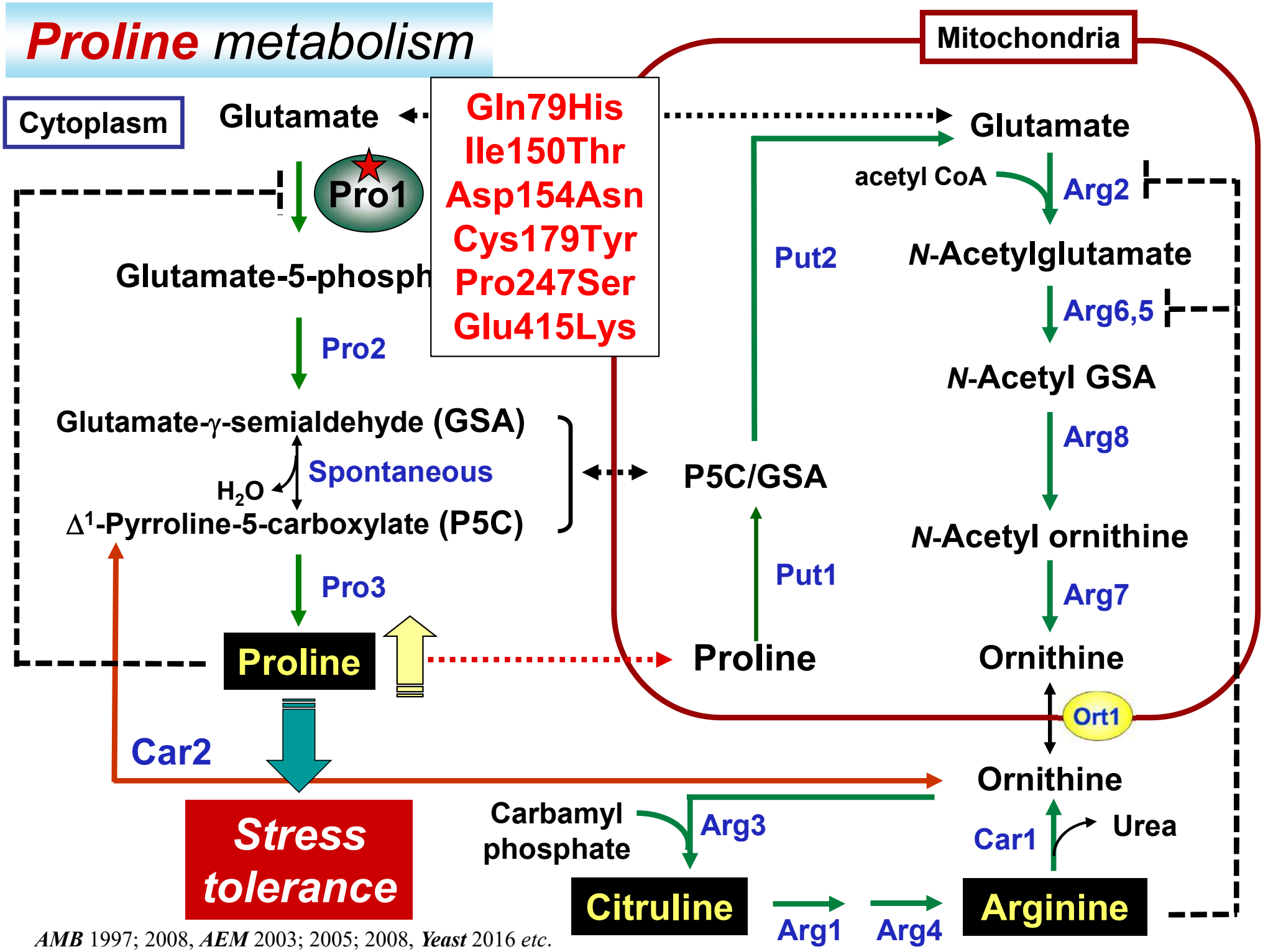
the rate-limiting enzyme
in proline synthesis



PCR random mutagenesis in ***PRO1*** → Isolation of **AZC-resistant** clones



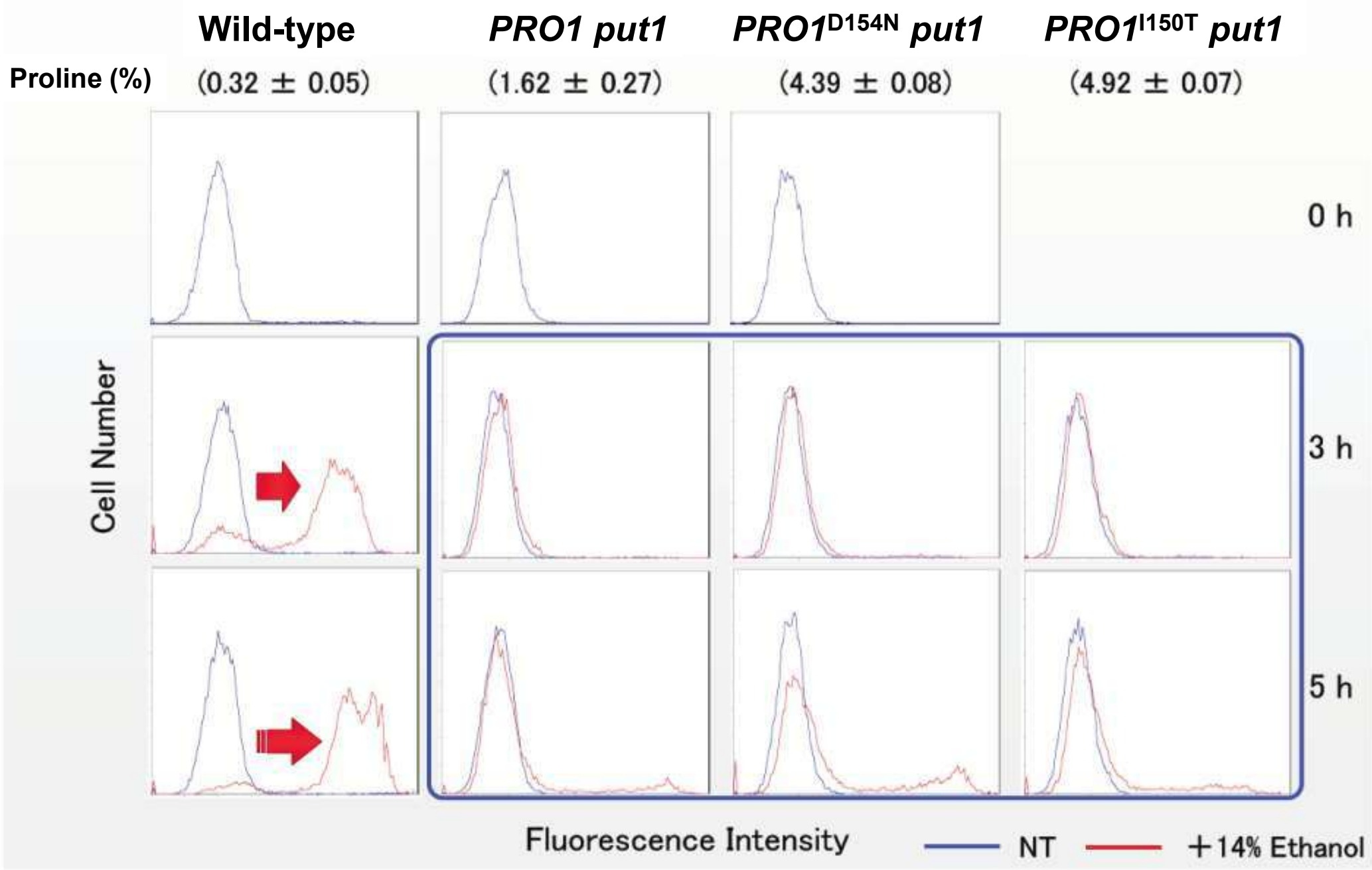




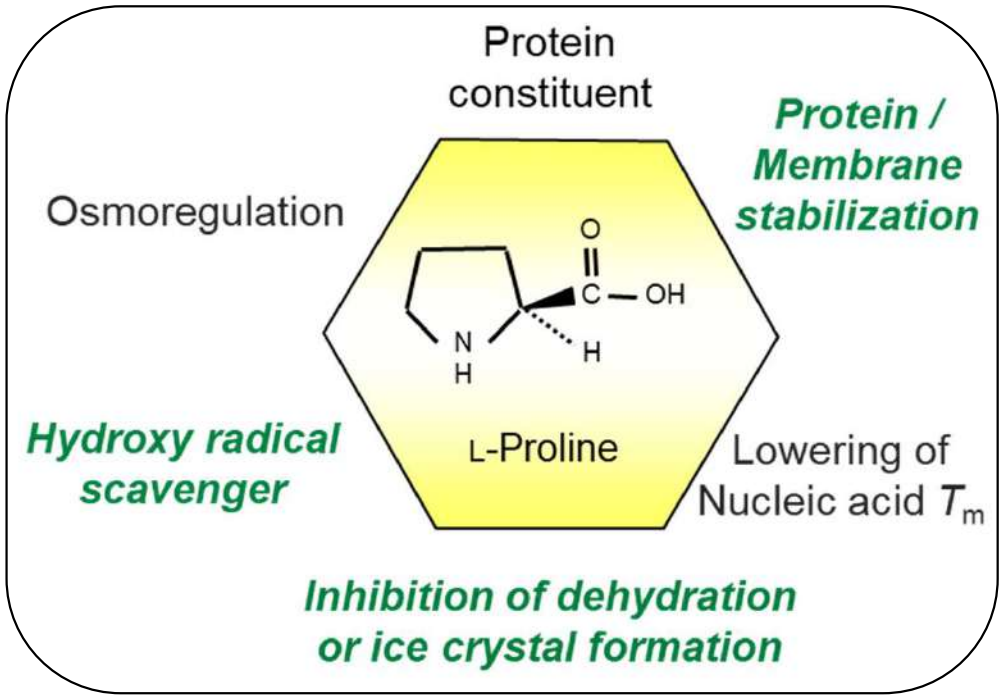
AMB 1997; 2008, *AEM* 2003; 2005; 2008, *Yeast* 2016 *etc.*

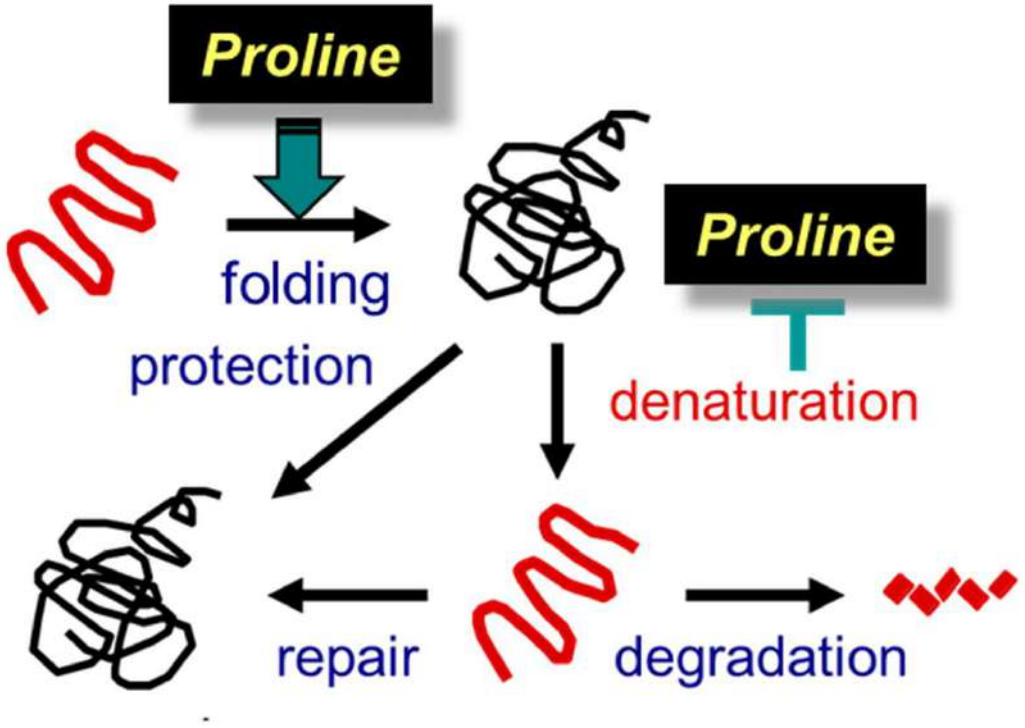
Proline *reduces intracellular ROS level.*

Takagi *et al*., ***Yeast***, **33**, 355, 2016.



Proline promotes folding and inhibits aggregation





Proline inhibits aggregation during protein refolding

DHARMARAJ SAMUEL,[1] THALLAMPURANAM KRISHNASWAMY S. KUMAR,[1,2] GOPAL GANESH,[1] GURUNATHAN JAYARAMAN,[1,3] PEY-WEN YANG,[1] MEI-MING CHANG,[1] VISHWA DEO TRIVEDI,[1,2] SUE-LEIN WANG,[1] KUO-CHU HWANG,[1] DING-KWO CHANG,[2] AND CHIN YU[1]

[1]Department of Chemistry, National Tsing Hua University, Hsinchu, Taiwan
[2]Institute of Chemistry, Academia Sinica, Taipei, Taiwan

The in vitro refolding of hen egg-white lysozyme is studied in the presence of various osmolytes. Proline is found to prevent aggregation during protein refolding. However, other osmolytes used in this study fail to exhibit a similar property. Experimental evidence suggests that proline inhibits protein aggregation by binding to folding intermediate(s) and trapping the folding intermediate(s) into enzymatically inactive, "aggregation-insensitive" state(s). However, elimination of proline from the refolded protein mixture results in significant recovery of the bacteriolytic activity. At higher concentrations (>1.5 M), proline is shown to form loose, higher-order molecular aggregate(s). The supramolecular assembly of proline is found to possess an amphipathic character. Formation of higher-order aggregates is believed to be crucial for proline to function as a protein folding aid. In addition to its role in osmoregulation under water stress conditions, the results of this study hint at the possibility of proline behaving as a protein folding chaperone.

Protein Sci., **9**, 344, 2000.

The Chemical Chaperone Proline Relieves the Thermosensitivity of a *dnaK* Deletion Mutant at 42°C

Madhab K. Chattopadhyay,[1]† Renée Kern,[1] Michel-Yves Mistou,[2] Abhaya M. Dandekar,[3] Sandra L. Uratsu,[3] and Gilbert Richarme[1]*

Molécules de Stress, Institut Jacques Monod, Université Paris 7, Paris,[1] and Unité de Biochimie et Structure des Protéines, INRA, Jouy en Josas,[2] France, and Department of Pomology, University of California-Davis, Davis, California[3]

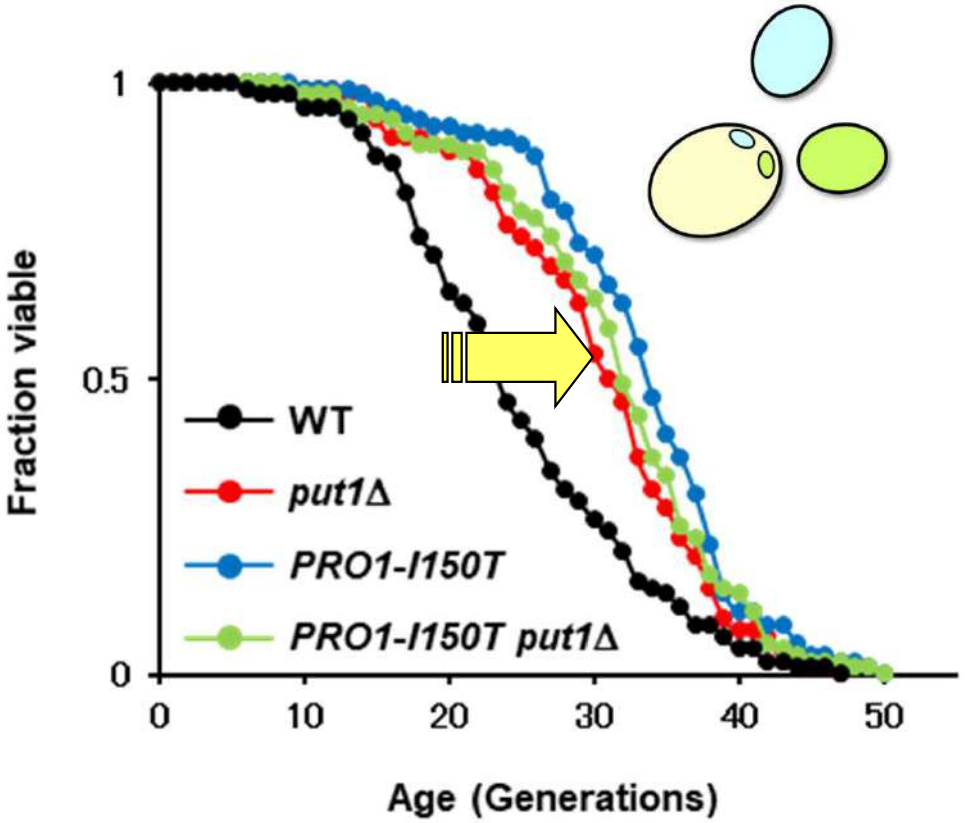
Since, like other osmolytes, proline can act as a protein stabilizer, we investigated the thermoprotectant properties of proline in vitro and in vivo. In vivo, elevated proline pools in *Escherichia coli* (obtained by altering the feedback inhibition by proline of γ-glutamylkinase, the first enzyme of the proline biosynthesis pathway) restore the viability of a *dnaK*-deficient mutant at 42°C, suggesting that proline can act as a thermoprotectant for *E. coli* cells. Furthermore, analysis of aggregated proteins in the *dnaK*-deficient strain at 42°C by two-dimensional gel electrophoresis shows that high proline pools reduce the protein aggregation defect of the *dnaK*-deficient strain. In vitro, like other "chemical chaperones," and like the DnaK chaperone, proline protects citrate synthase against thermodenaturation and stimulates citrate synthase renaturation after urea denaturation. These results show that a protein aggregation defect can be compensated for by a single mutation in an amino acid biosynthetic pathway and that an ubiquitously producible chemical chaperone can compensate for a defect in one of the major chaperones involved in protein folding and aggregation.

J. Bacteriol., **186**, 8149, 2004.

Proline *metabolism regulates* ***cell lifespan***

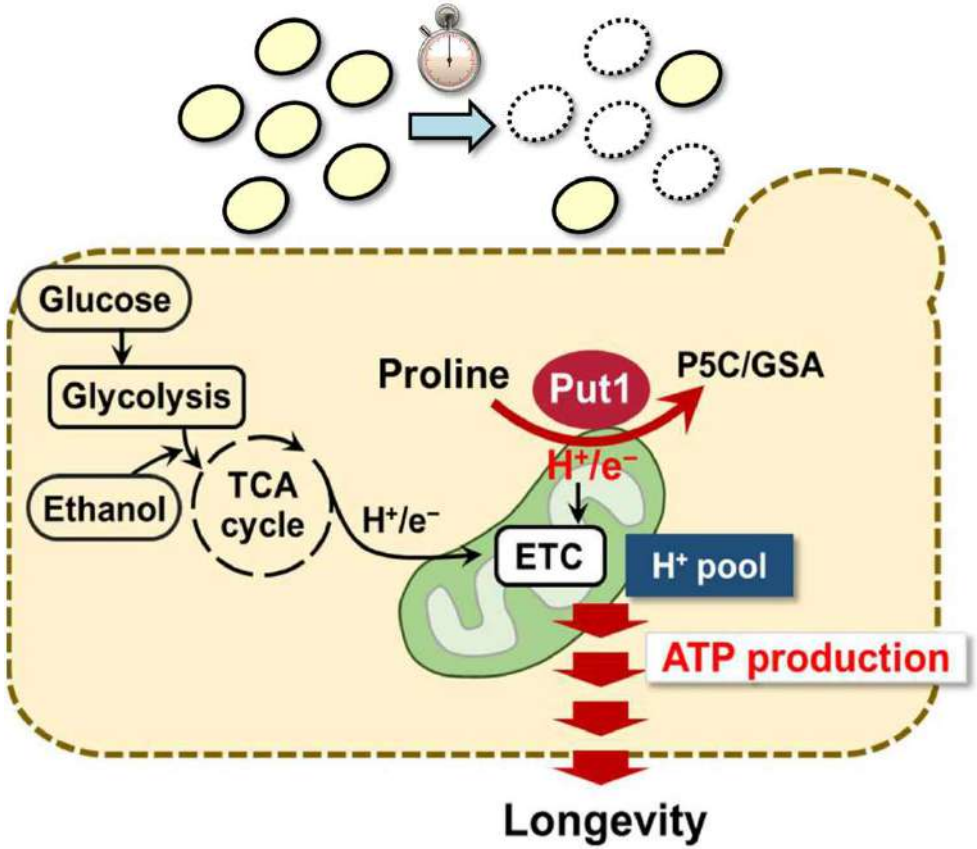
Mukai *et al*., ***Microbial Cell***, **6**, 482, 2019; Nishimura *et al*., ***Microorganisms***, **9**, 1650, 2021.

Replicative *lifespan*



Extended lifespan with increased **proline** content

Chronological *lifespan*



Reduced lifespan due to the lack of **proline** oxidation

*Application to **industrial yeasts***

***Replicative** lifespan*

Beer yeast

(For harvesting and reusing)

https://www.vectorstock.com/royalty-free-vector/beer-brewing-process-infographic-in-flat-style-vector-5771537

***Chronological** lifespan*

Baker's yeast

Air-dry stress

(For dried yeast making)

Freezing stress

(For frozen dough baking)

Proline *increases fermentation ability in frozen dough.*

Kaino *et al.*, ***Appl. Environ. Microbiol.***, **74**, 5845, 2008.

Collaboration with Dr. Shima (NFRI)

bread dough

bread-making flour	100	g
yeast (66% moisture basis)	4	g
sucrose	5	g
NaCl	2	g
water	68	ml

CO_2 gas production

↓

prefermentation (30℃, 120 min)

↓

freezing (-20℃, 9 days)

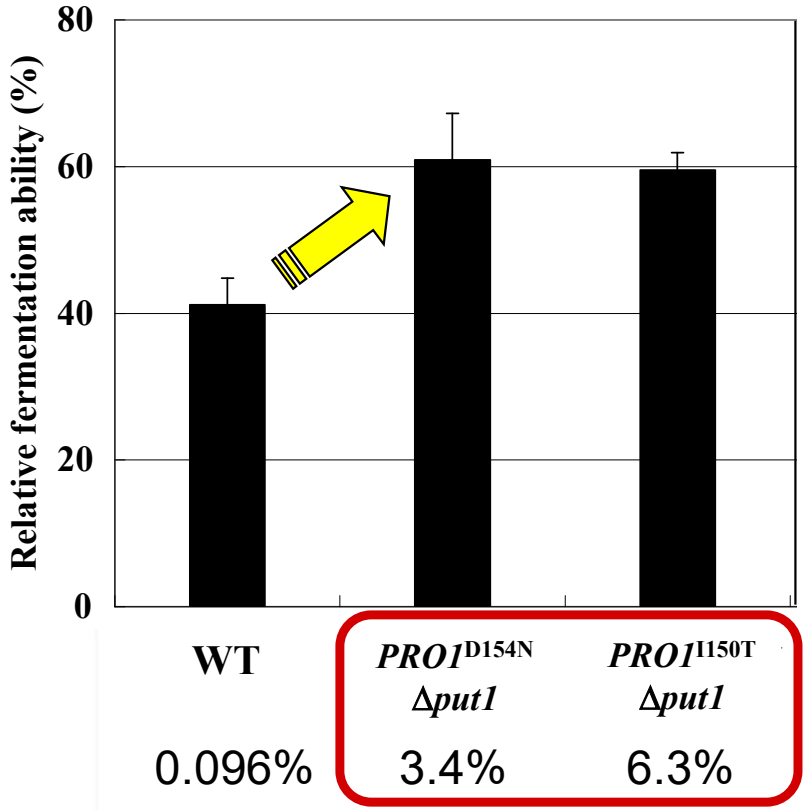
↓

thawing (30℃, 30 min)

↓

CO_2 gas production

Freezing stress tolerance test



also in sweet dough and after air-drying.

