



June 7, 2017

Rediscovery of *Lactobacillus pastorianus* Van Laer 1892, a beer spoilage *Lactobacillus* species named in honor of Louis Pasteur, and studies on its extraordinarily unique culturability

The logo for Asahi, featuring the word 'Asahi' in a stylized, blue, italicized font.

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About 150 years ago



**Franco-German War
(1870-1871)**

Better beer for France



Louis Pasteur
Father of Microbiology

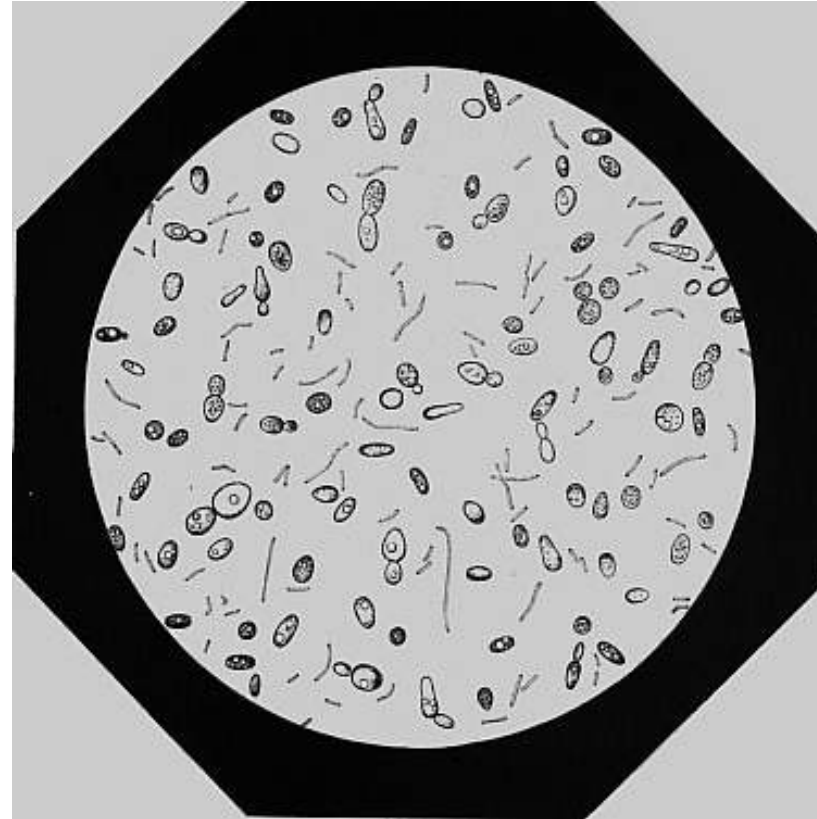


Etudes sur la bière (1876)

Discovery of beer spoilage lactic acid bacteria

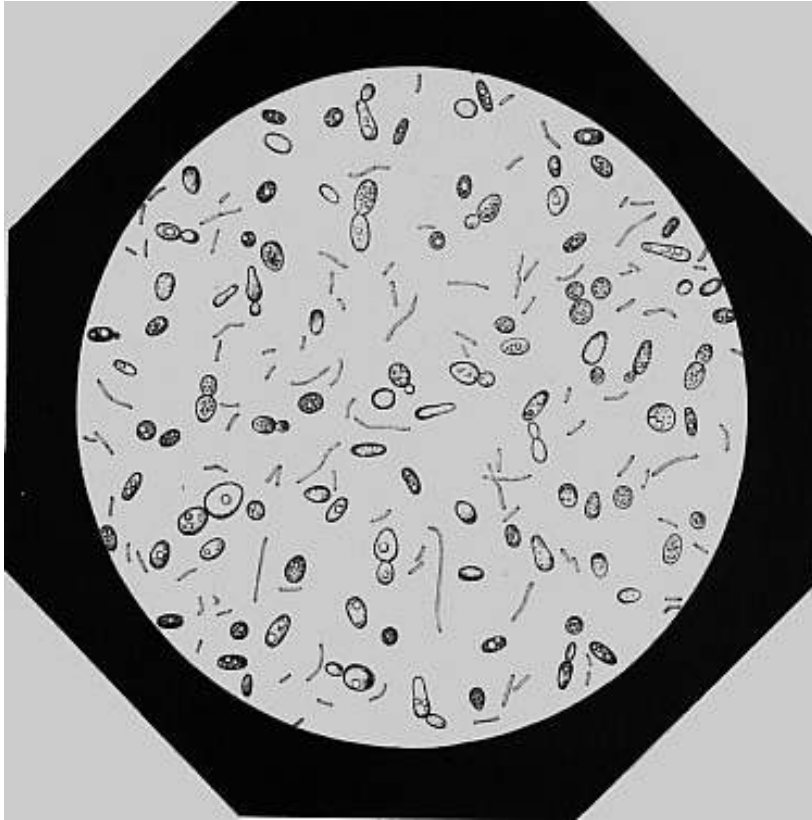


Louis Pasteur
Father of Microbiology



A drawing of spoiled beer
Etudes sur la bière (1876)

Historic achievements influenced by Pasteur