Sasano *et al.*, ***Int. J. Food Microbiol.***, **138**, 181, 2010; **152**, 40, 2012.

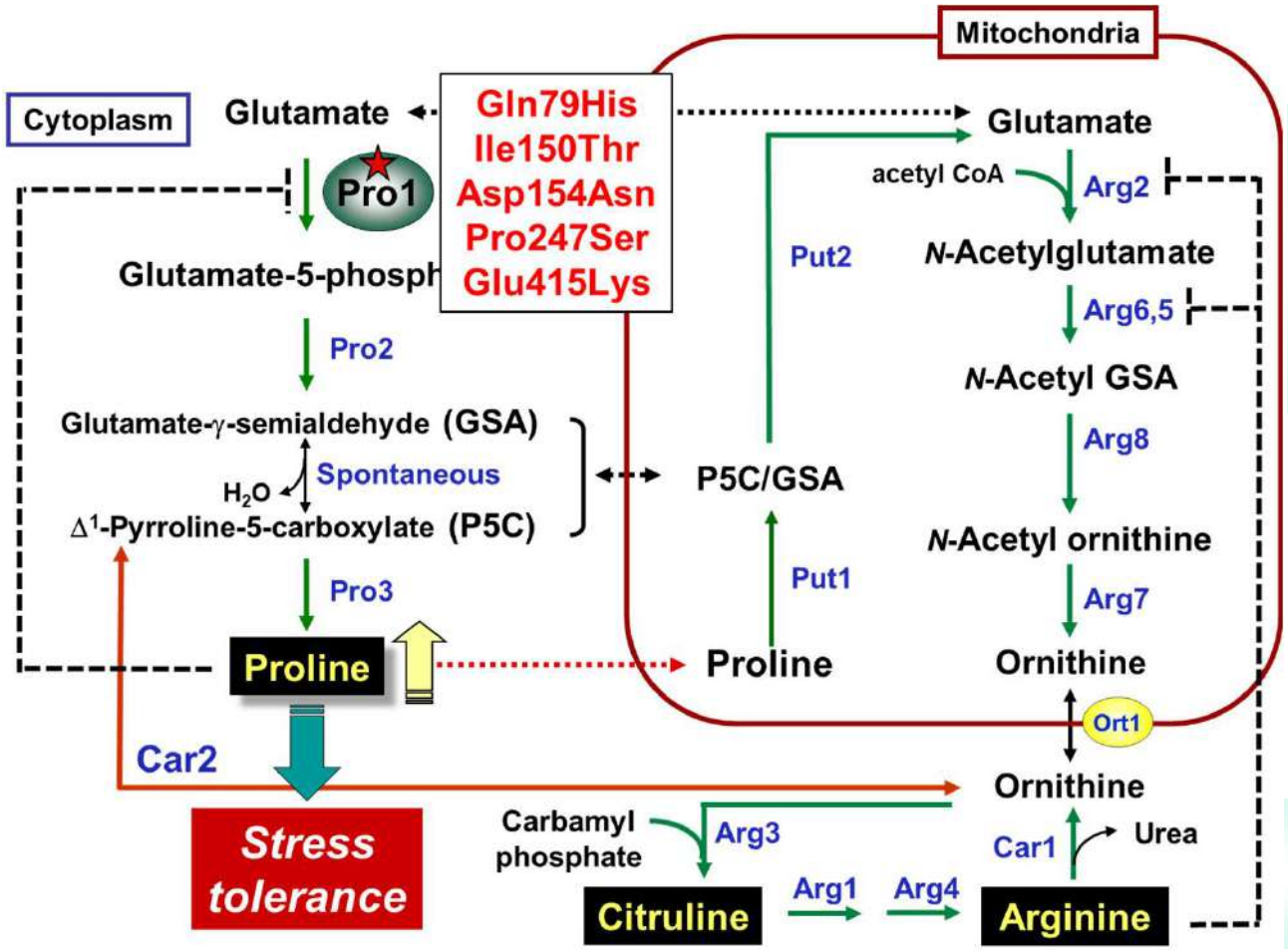
Proline

< So far >

Appl. Microbiol. Biotechnol., **47**, 405, 1997; *ibid.*, **79**, 273, 2008; *ibid.*, **81**, 211, 2008; ***FEMS Microbiol. Lett.***, **184**, 103, 2000; ***Appl. Environ. Microbiol.***, **69**, 212, 2003; *ibid.*, **69**, 6527, 2003; *ibid.*, **71**, 8656, 2005; *ibid.*, **73**, 4011, 2007; *ibid.*, **74**, 5845, 2008; ***J. Biosci. Bioeng.***, **94**, 2002; *ibid.*, **100**, 538, 2005; *ibid.*, **103**, 277, 2007; *ibid.*, **116**, 576, 2013; ***Biosci. Biotechnol. Biochem.***, **73**, 2131, 2009; *ibid.*, **76**, 454, 2012; ***Int. J. Food Microbiol.***, **152**, 40, 2012; *ibid.*, **238**, 233, 2016; ***J. Gen. Appl. Microbiol.***, **62**, 132, 2016; ***Yeast***, **33**, 353, 2016; ***FEBS Lett.***, **590**, 2906, 2016; ***Microbial Cell***, **3**, 522, 2016; *ibid.*, **6**, 482, 2019; ***J. Ind. Microbiol. Biotechnol.***, **47**, 715, 2020; ***Microorganisms***, **9**, 1650, 2021; *ibid.*, **9**, 1902, 2021.

★ Proline protects yeast cells from various stresses as a ROS scavenger *!!*

★ Removal of feedback inhibition of γ-glutamyl kinase (Pro1) enhances proline accumulation and stress tolerance *!!*



< Current projects >

- Regulation of cell lifespan
  (Mitochondria energy metabolism)
- Inhibition of proline utilization
  (Arginine-mediated mechanism)

GOLDEN -Rabbit-

Kaguyama 'Sweet' beer

Breeding of novel yeast strains with '**proline engineering**'

*What is **sake** ?*

Sake, Japanese rice wine, is a traditional alcoholic beverage made from rice through fermentation and filtration. Sake is labeled with the word ***Seishu*** (清酒: refined alcohol).

Shōryaku-ji (Nara)

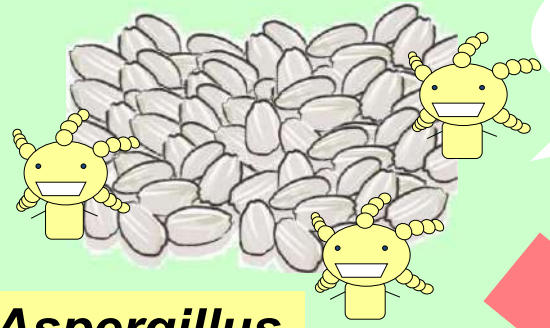
Kounoike (Itami)

Sake (18-20% Ethanol)

multiple parallel fermentations

How to make **sake** !!

Rice starch



Degrade starch into sugars !!

Aspergillus oryzae

EtOH

Yummy

Saccharomyces cerevisiae

EtOH

EtOH **Sugars**

EtOH

Produce ethanol !!

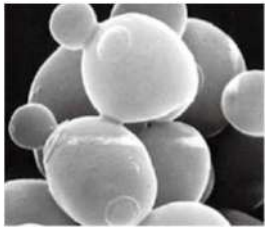
Yummy

Yummy

SAKE

Ethanol
makes us feel drunk

Aromas and flavors
smell like fruits

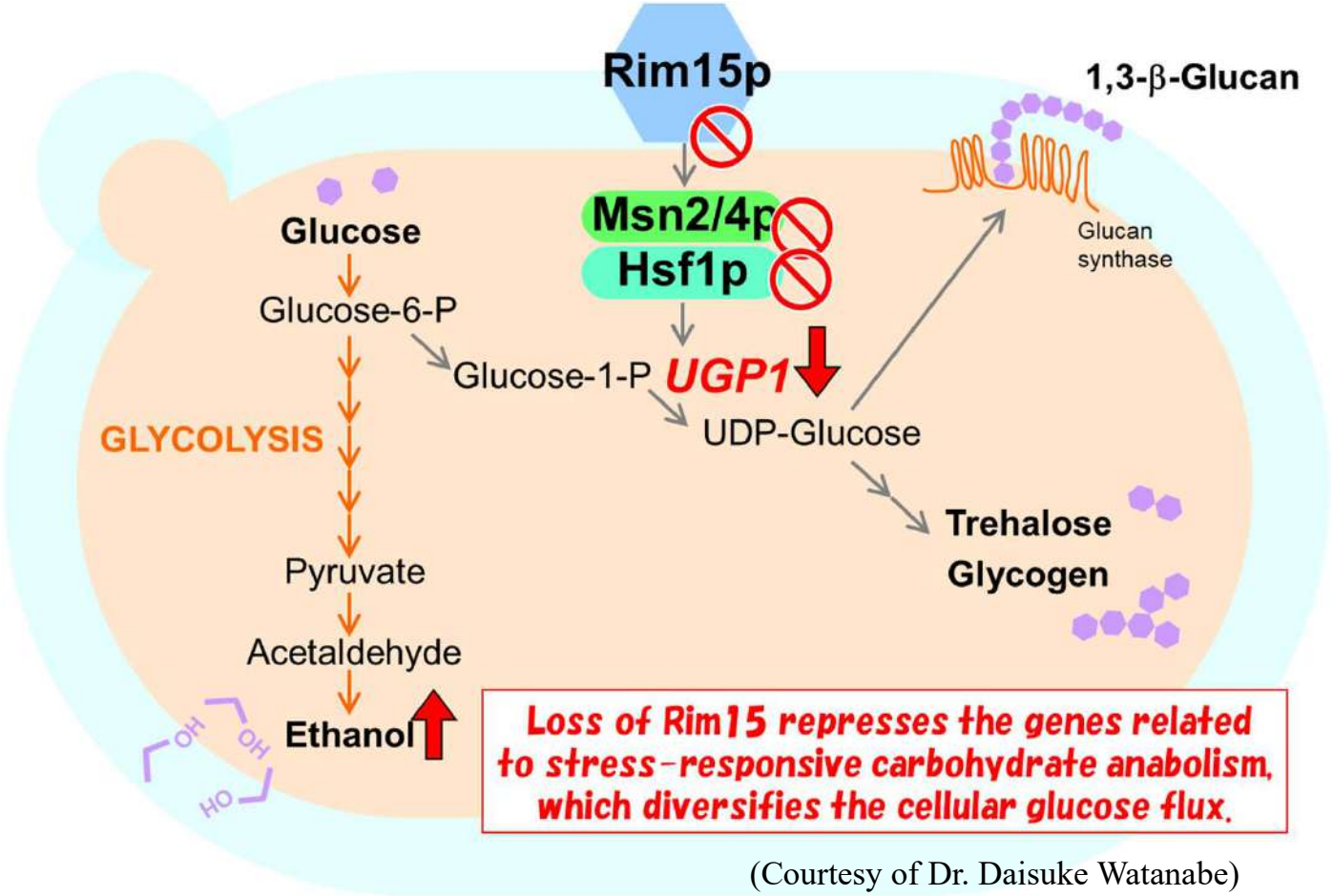


What is **sake yeast** ?

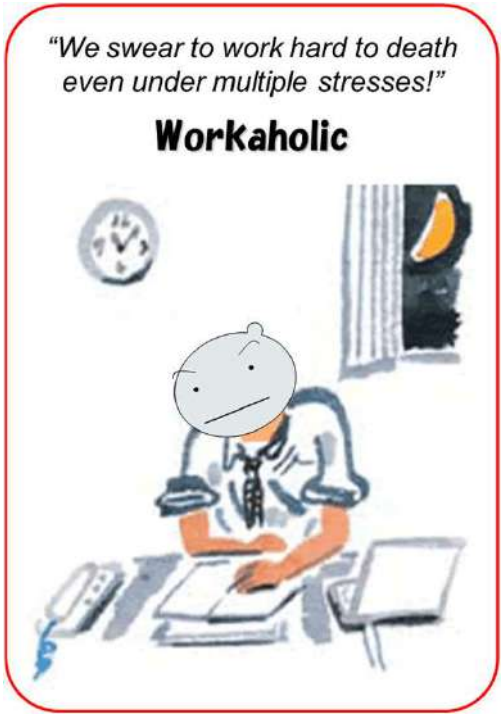
Appl. Environ. Microbiol., **77**, 934, 2011; **78**, 385, 2012; **78**, 4008, 2012; **82**, 340, 2016.
J. Biosci. Bioeng., **112**, 44, 2011; **112**, 577, 2011; **116**, 591, 2013; **123**, 183, 2017. *etc*.

We expected that sake yeast cells have “special” genes or mutations to enhance ethanol stress tolerance, like a “**superhero**”, however, sake yeast cells are like “**workaholic**”, because they gave up the genes to protect themselves, such as ***MSN4*** and ***RIM15***, which inhibit ethanol production.

Dr. Daisuke Watanabe



(Courtesy of Dr. Daisuke Watanabe)



Proline *increased fermentation ability in sake brewing.*

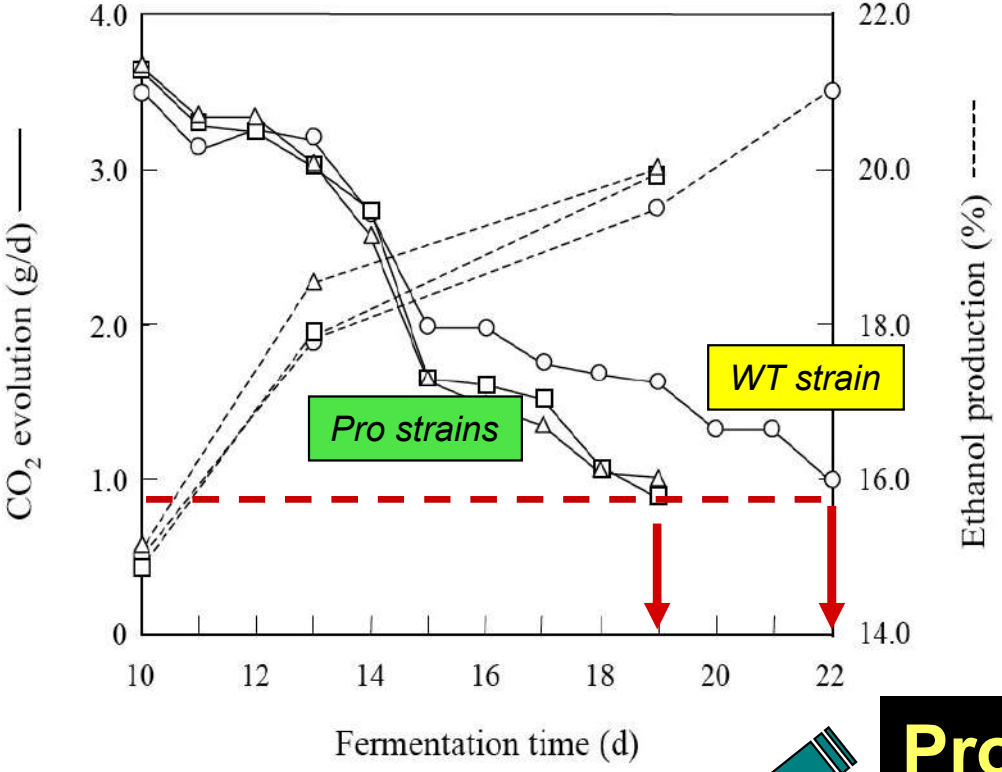
Self-cloning **sake yeast** with **proline** accumulation

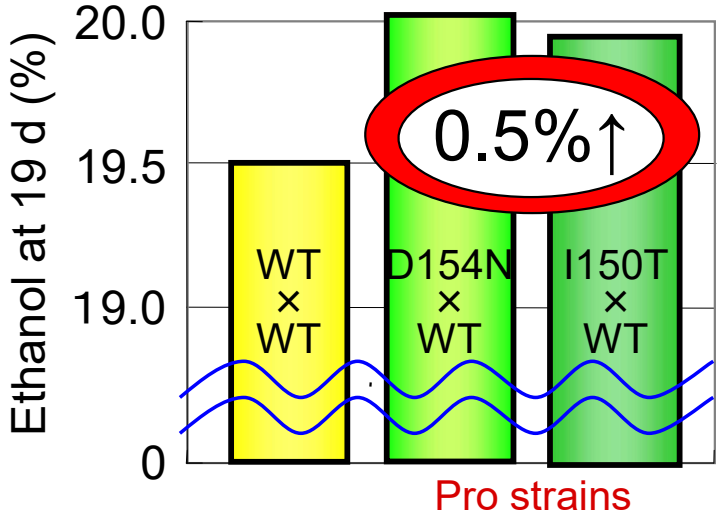
Takagi *et al.*, ***J. Biosci. Bioeng.***, **103**, 377, 2007.

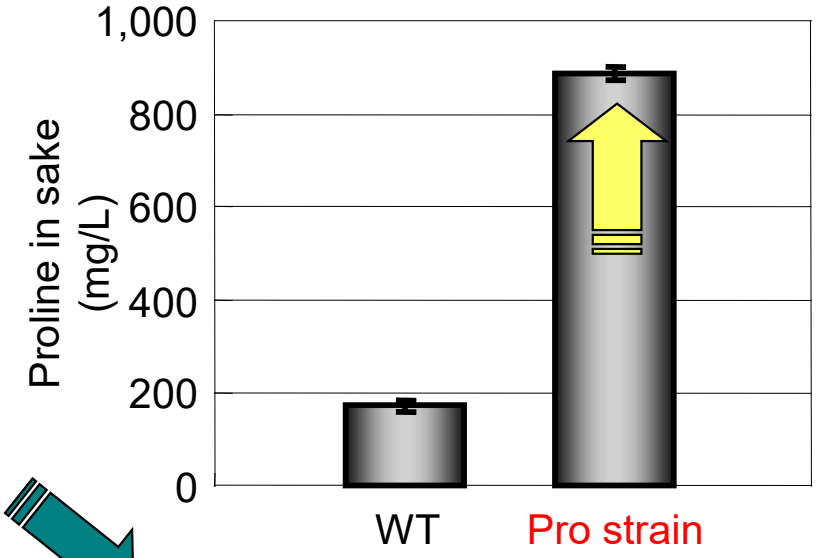
Collaboration with Dr. Shimoi (NRIB)

Brewing condition

- Steamed rice: 160 g
- Koji rice: 40 g
- Water added in three steps: 260 ml
- Temp.: 15℃ (n=3)







Proline

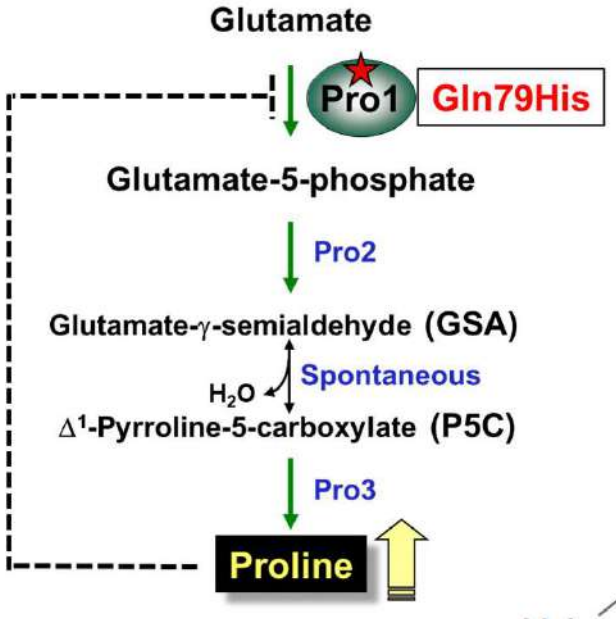
Improves ethanol productivity ?

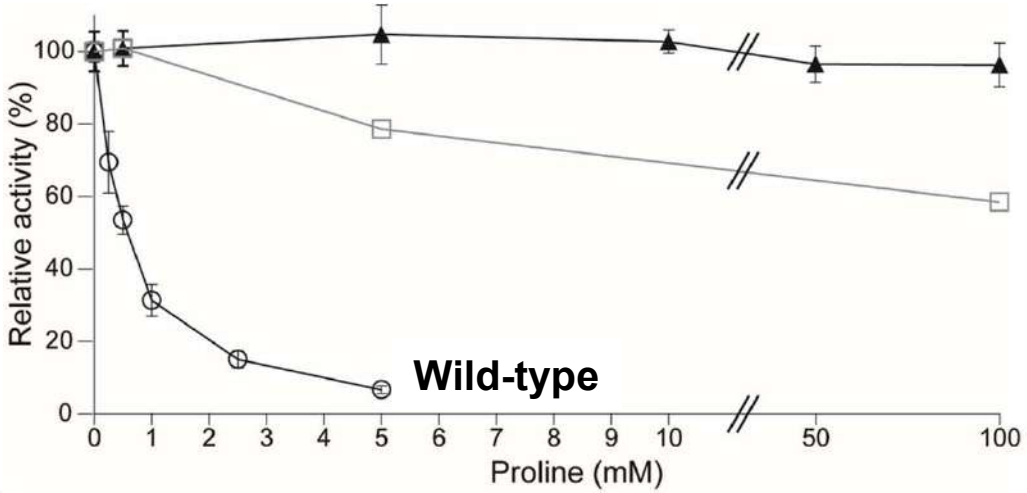
Reduces fermentation time ?

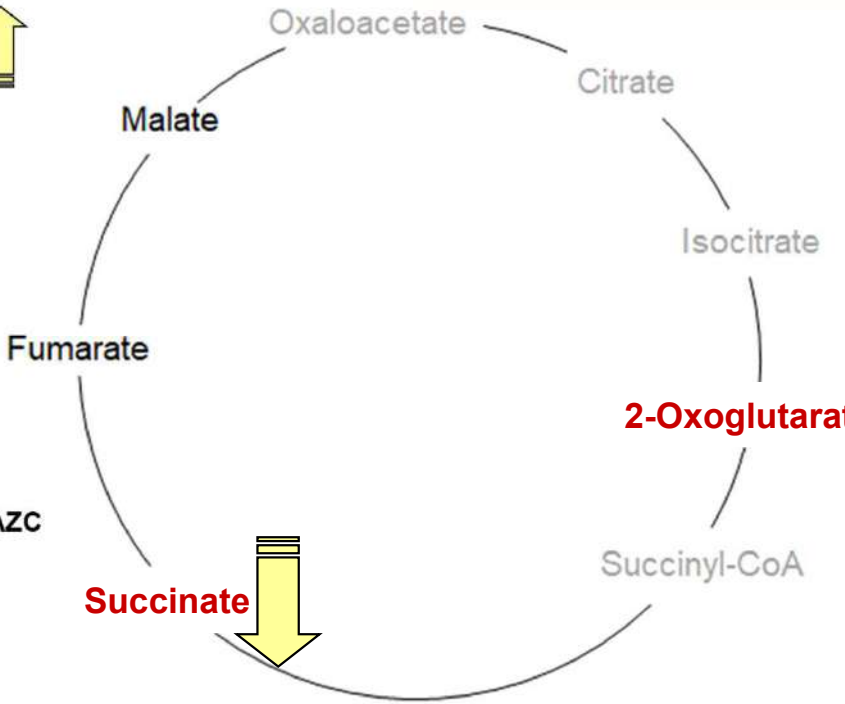
Proline may contribute to the diversity of sake taste.

Proline-accumulating **sake yeast** mutant **K-9-AZC**

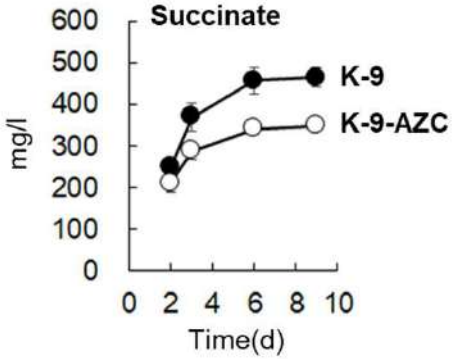
Collaboration with Gekkeikan Sake

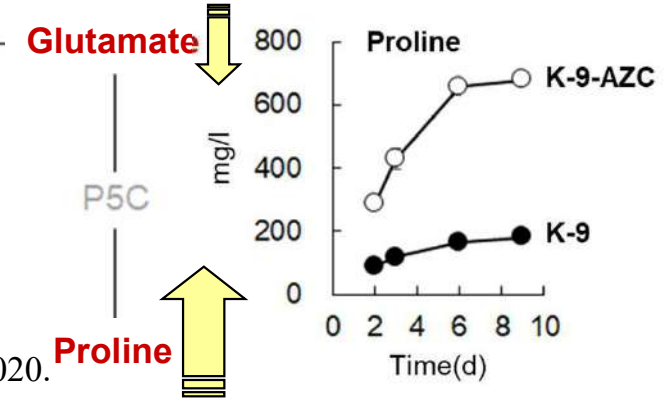






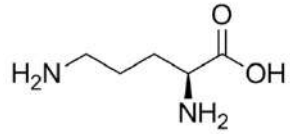
K-9-AZC increases proline and decreases succinate in sake.





Murakami *et al.*, ***J. Ind. Microbiol. Biotech.***, **47**, 715, 2020.

“Healthy image” sake containing a high level of ornithine



L-Ornithine

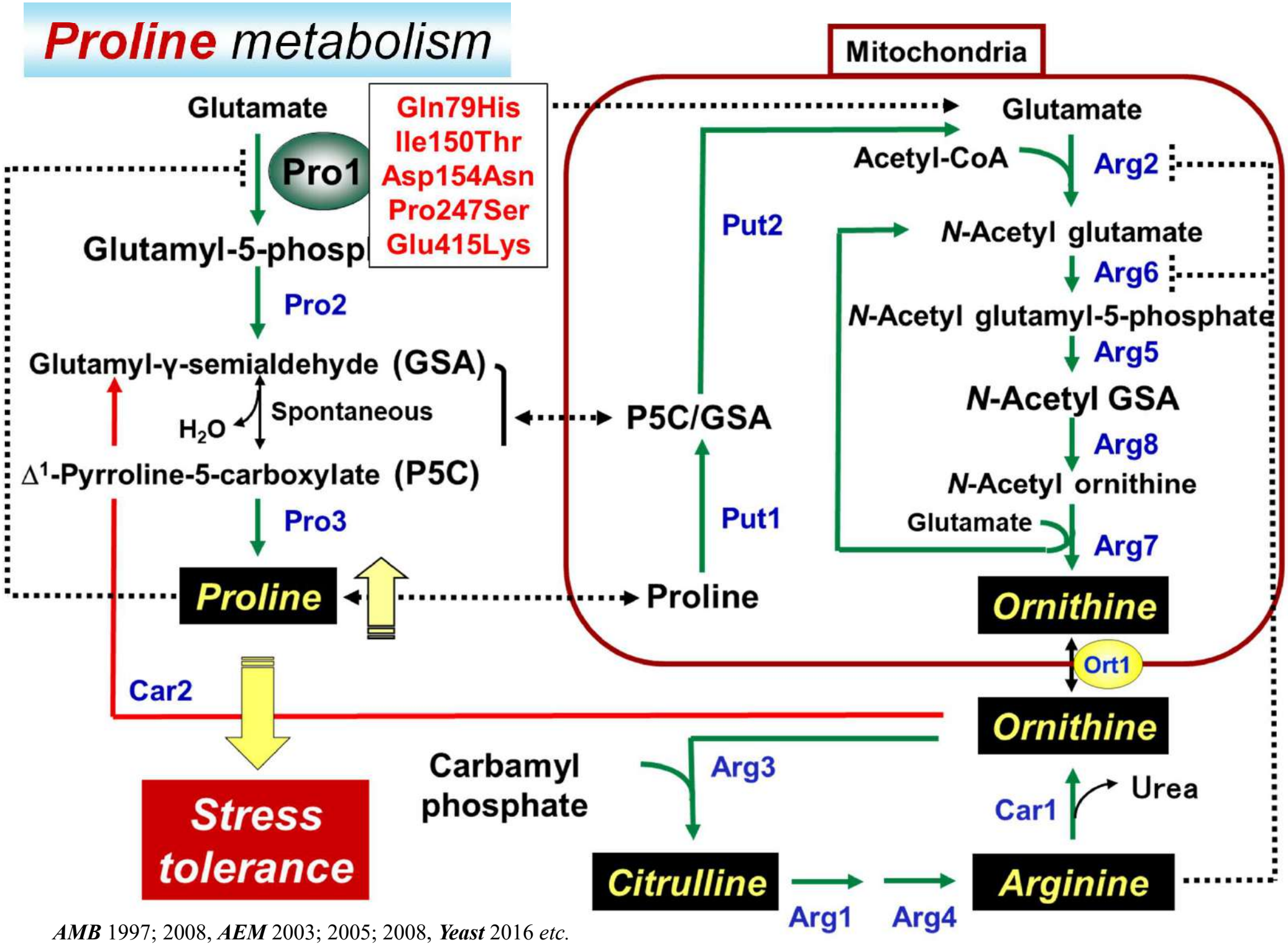
Shōryaku-ji (正暦寺)

Birthplace of Japanese sake

Bodaimoto (菩提酛)

Collaboration of yeast and lactic acid bacteria

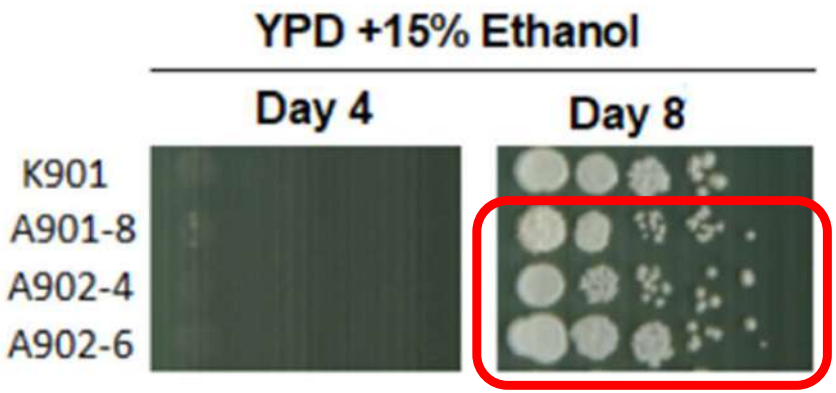
奈良県産業振興総合センター
Nara Prefecture Institute of Industrial Development
Masataka Ohashi
(Nara Pref Inst Ind Dev)



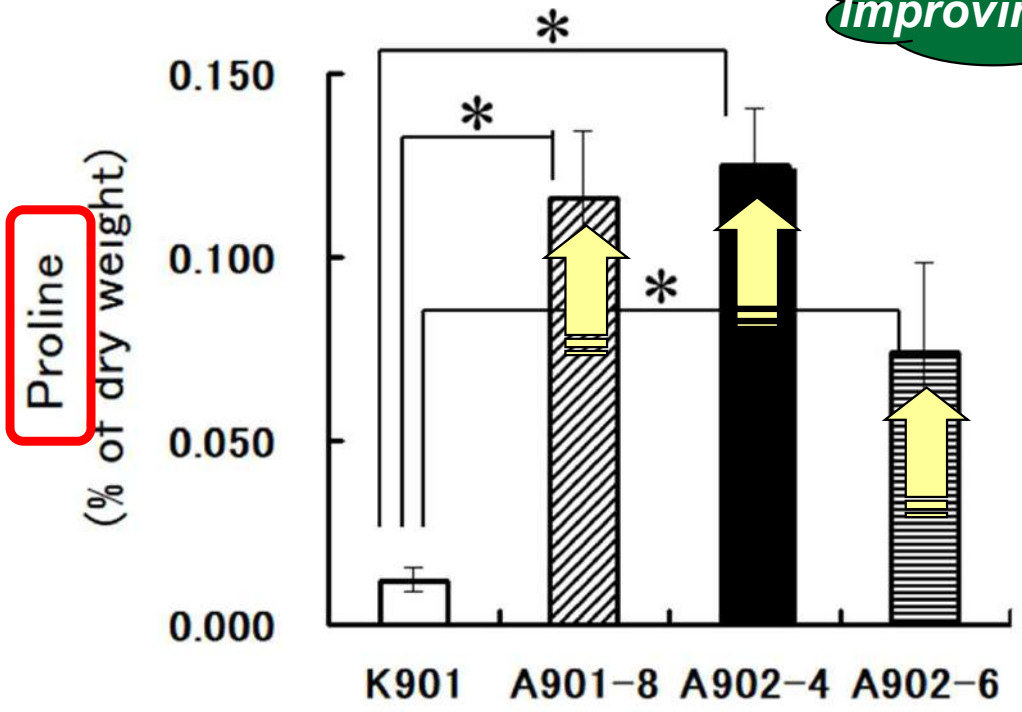
AMB 1997; 2008, *AEM* 2003; 2005; 2008, *Yeast* 2016 *etc.*

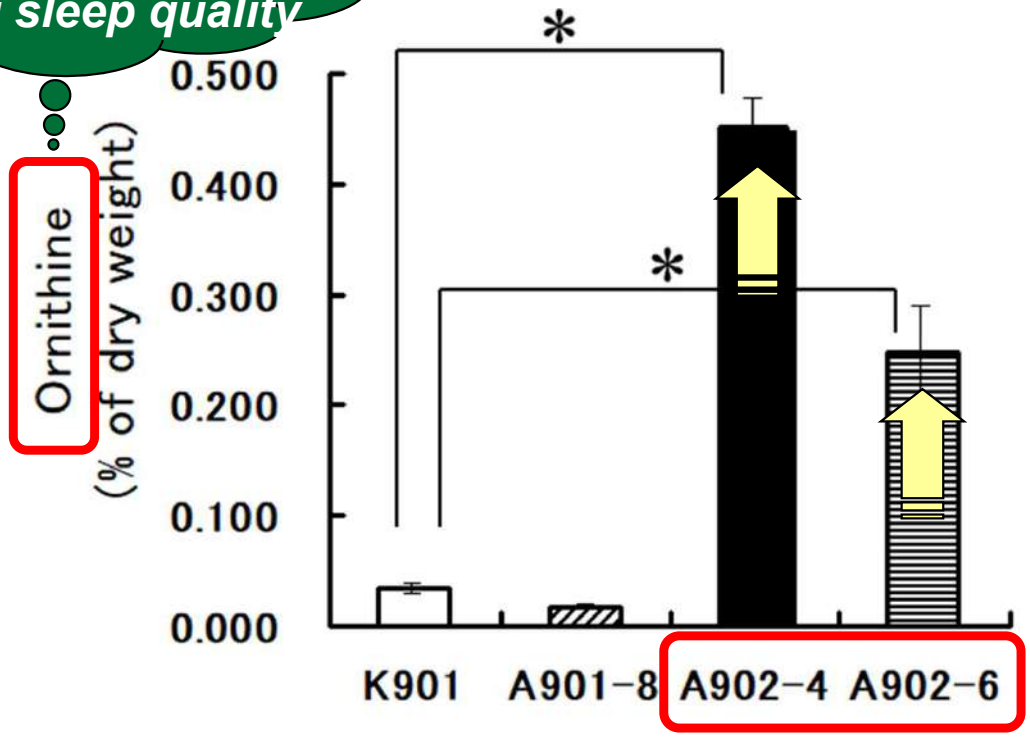
*Isolation of sake yeast mutants with **proline** accumulation*

Ohashi *et al.*, ***Metab. Eng.***, **62**, 1, 2020.



Reducing fatigue
Improving sleep quality



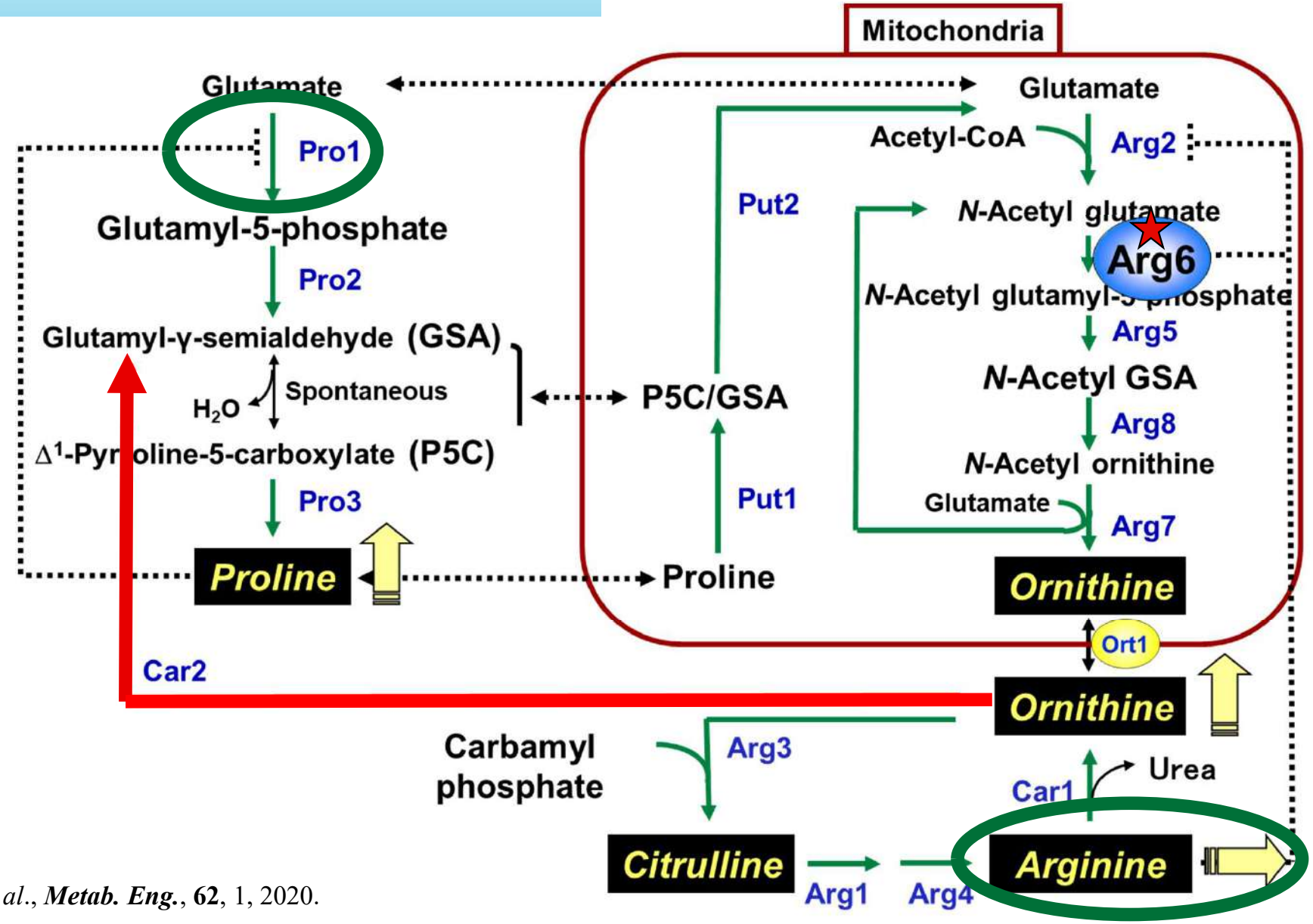


Strain A902-4 accumulated not only **proline** but also **ornithine**

*What is the **Proline** and **Ornithine** accumulation mechanism ?*

Thr340Ile variant Arg6 (*N*-acetyl glutamate kinase)

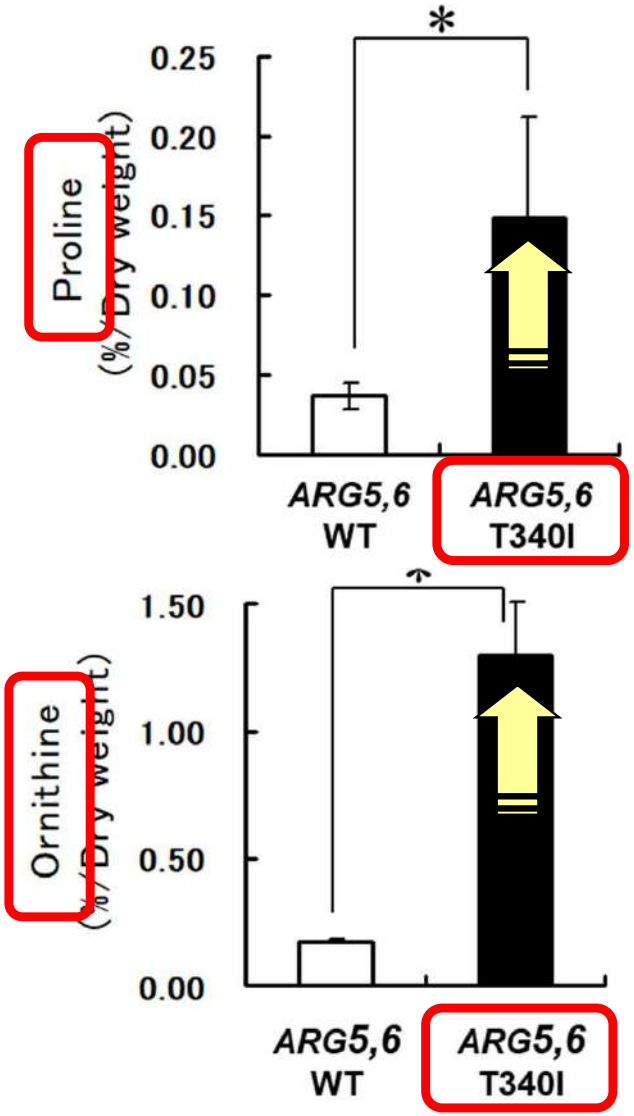
A homo allelic mutation in *ARG6*



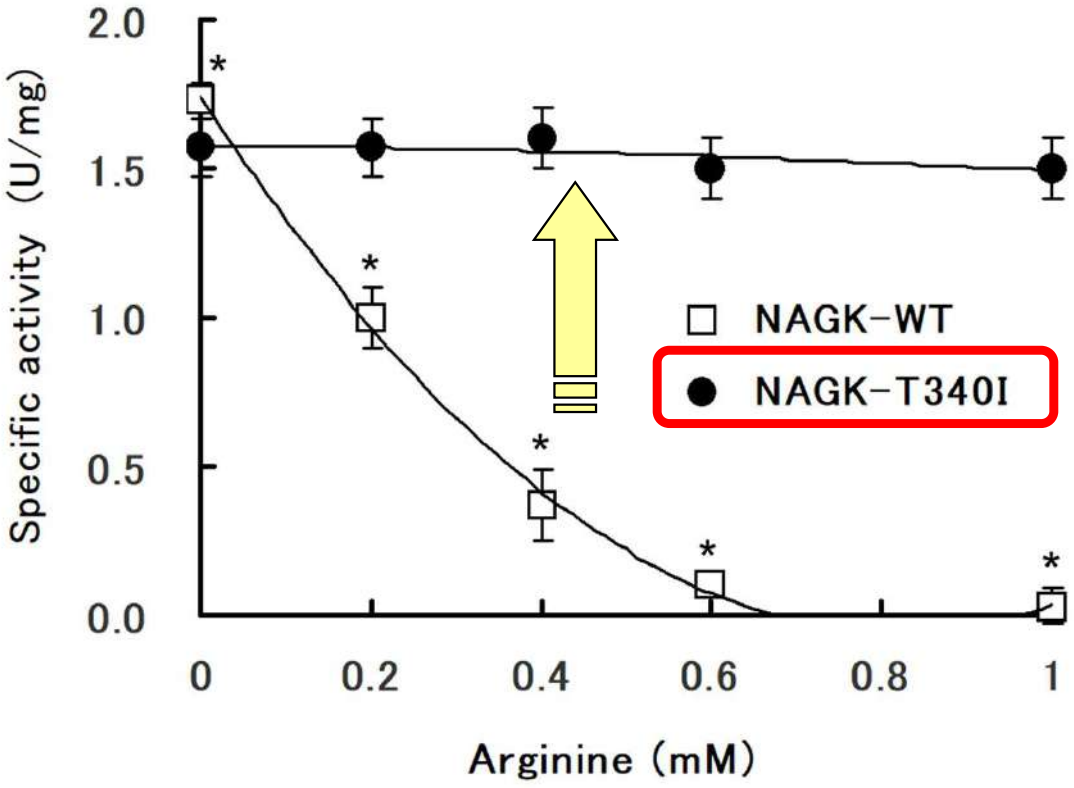
Ohashi *et al*., ***Metab. Eng.***, **62**, 1, 2020.

*Functional analysis of the **NAGK variant***

Ohashi *et al*., ***Metab. Eng.***, **62**, 1, 2020.



***N*-acetyl glutamate kinase (NAGK)**



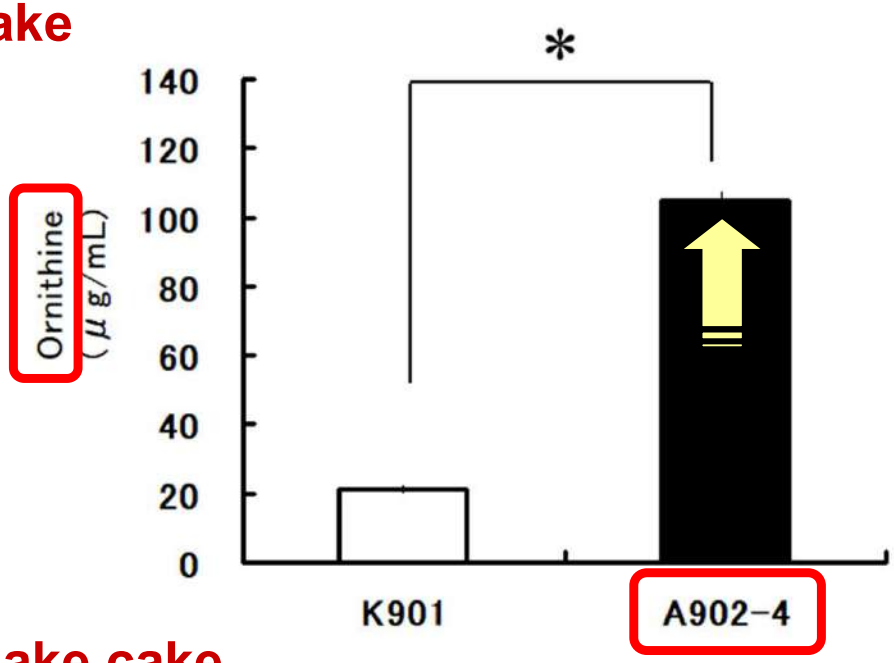
Increases **proline** and **ornithine** levels

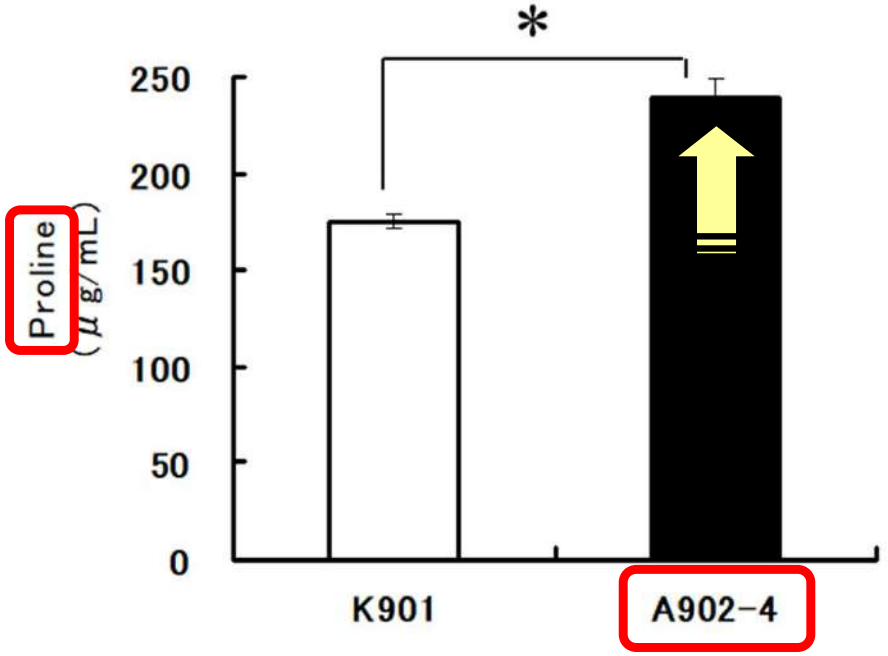
Is insensitive to feedback inhibition by **arginine**

Sake fermentation test

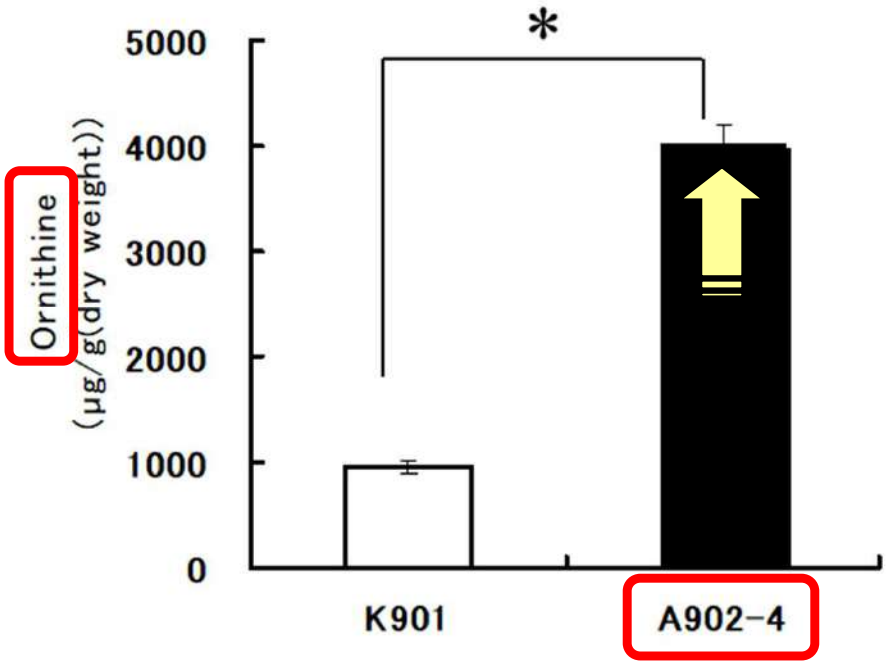
Healthy image *Sake*

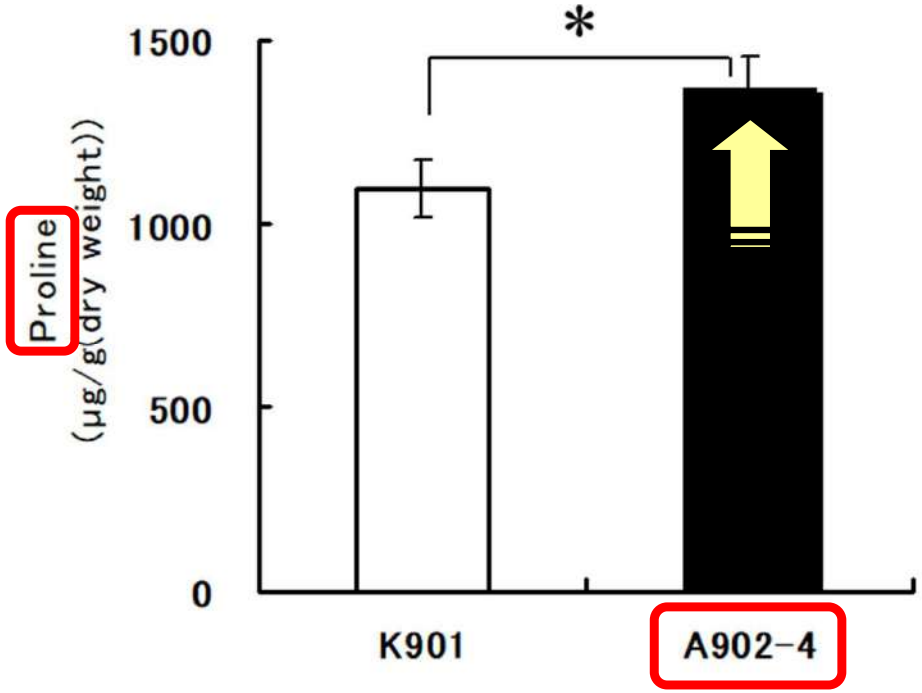
Sake





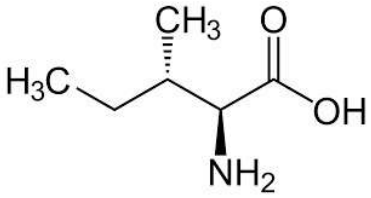
Sake cake





Improvement of isoleucine and 2-methyl-1-butanol production in sake yeast

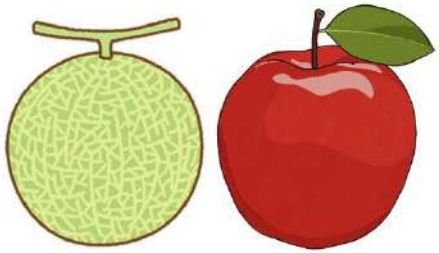
Dr. Akira Nishimura



L-Isoleucine

Dr. Kengo Matsumura

GEKKEIKAN SAKE
KYOTO SINCE 1637



2-Methylbutyl acetate

Dr. Shota Isogai

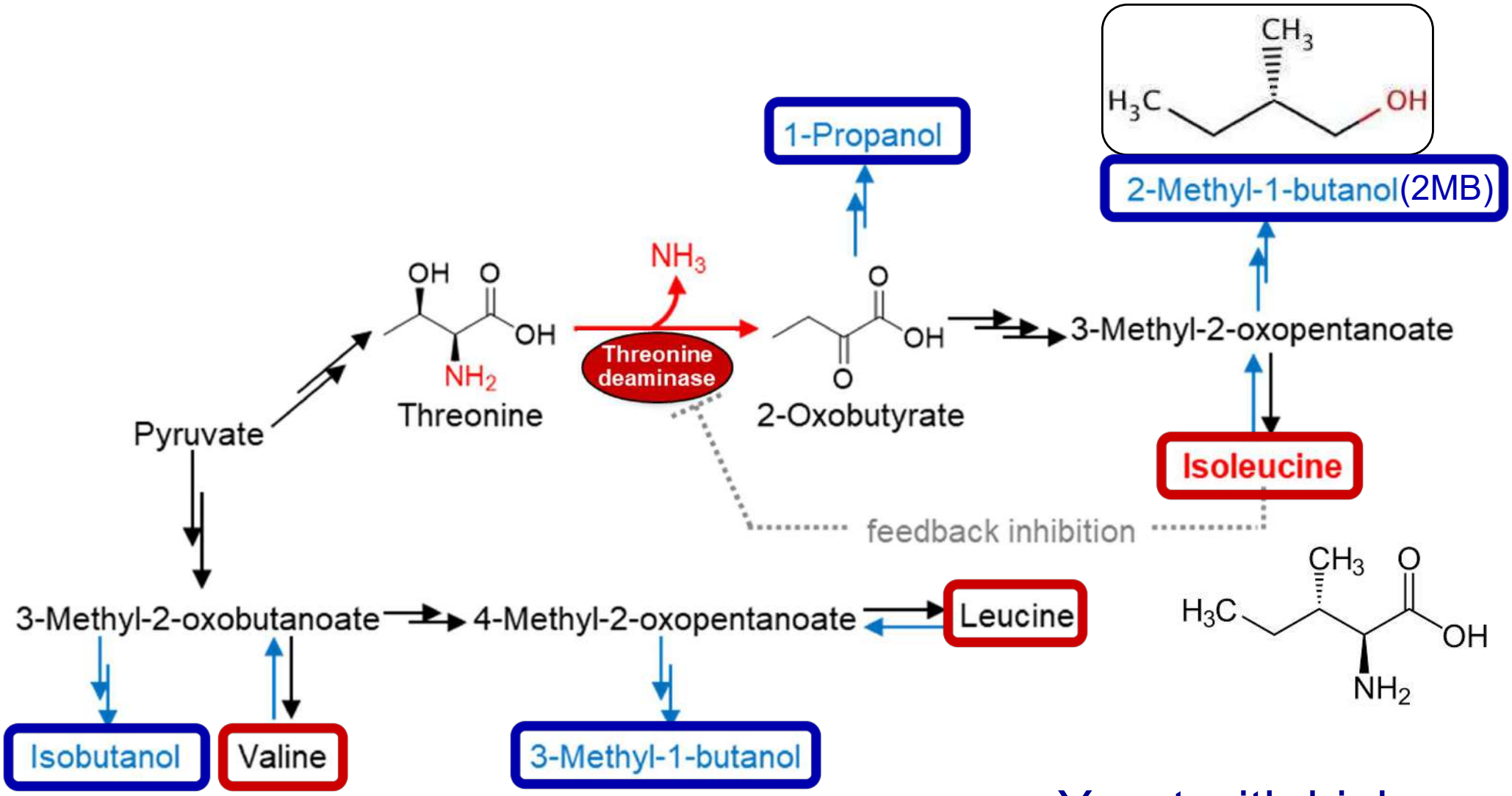
Dr. Atsushi Kotaka

Naoyuki Murakami

Dr. Hiroki Ishida ***Natsuki Hotta***

Isogai *et al*., ***Appl. Environmental. Microbiol***, **88**, e02130-21, 2022.

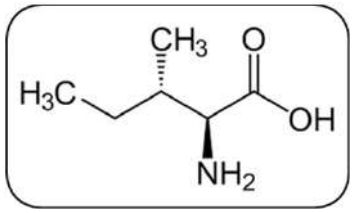
*Metabolic pathways of **BCAAs** and **fusel alcohols***

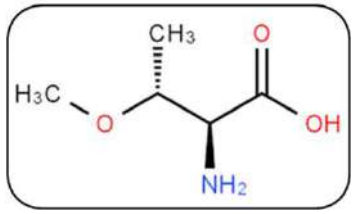


2-Methyl-1-butanol (2MB) and its acetate ester are fruit-like flavors.

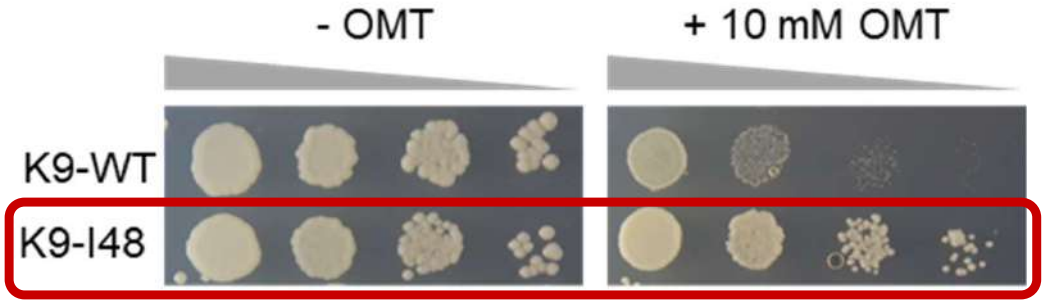
Yeast with higher **Ile** may produce a distinctive *sake*.

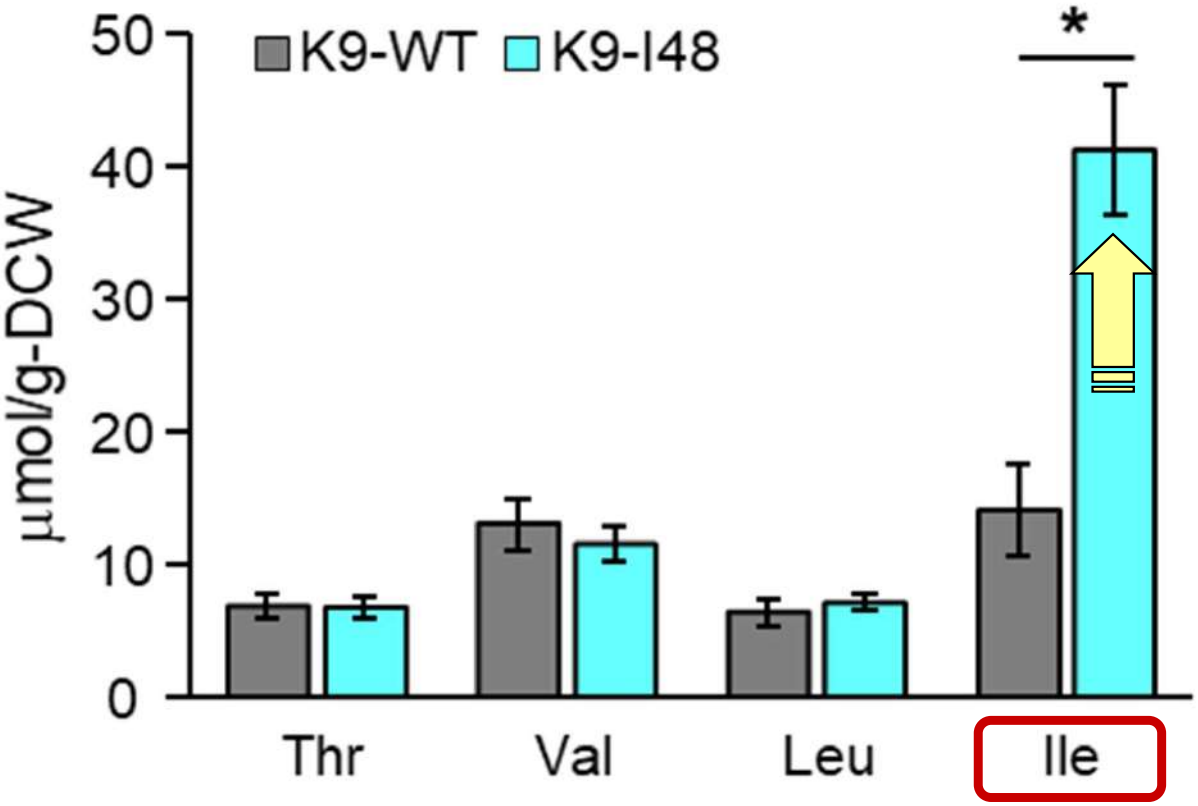
Sake yeast *mutant with* ***Ile*** *accumulation*





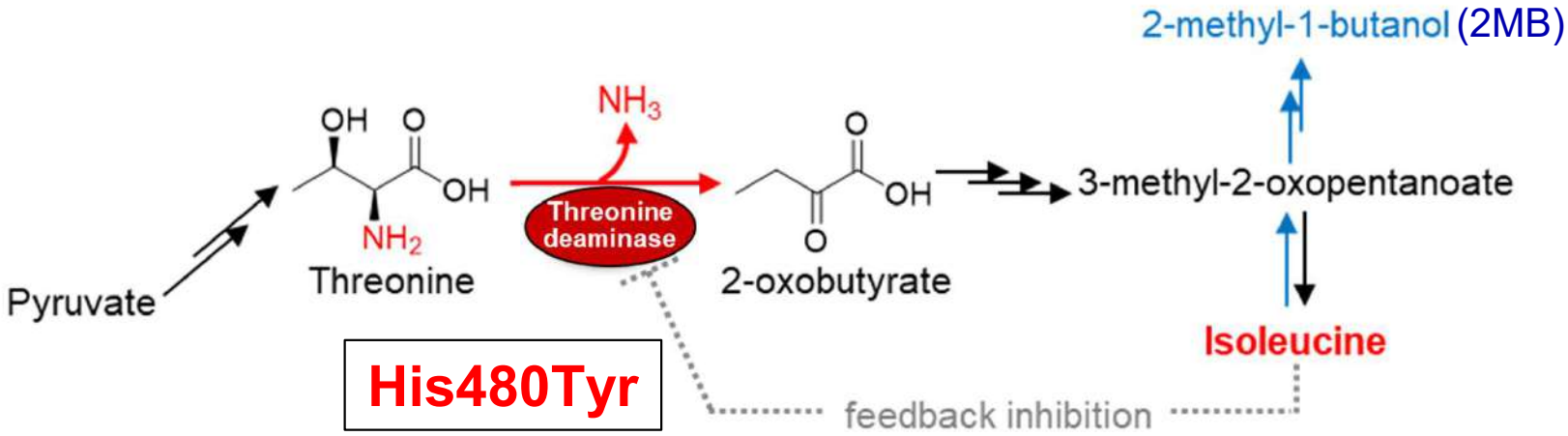
***O*-Methyl threonine (OMT)**



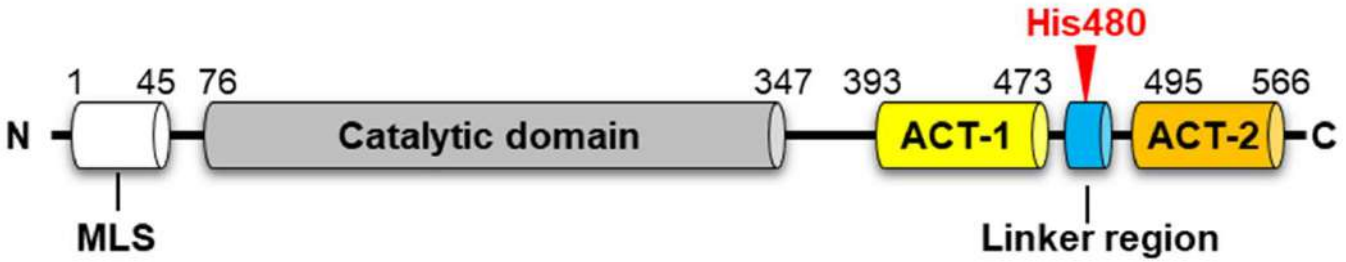


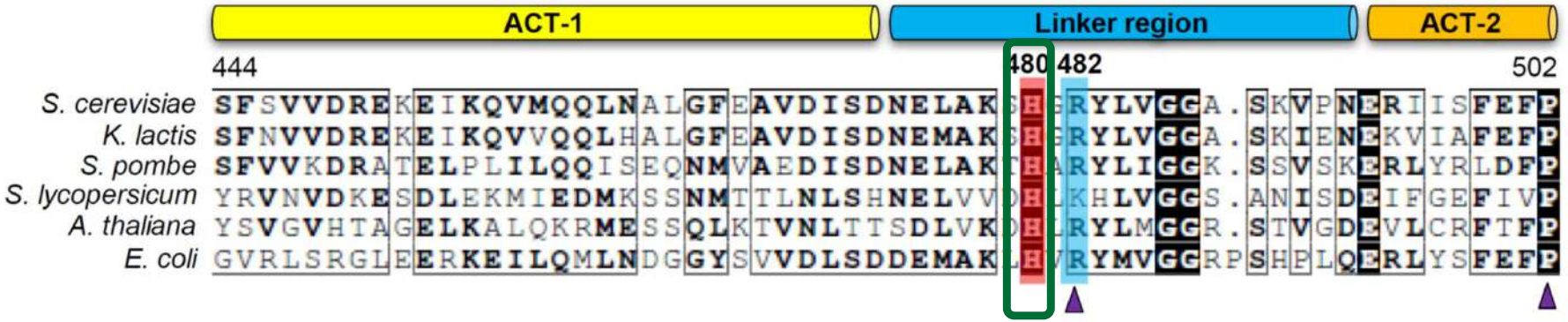
K9-I48 accumulated high amount of **Ile** in the cells.

*Mutation of the **ILV1** gene in **K9-I48***

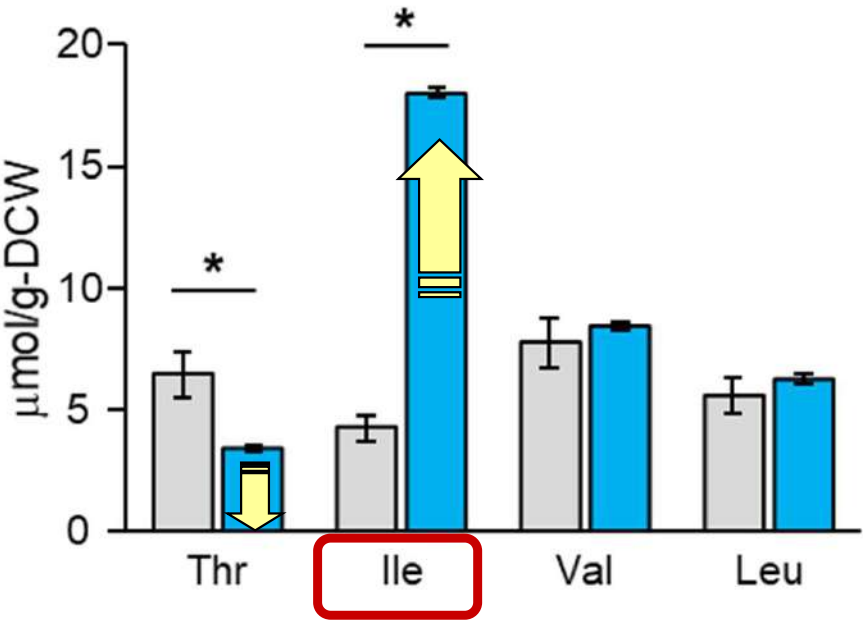


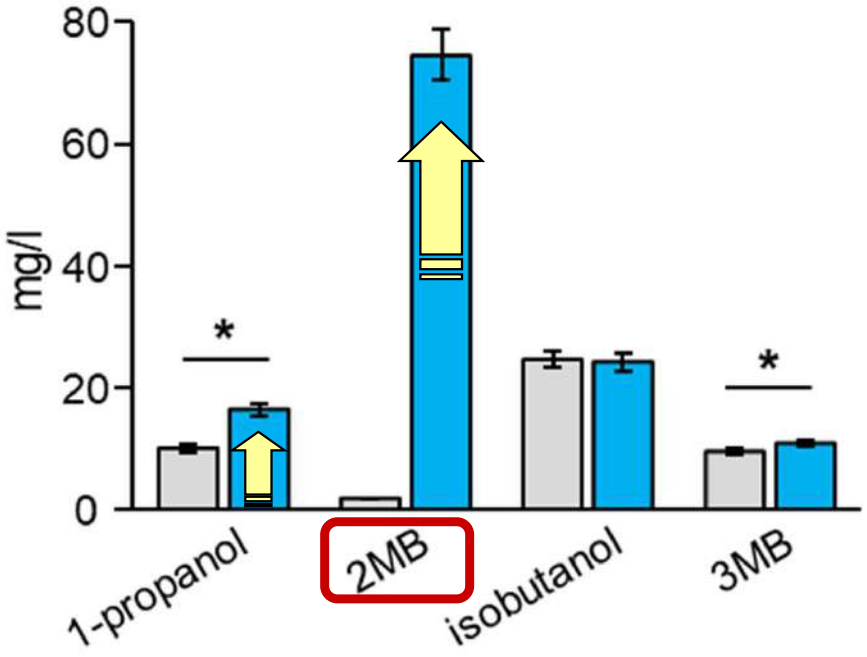
Threonine deaminase (TD; Ilv1)

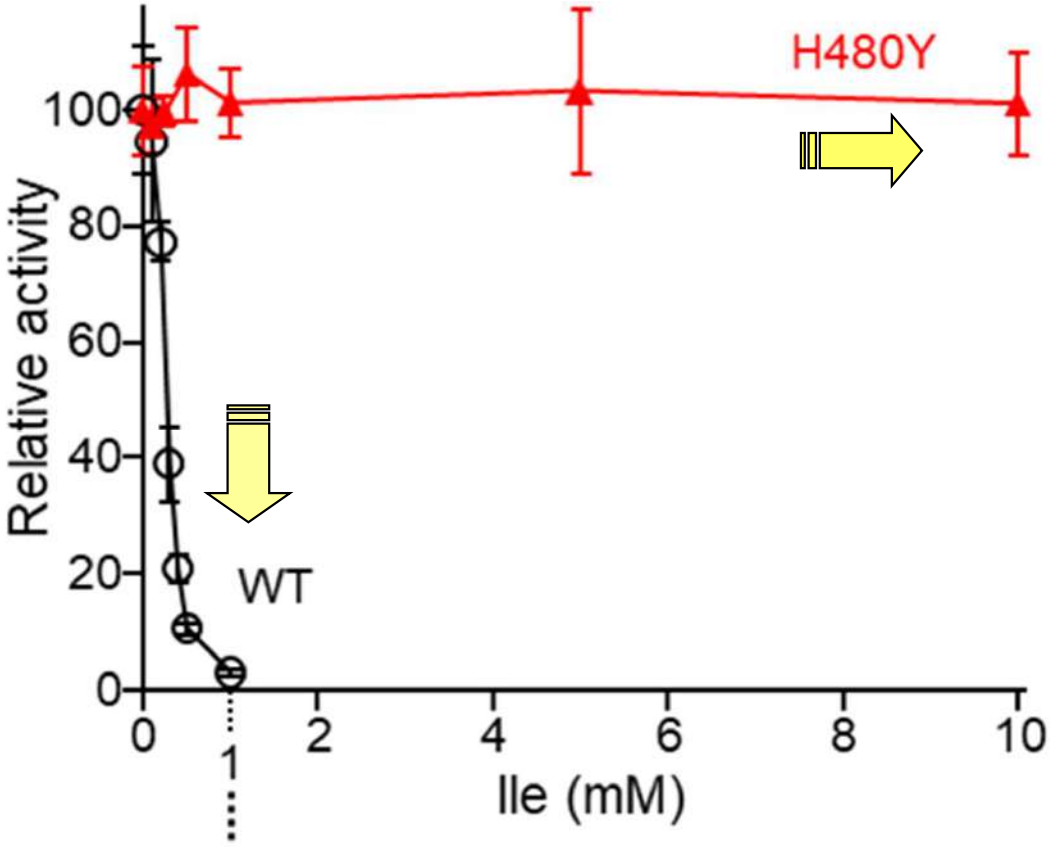




*Functional analysis of the **TD variant***



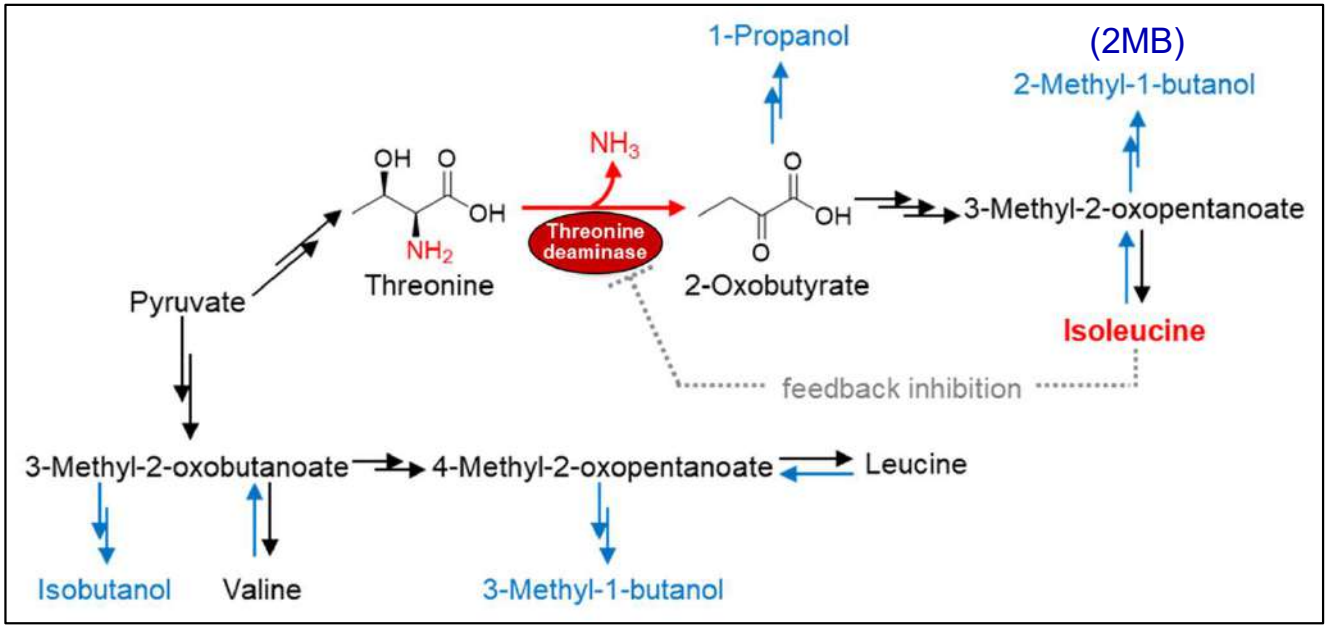




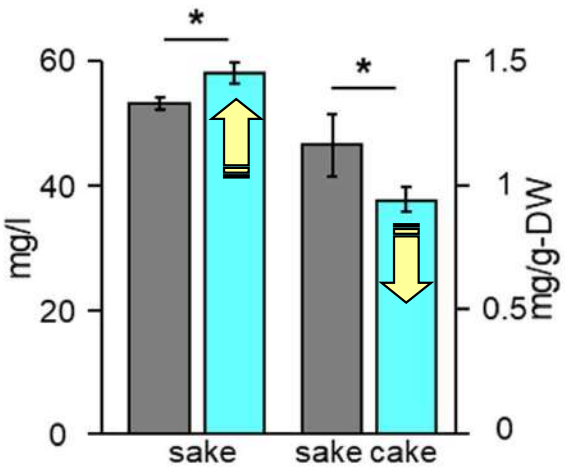
Increases **proline** and **ornithine** levels

Is insensitive to feedback inhibition by **arginine**

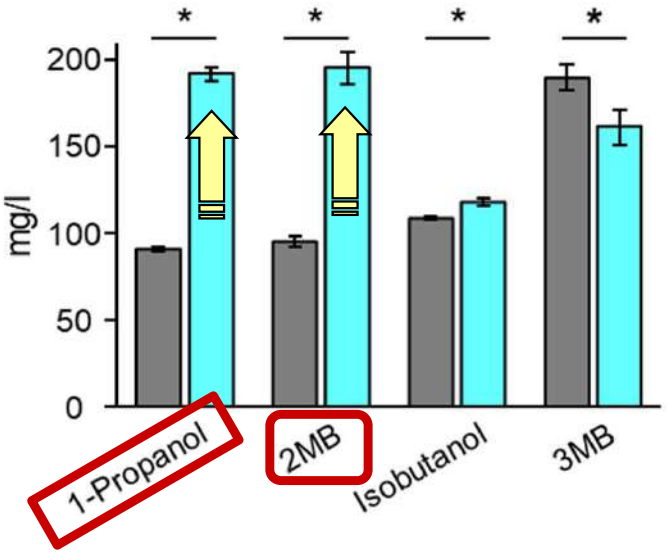
*Characteristics of **sake** brewed with **K9-I48***



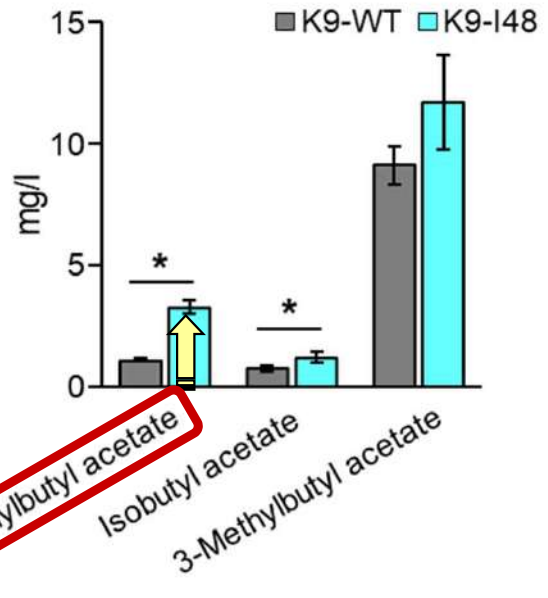
Isoleucine



Fusel alcohol



Acetate ester



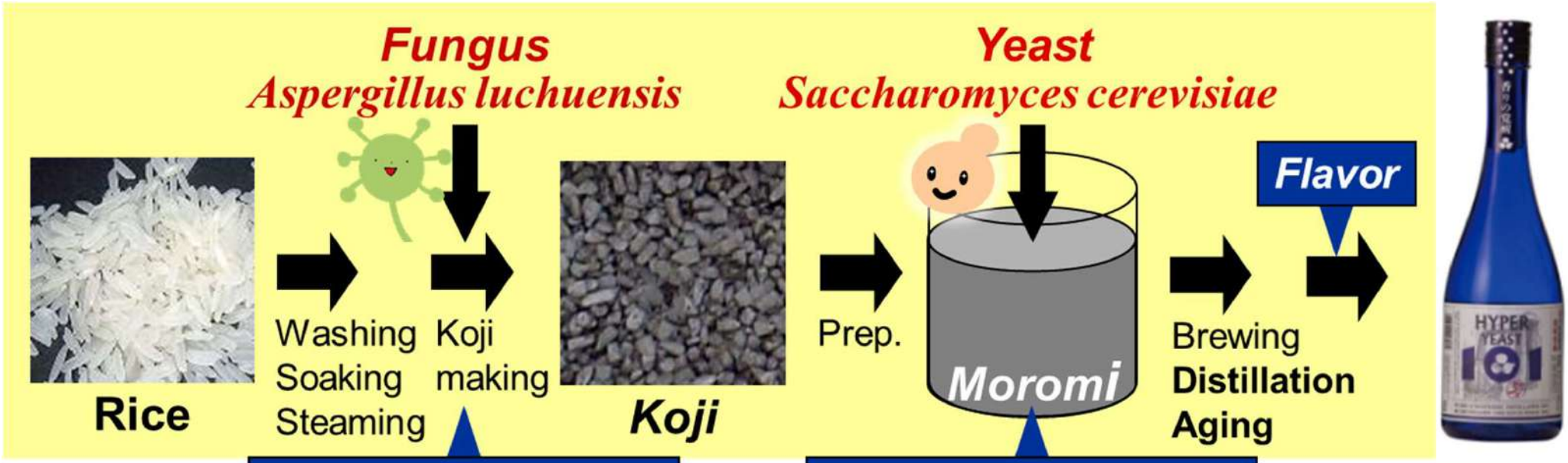
Sake brewed with **K9-I48** contained more **2MB** and **its acetate ester**.

The ***ILV1*** mutants are promising for the creation of new, distinctive *sake* flavors.

*What is **awamori** ?*

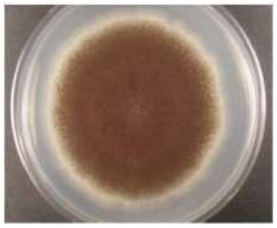
Awaomori（泡盛）, one type of ***shochu***, is a traditional spirits made from steamed rice in Okinawa. The foam (***Awa***) would rise up (***Mori***) during distillation.

***Awamori* (25-45% Ethanol)**



multiple parallel fermentations

How to make **awamori** !!



Rice starch

Degrade starch into sugars !!

Aspergillus luchuensis

Citric acid Ferulic acid

EtOH

Yummy

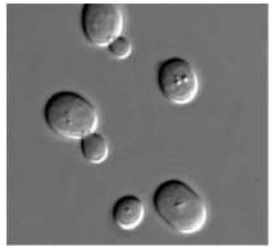
Saccharomyces cerevisiae

EtOH

EtOH

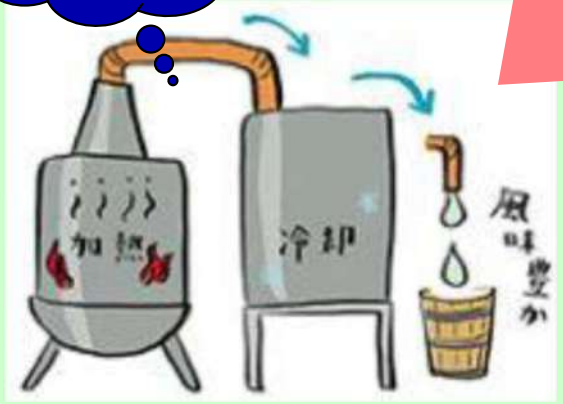
Sugars

EtOH



Produce ethanol !!

Distillation



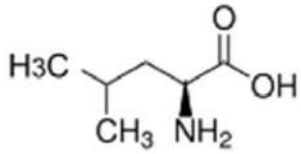
Aging

Ethanol *makes us feel drunk*

AWAMORI

Aromas & flavors *smell like fruits*

“Aromatic” awamori containing a high level of isoamyl acetate



L-Leucine

I love Okinawa

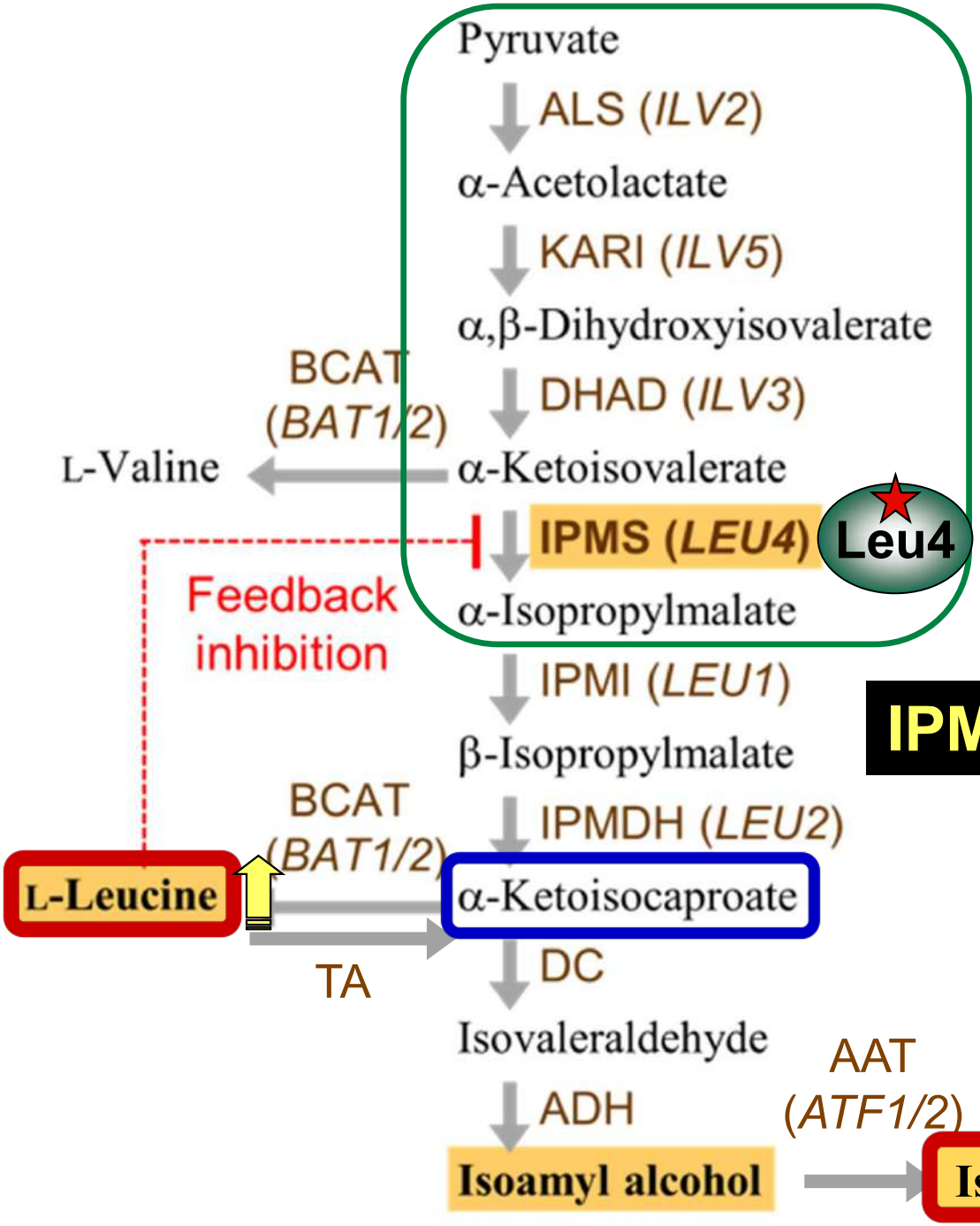
Masatoshi Tsukahara
(BioJet)

*Synthetic pathway of **leucine** and **isoamyl acetate***

Takagi *et al.*, ***J. Biosci. Bioeng.***, **119**, 140, 2015.

Abe *et al.*, ***Front. Genet.***, **10**, 490, 2019.

Tsukahara *et al.*, ***Biosci. Biotech. Biochem.***, **87**, 240, 2023.



α-Isopropylmalate synthase
(**IPMS**: the ***LEU4*** product)
is subject to Leu feedback inhibition.

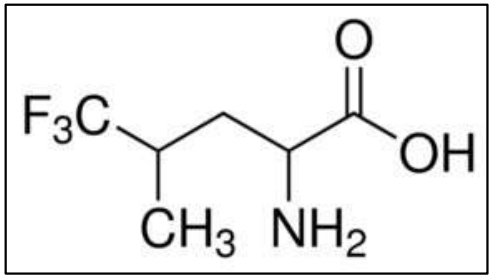
IPMS is the rate-limiting enzyme.

吟醸香 Ginjoko
(Banana-like flavor)

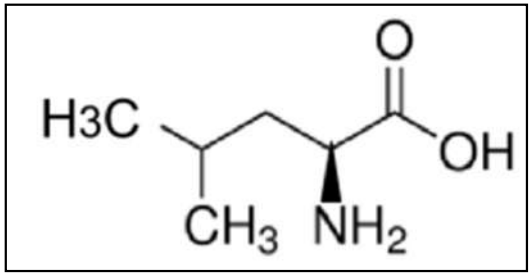
*Isolation of yeast mutants with **leucine** accumulation*

Mechanisms of TFL resistance

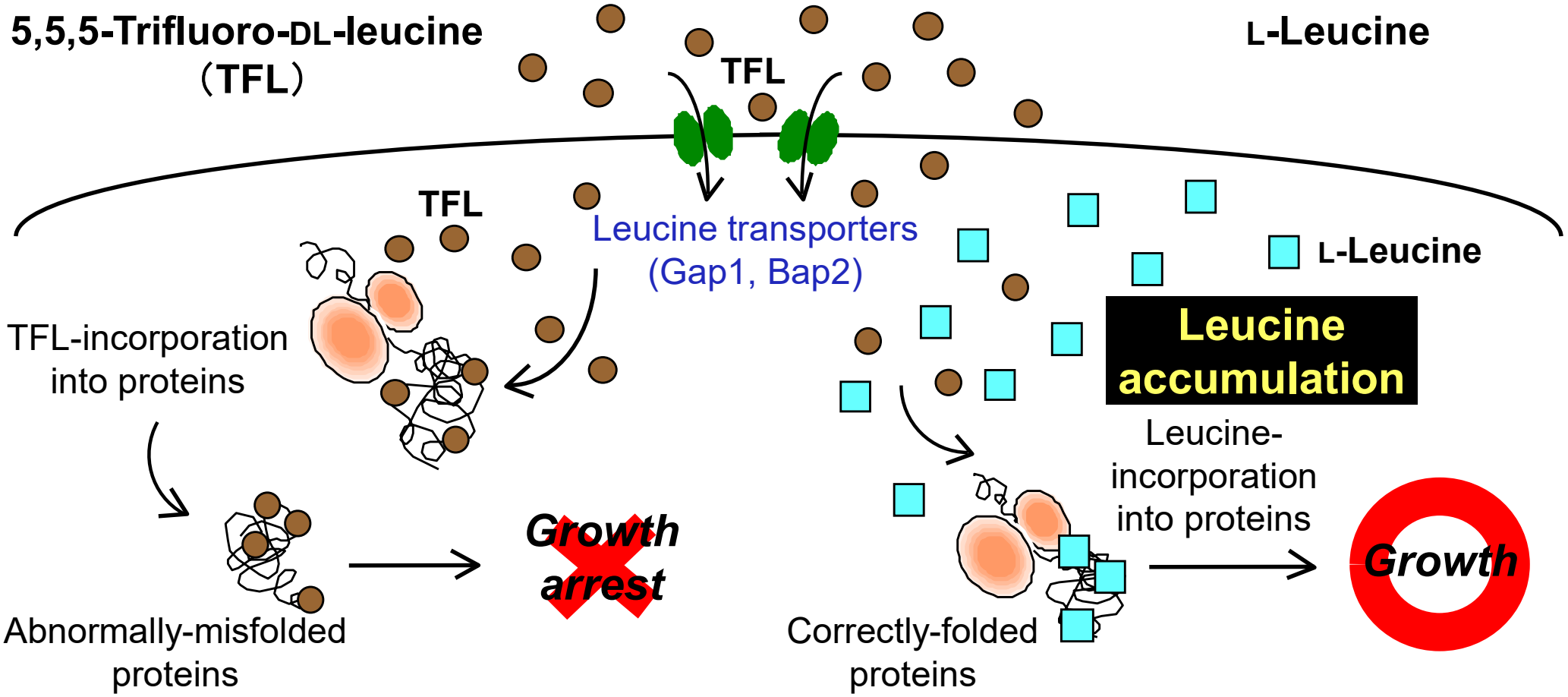
① **Leucine accumulation**
② TFL detoxification
③ Transporters inactivation



5,5,5-Trifluoro-DL-leucine (TFL)



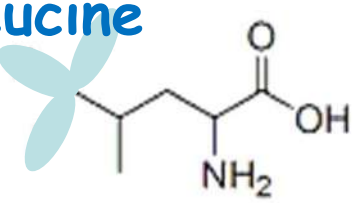
L-Leucine

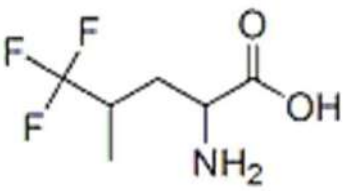


*Isolation of **leucine** overproducing mutants*

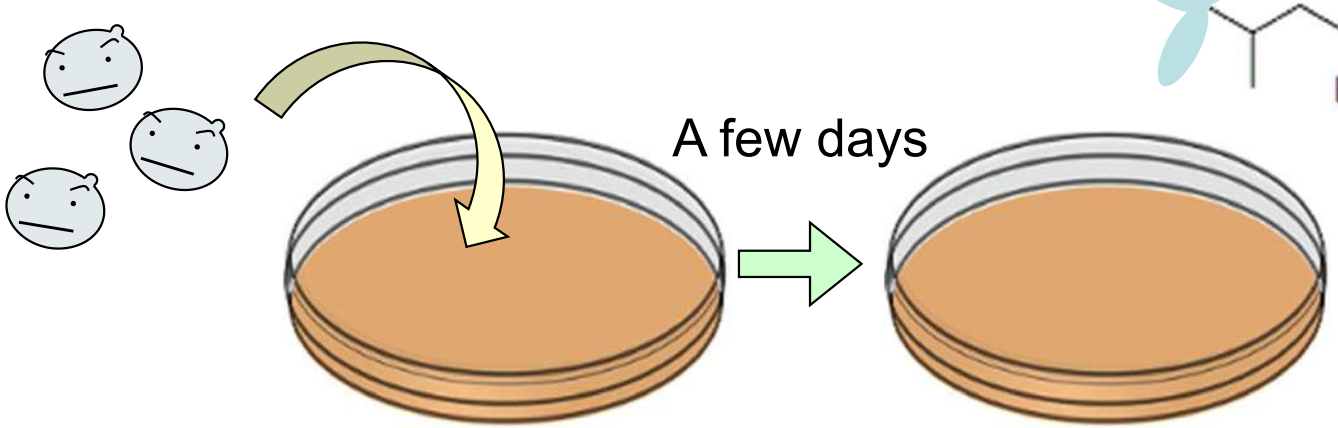
Hibiscus yeast

L-Leucine



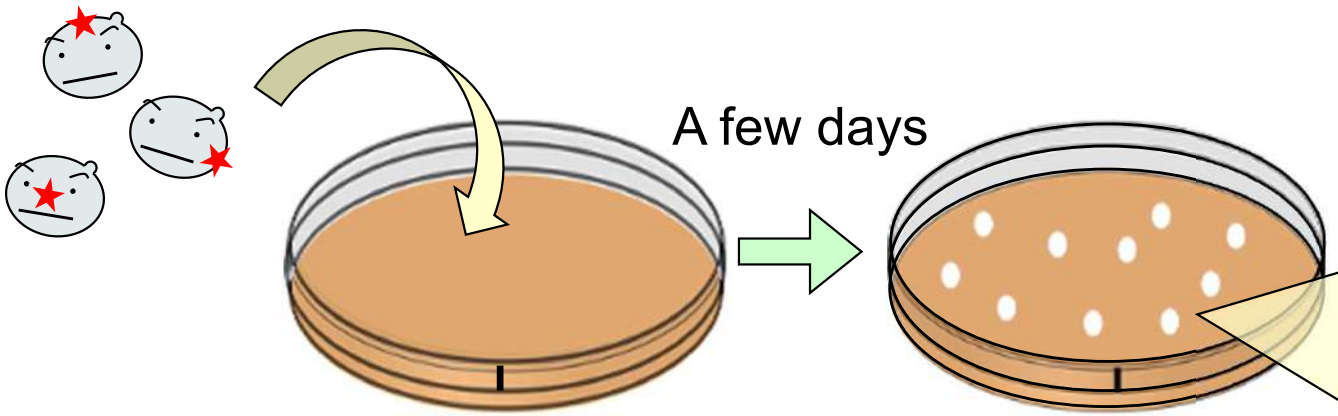


5,5,5-Trifluoro-DL-leucine (TFL)



Minimal medium agar plate containing **TFL** (150 μg/mL)

Mutagen-treated Hibiscus yeast



TFL-resistant colonies

Measure cellular **leucine** level

↓

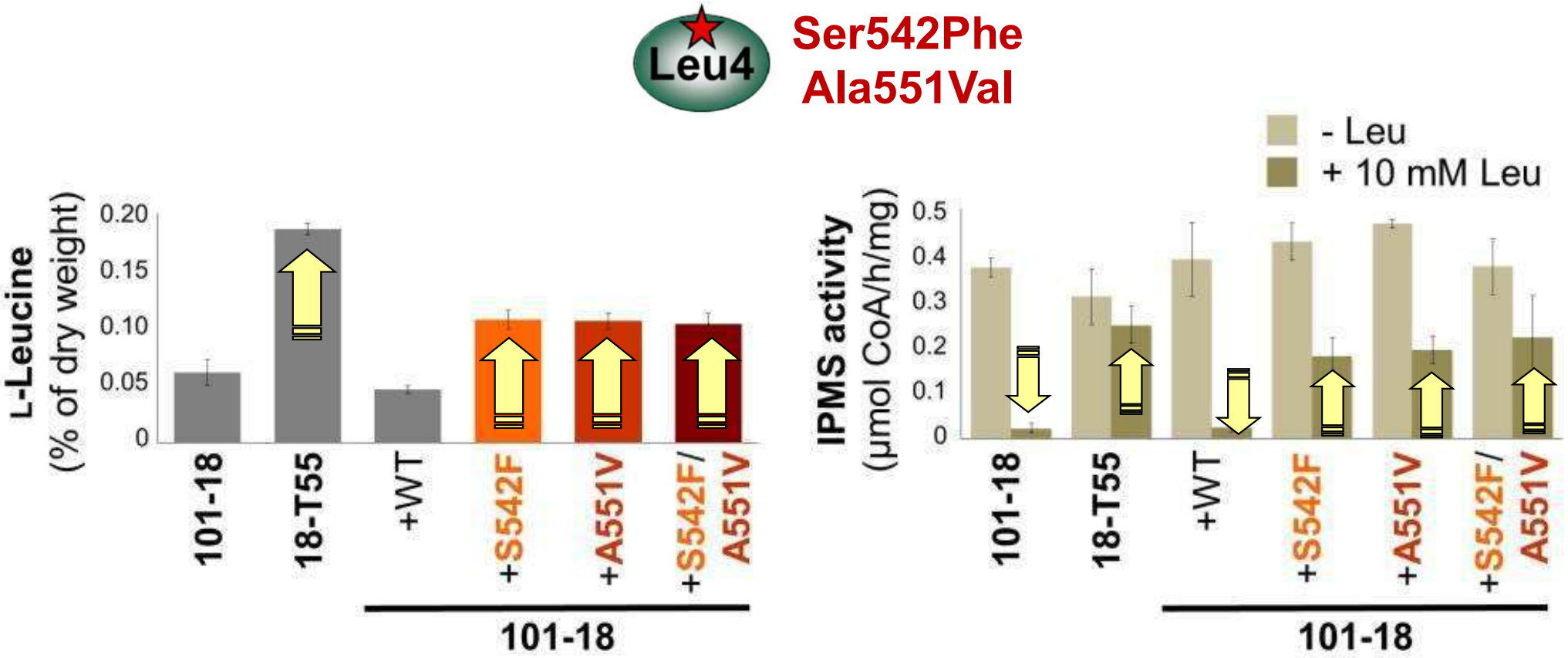
Select **leucine** overproducers

↓

Test ethanol productivity

Awamori yeast mutants that accumulate ***leucine***

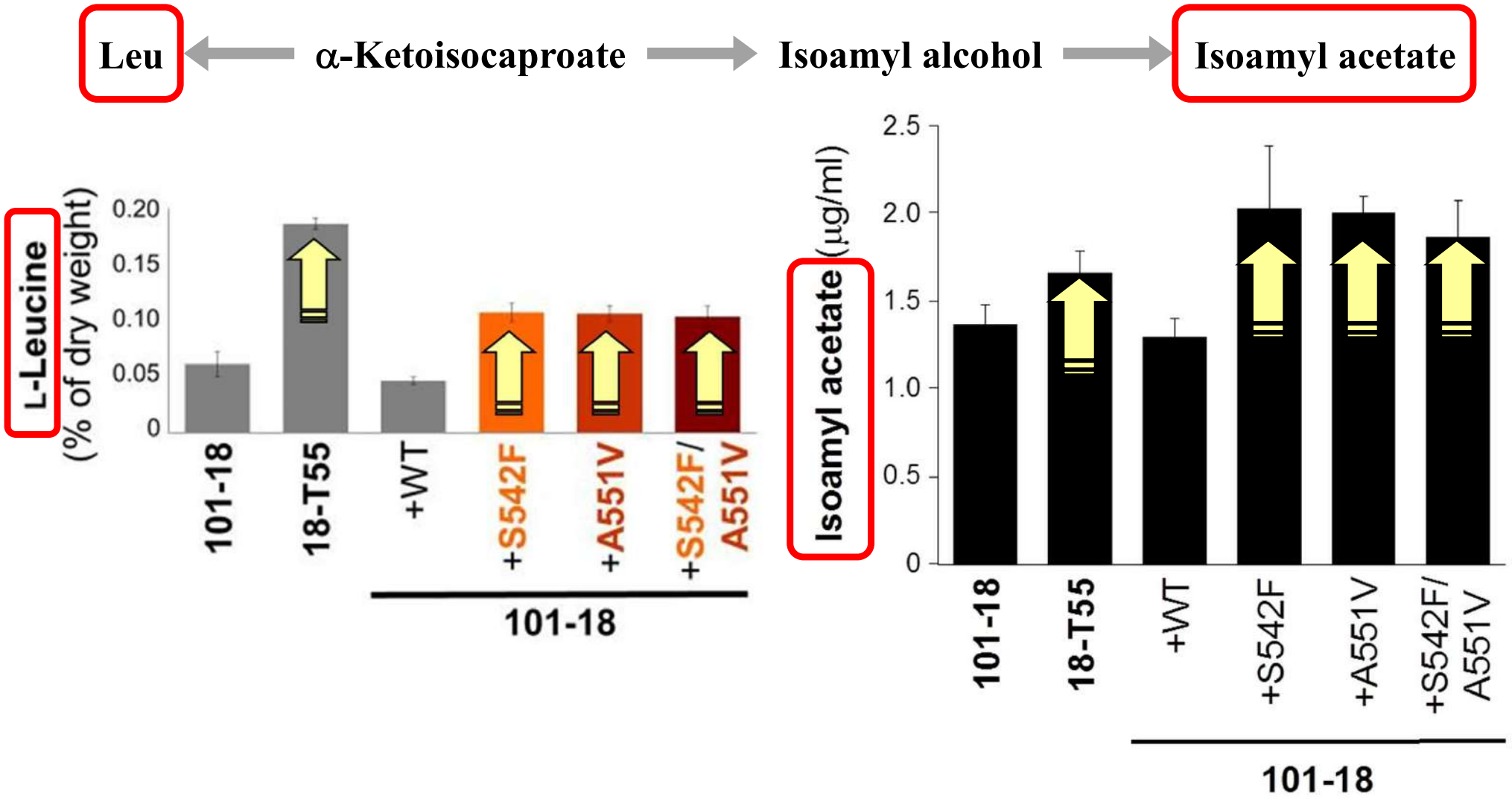
Takagi *et al.*, ***J. Biosci. Bioeng.***, **119**, 140, 2015.



The IPMS variants increase intracellular leucine content.

The IPMS variants are less sensitive to feedback inhibition.

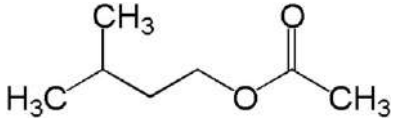
*Functional analysis of the **IPMS variants***



The IPMS variants increase leucine and isoamyl acetate levels.

Takagi *et al*., ***J. Biosci. Bioeng.***, **119**, 140, 2015.

“Aromatic” awamori containing a high level of isoamyl acetate



Isoamyl acetate

2024.2 2018.1 2019.10

SHIMA BANANA by Higa Shuzo

HANAHANA by Kamiya Shuzosho

SHŌ-KAMIMURA by Kamimura Shuzo

BioJet

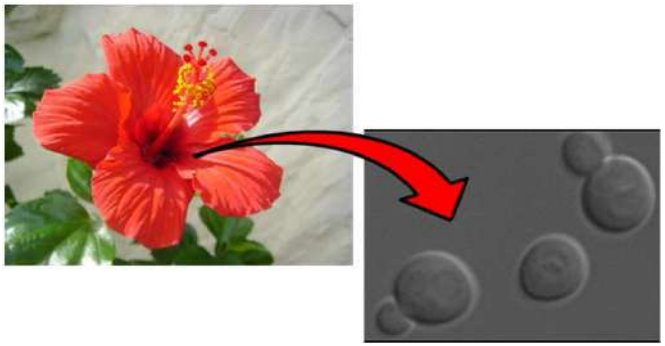
Masatoshi Tsukahara *(BioJet)*

Abe *et al*., ***Front. Genet.***, **10**, 490, 2019.

Tsukahara *et al*., ***Biosci. Biotech. Biochem.***, **87**, 240, 2023.

*A novel **S. cerevisiae** isolated from **hibiscus** flowers*

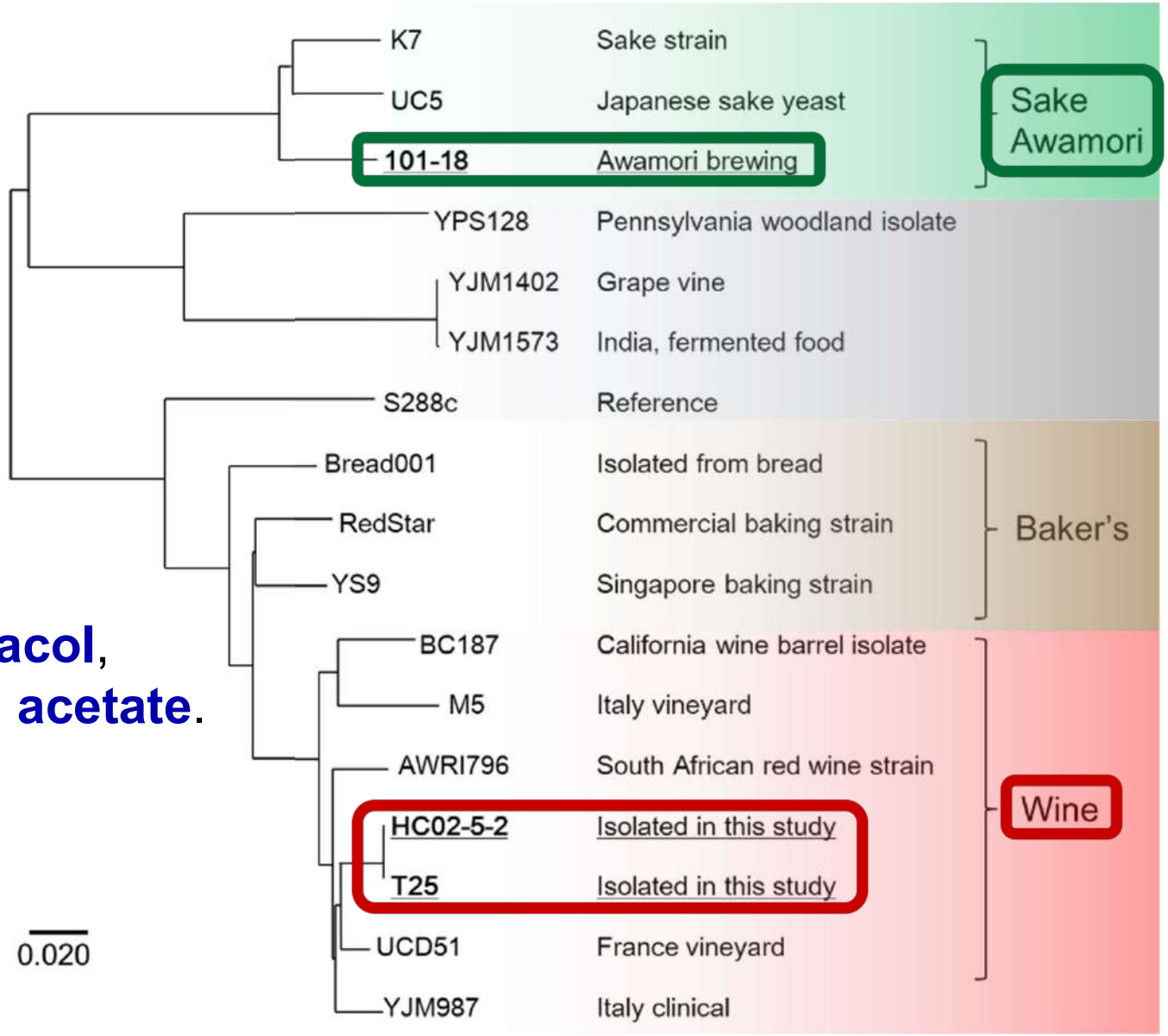
Abe *et al.*, ***Front. Genet.***, **10**, 490, 2019.



S. cerevisiae
HC02-5-2
(**HB yeast**)

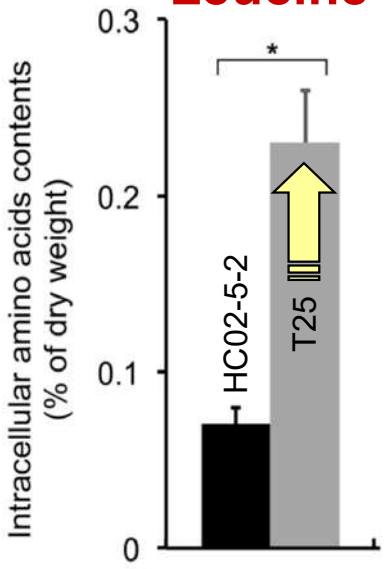
It produces high levels of **ethanol** and **4-vinyl guaiacol**, but a low level of **isoamyl acetate**.

Hibiscus yeast is contiguous to wine yeast strains.

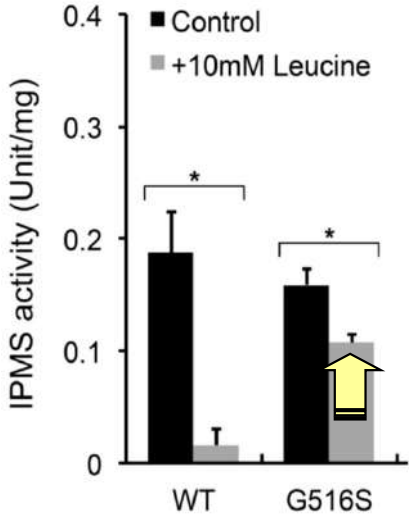


A new **hibiscus yeast** mutant for awamori brewing

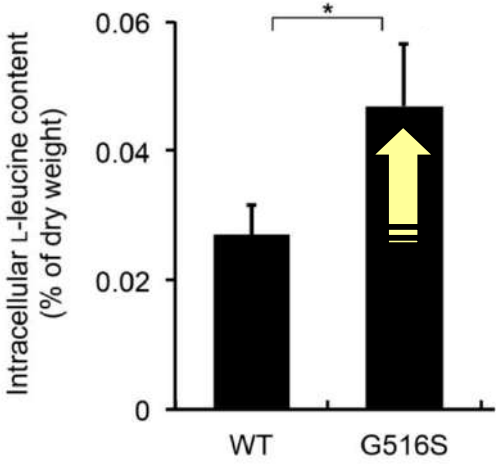
Leucine



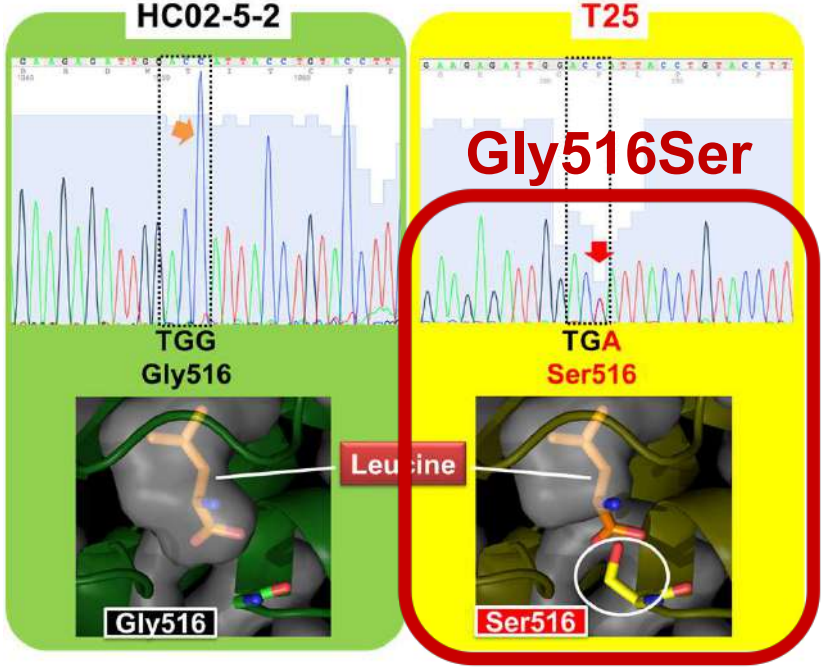
IPMS



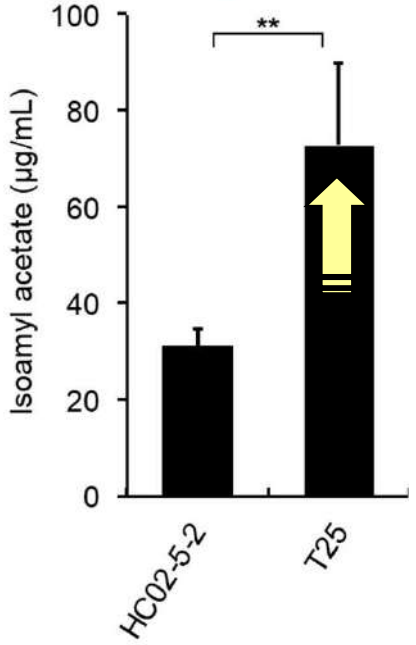
Leucine



LEU4/IPMS

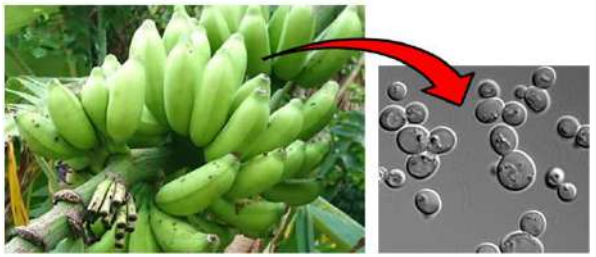


Isoamyl acetate

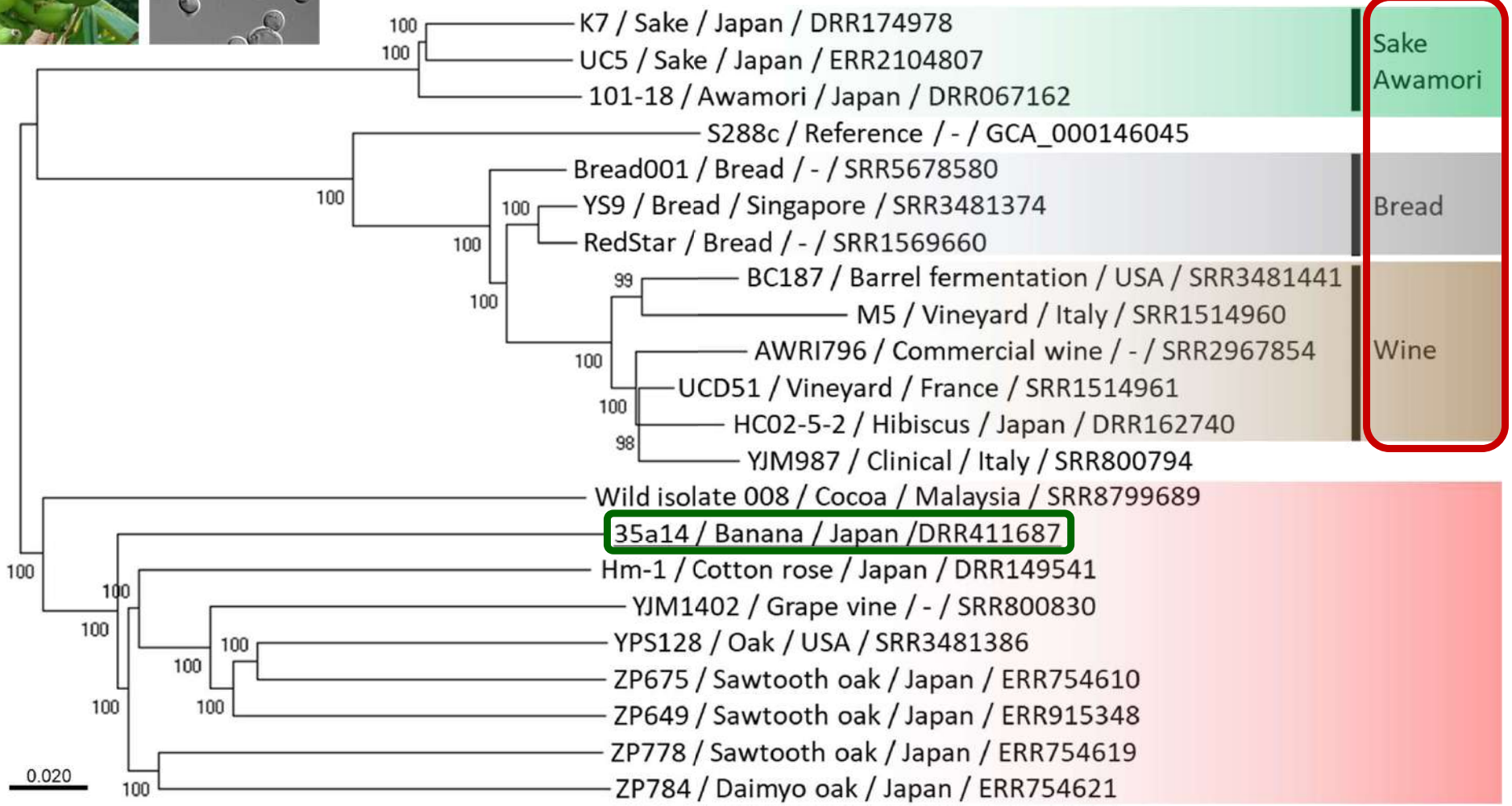


Abe *et al.*, ***Front. Genet.***, **10**, 490, 2019.

*A novel **S. cerevisiae** isolated from **banana** stems*

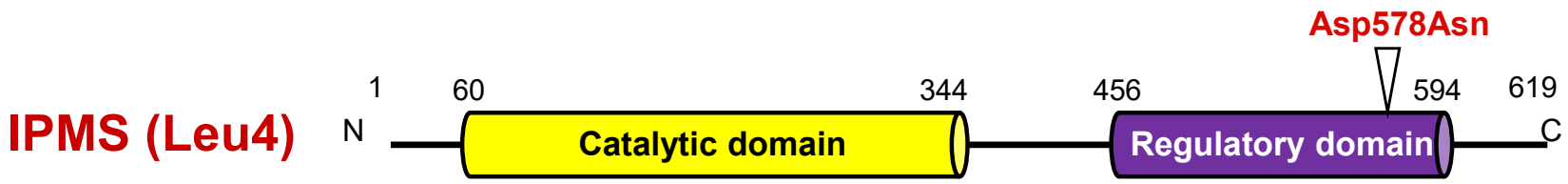


***S. cerevisiae* 35a-14** produces high levels of **ethanol** & **4-vinyl guaiacol**, but a low level of **isoamyl acetate**.

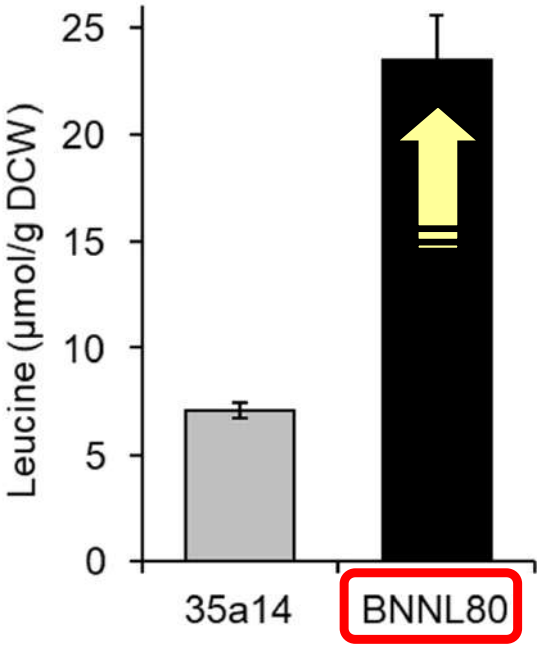


Banana yeast did NOT belong to any industrial yeast groups.

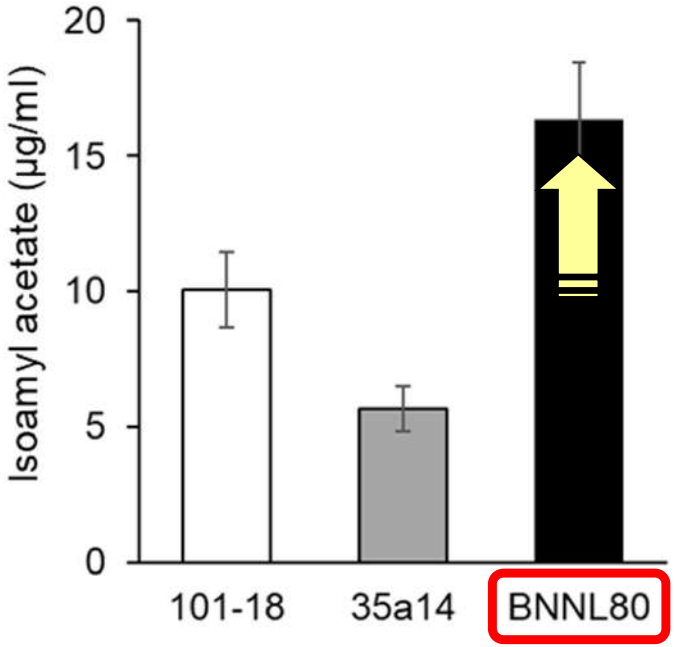
*A new **banana yeast** mutant for awamori brewing*



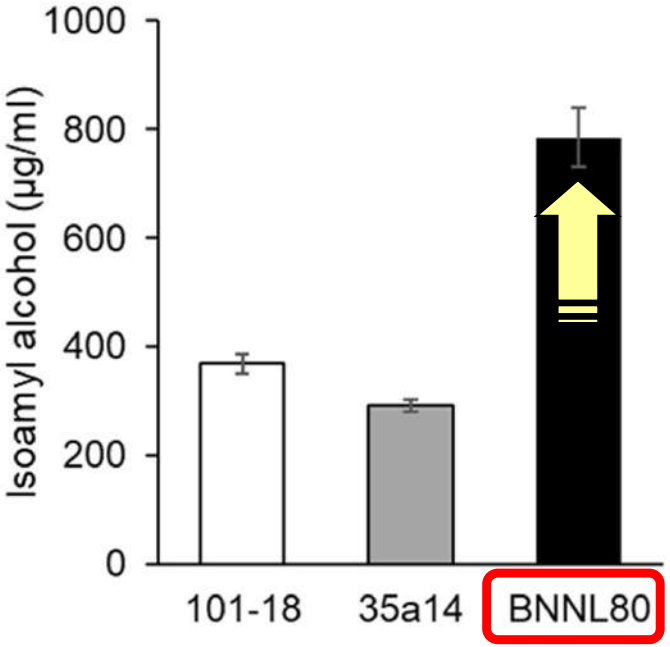
Leucine



Isoamyl acetate



Isoamyl alcohol



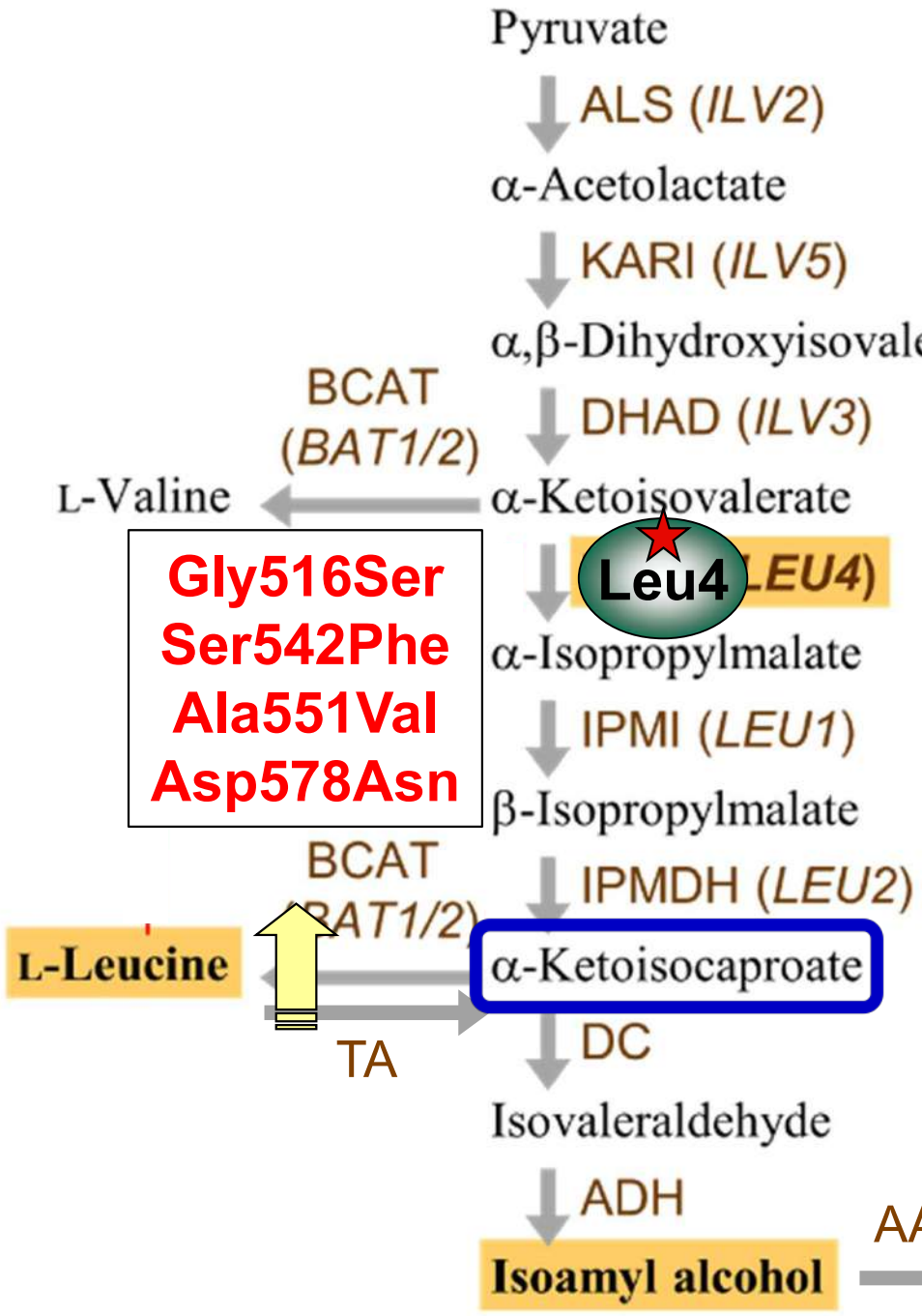
Strain **BNNL80** produced more **Leu** and **isoamyl acetate**.

Leucine metabolism

Takagi *et al.*, ***J. Biosci. Bioeng.***, **119**, 140, 2015.

Abe *et al.*, ***Front. Genet.***, **10**, 490, 2019.

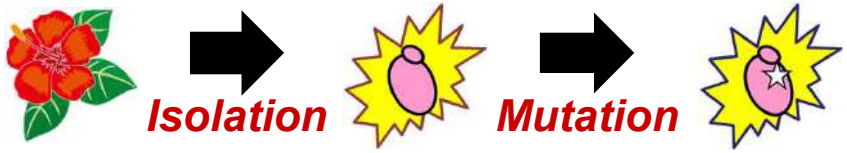
Tsukahara *et al.*, ***Biosci. Biotech. Biochem.***, **87**, 240, 2023.

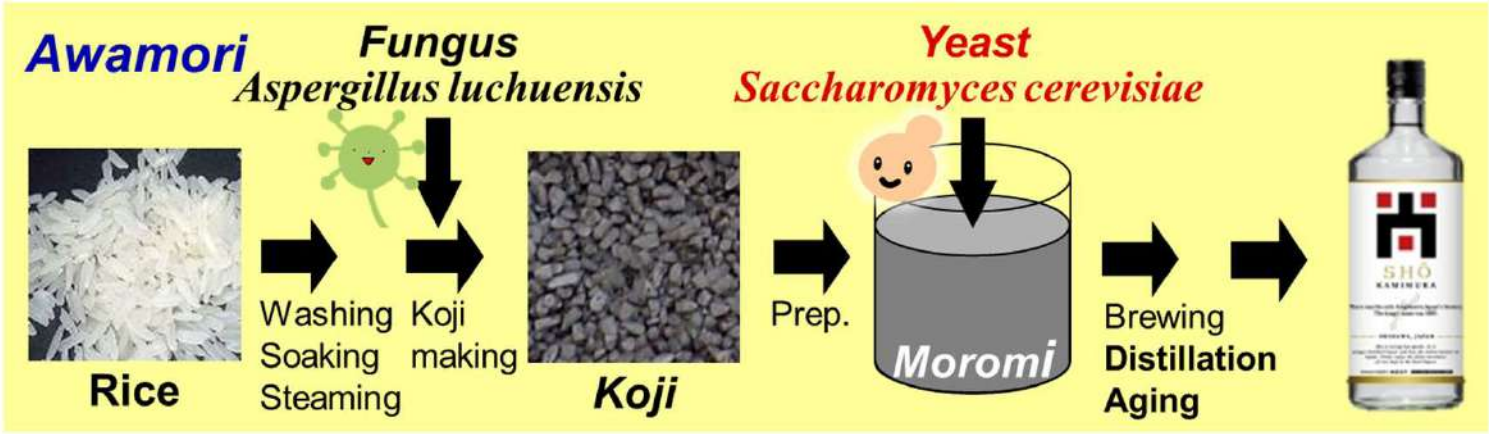


2018.1	2019.10	2024.2
HANA HANA by Kamiya Shuzosho	***SHŌ-KAMIMURA*** by Kamimura Shuzo	***SHIMA BANANA*** by Higa Shuzo

Yeast breeding

Yeast isolation from nature
Whole-genome analysis
Metabolism-focused mutation





How to g
"Awam

●個人：10 万円、法人：100 万円

Awamori

Chopsticks

1) Visit **SI** · '***HYPER YEAST***

●個人：5 万円、法人：50 万円

Business card case

Stainless tumbler

2) Buy '***I*** ***A***' and '***SHIMA***

●個人：3 万円、法人：30 万円

3) Donate (NAIST

Local pottery

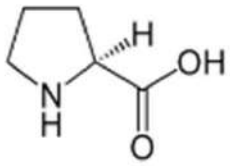
Wrapping cloth

4) Become which is

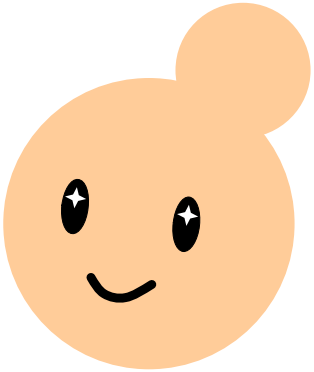
Hiroshi '***Hiro***' *Takagi*

New taste and flavor wine/beer brewed with proline-utilizing yeast

Wine



L-Proline



Proline-Utilizing Yeast !!

Beer

Kyria **Boundy-Mills**

UCDAVIS
UNIVERSITY OF CALIFORNIA

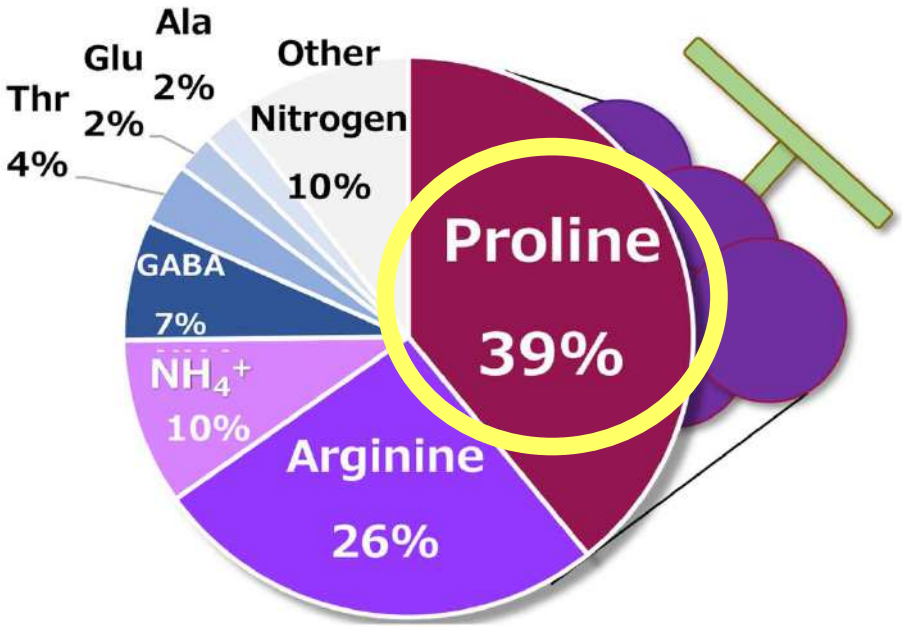
phaff yeast culture collection

Glen Fox

Nitrogen availability *is a key index of wine making*

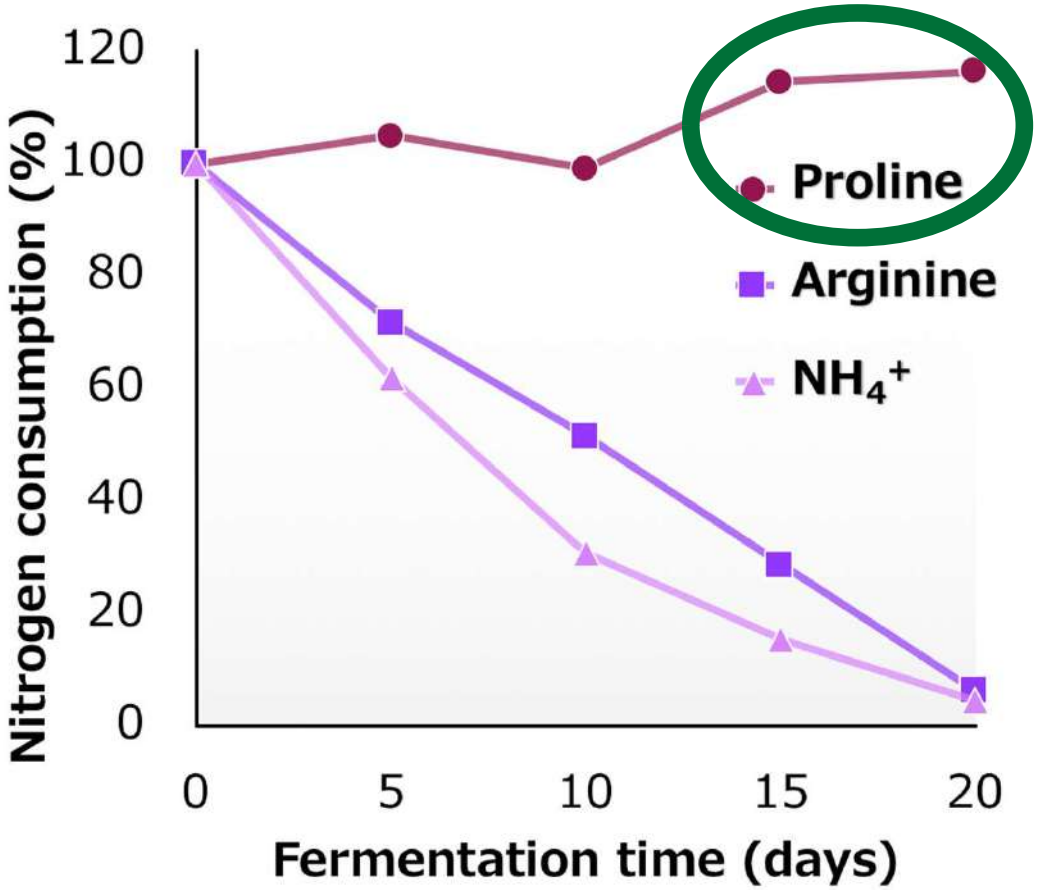
Valero *et al.*, ***J. Sci. Food Agric.***, **83**, 830, 2003.

Nitrogen source ratio in grapes



Proline is the most abundant in grapes.

Nitrogen source consumption

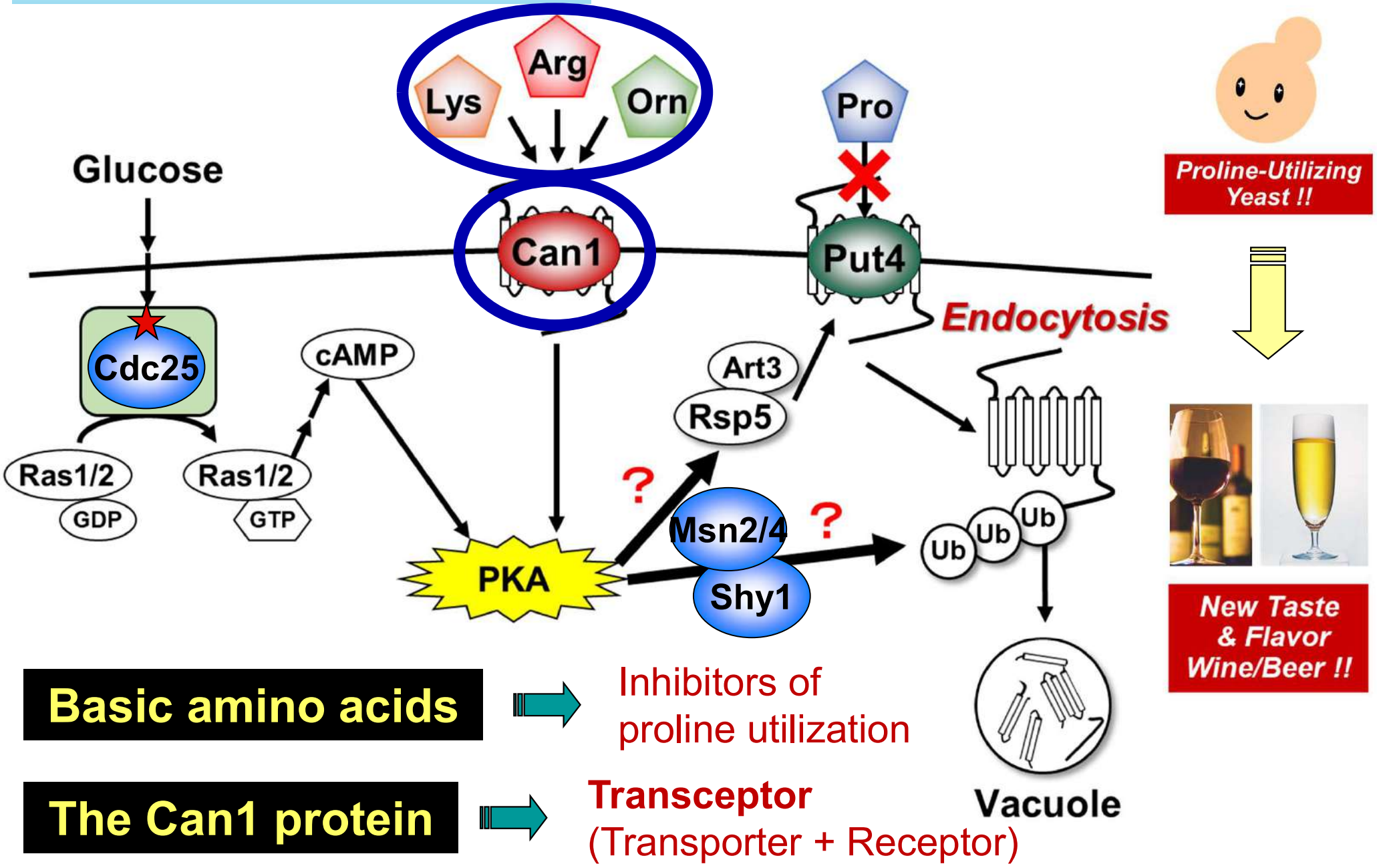


Proline-utilizing yeast may improve fermentation ability and wine quality.

Proline is not utilized during fermentation.

*Inhibitory mechanism of **proline** utilization*

Biochem. Biophys. Res. Commun., **531**, 416, 2020; ***Biosci. Biotechnol. Biochem.***, **86**, 1300, 2022; *ibid.*, **86**, 1318, 2022; *ibid.*, **87**, 358, 2023; *ibid.*, **87**, 458, 2023; ***Yeast***, **37**, 531, 2020; *ibid.*, **40**, 333, 2023; ***J. Biosci. Bioeng.***, **136**, 438, 2023; ***J. Fungi***, **9**, 1137, 2023.

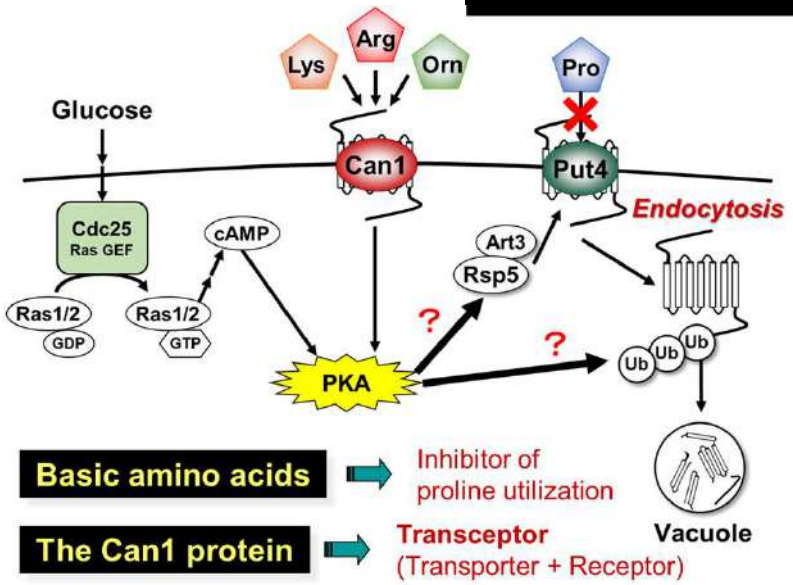


Basic amino acids → Inhibitors of proline utilization

The Can1 protein → **Transceptor** (Transporter + Receptor)

NAIST-UC Davis International Collaborative Laboratory

Yeast Amino Acid and Brewing Sciences



Proline Utilization Mechanism

Development of Proline-Utilizing Yeast

Whole-Genome & Amino Acid Analyses

New Taste & Flavor Beer/Wine !!

Mutation Genome Editing

Proline-Utilizing Yeast !!

Beer Brewing/Wine Making

Isolation

Cultivation

Screening

phaff yeast culture collection

Kyria Boundy-Mills

Screening from Yeast Culture Collection

Ryoya Tanahashi

Screening of proline-utilizing strains and application to brewing

“Nara” original craft beer brewed with my lovely yeasts

Yamato Sanzan (大和三山)

A national scenic beauty spot

Naramachi

Drying

Temperature

Stress!!

Freezing

Osmolarity

Tolerance

Ethanol

Proline

Phenetyl alcohol

Unebiyama

2022.7.7

Kaguyama

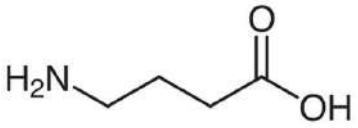
2021.7.15

Miminashiyama

2022.11.22

GOLDEN -Rabbit-

“Healthy image” craft beer containing a high level of GABA



GABA (γ-Aminobutyric acid)

NAIST Pond

10 Fields Factory

Adventures in brewing exotic Japanese alcoholic beverages

Yeast research

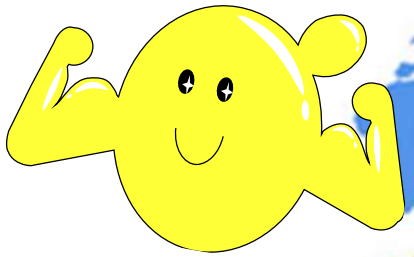
Originality
Priority

Functional amino acids

Taste/Flavor

Nutrition/Health

Happy Yeast



正暦寺「日本清酒発祥之地」の碑

Nara

Okinawa

North America

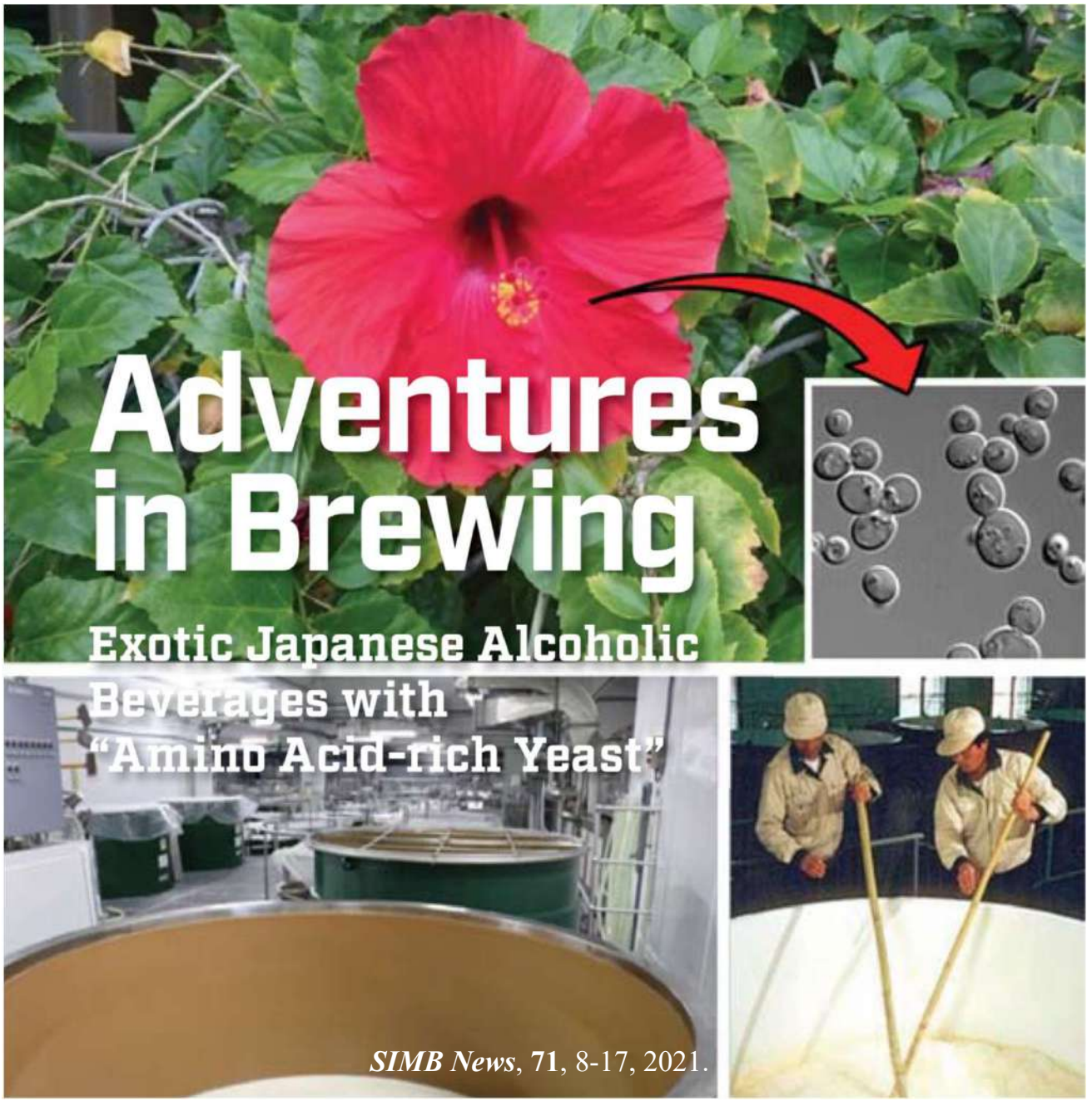
Worldwide innovation of alcoholic beverages

*World-class Japanese **yeast science** and **brewing technology***

Western yeast scientists are NOT familiar with **multiple parallel fermentation** (the role of ***koji* mold**) compared to simple fermentation (wine) and multiple sequential fermentation (beer) brewing.

Overseas researchers and companies have little interest in "**breeding**" **yeast** strains (obtaining mutants).

Microbial isolation and breeding are useful technologies for brewing.



*Why is **fermentation** (**Hakko**) attracting attention around the world ?*

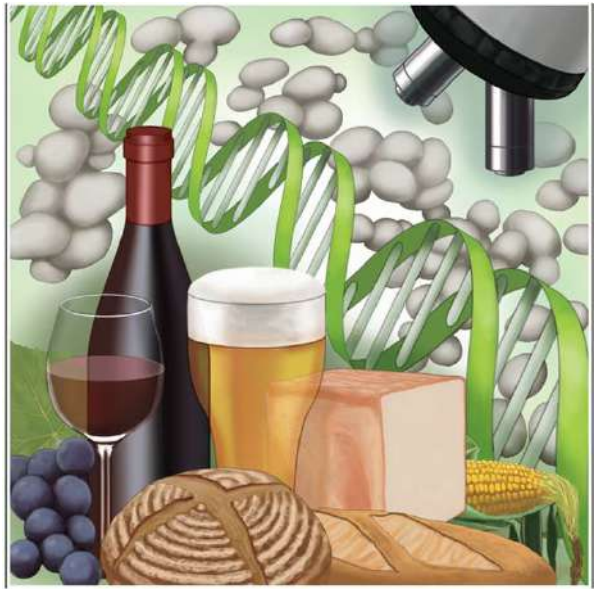
発酵

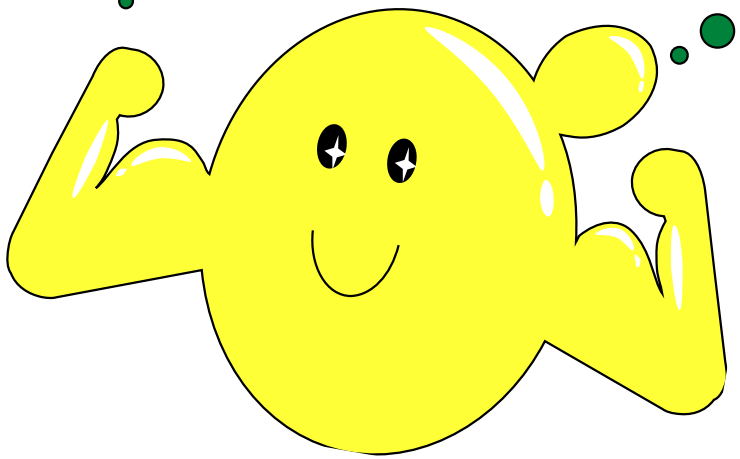
醸造

Wellbeing

Gastronomy

Sustainability



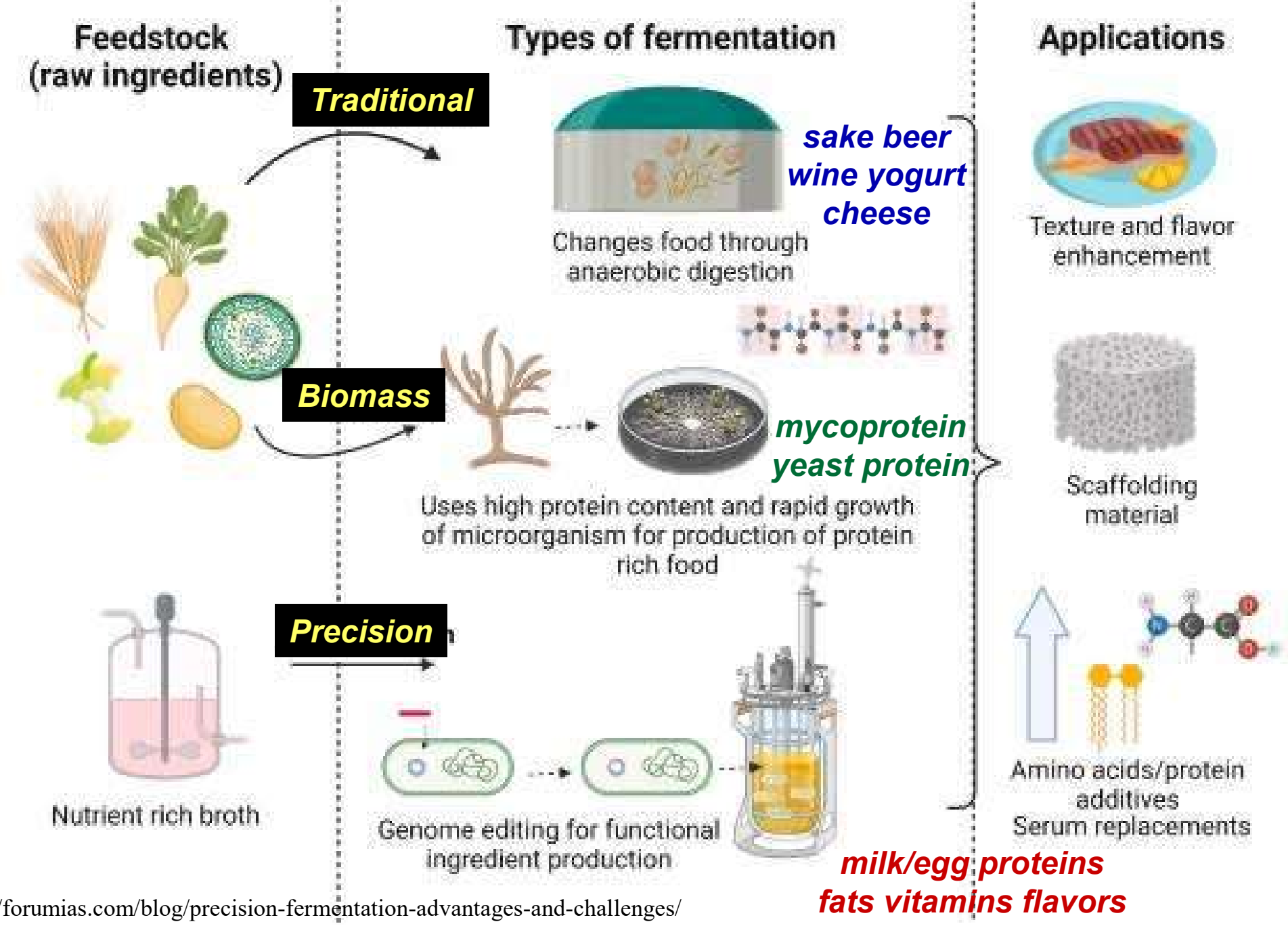


Happy Yeast

unesco

Intangible Cultural Heritage

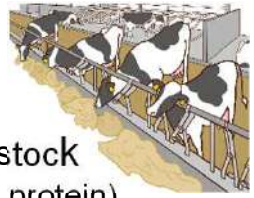
Fermentation *for alternative proteins*



https://forumias.com/blog/precision-fermentation-advantages-and-challenges/

Development of **yeast-based meat** with high added value

Population increase
Climate change



Livestock
(animal protein)

Food crisis
(Protein crisis)

Environmental load
Animal ethics

Growing demand for alternative proteins

1) **Plants**
2) **Animal cells**
3) **Insects**
4) **Microbes**

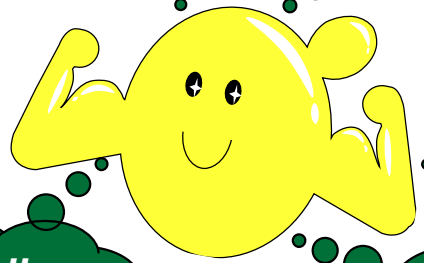
- Requires farmland, water, time
- High production cost
- Psychological resistance
- Difficult to reproduce the meat taste and texture

The microbe ***Yeast***, which is GRAS, widely used in the production of fermented/brewed foods

Rich functional metabolites | High proliferative ability | Low environmental load | Advanced breeding and culture technology

Attractive protein source with excellent **safety**, **functionality** and **economy**

Torula yeast
Candida utilis



- *Identification of functional metabolites*
- *Breeding of yeasts with increased functional metabolites*
- *Optimization of culture conditions*
- *Preparation and evaluation of yeast-based meat*
- *Non-genetically modified genome editing technology*

Yeast strains with increased **functional metabolites** that contribute to **taste*** and **nutrition/health**** of meat

*Glutamate, Inosinic acid, Oleic acid *etc*.

**Essential amino acids, Glutathione *etc*.

Development of **yeast-based meat** with taste, flavor and texture similar to meat, and high health functionality

Real scientists !!

Tsubasa Tanikawa
Kazuki Ichikawa
Kazuki Nakagami
Fumika Morita
Tetsuma Takemoto
Hayate Nakazawa
Atsuki Taniguchi
Yukiko Sugimoto
Dr. Noreen Suliani binti Mat Nanyan
Dr. Ryoya Tanahashi
Dr. Shota Isogai
Dr. Daisuke Watanabe
Dr. Ryo Nasuno
Dr. Akira Nishimura

Naoyuki Murakami
Dr. Atsushi Kotaka
Natsuki Hotta
Dr. Kengo Matsumura
Dr. Hiroki Ishida
Dr. Yoji Hata

Dr. Masataka Ohashi

Dr. Kyria Boundy-Mills
Dr. Irnayuli Sitepu
Dr. Glen Fox

科研費 KAKENHI
Grant-in-Aid for Challenging Exploratory Research

NARO 農研機構 National Agriculture and Food Research Organization
Research program on development of innovative technology

Real scientists !!

Keisuke Hashida
Yukiko Sugimoto
Dr. Shota Isogai
Dr. Yoichi Toyokawa
Dr. Ryo Nasuno
Dr. Daisuke Watanabe

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URL http://www.zanpa.co.jp

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奈良県産業振興総合センター
Nara Prefecture Institute of Industrial Development

Dr. Masataka Ohashi

合名会社 新里酒造

八重瀬が育む旨い酒。
神谷酒造所

Dr. Masatoshi Tsukahara

沖縄県 OKINAWA PREFECTURE

The Ryukyu Awamori Research Study Support Project

Adaptable and Seamless Technology Transfer Program through target-driven R&D, JST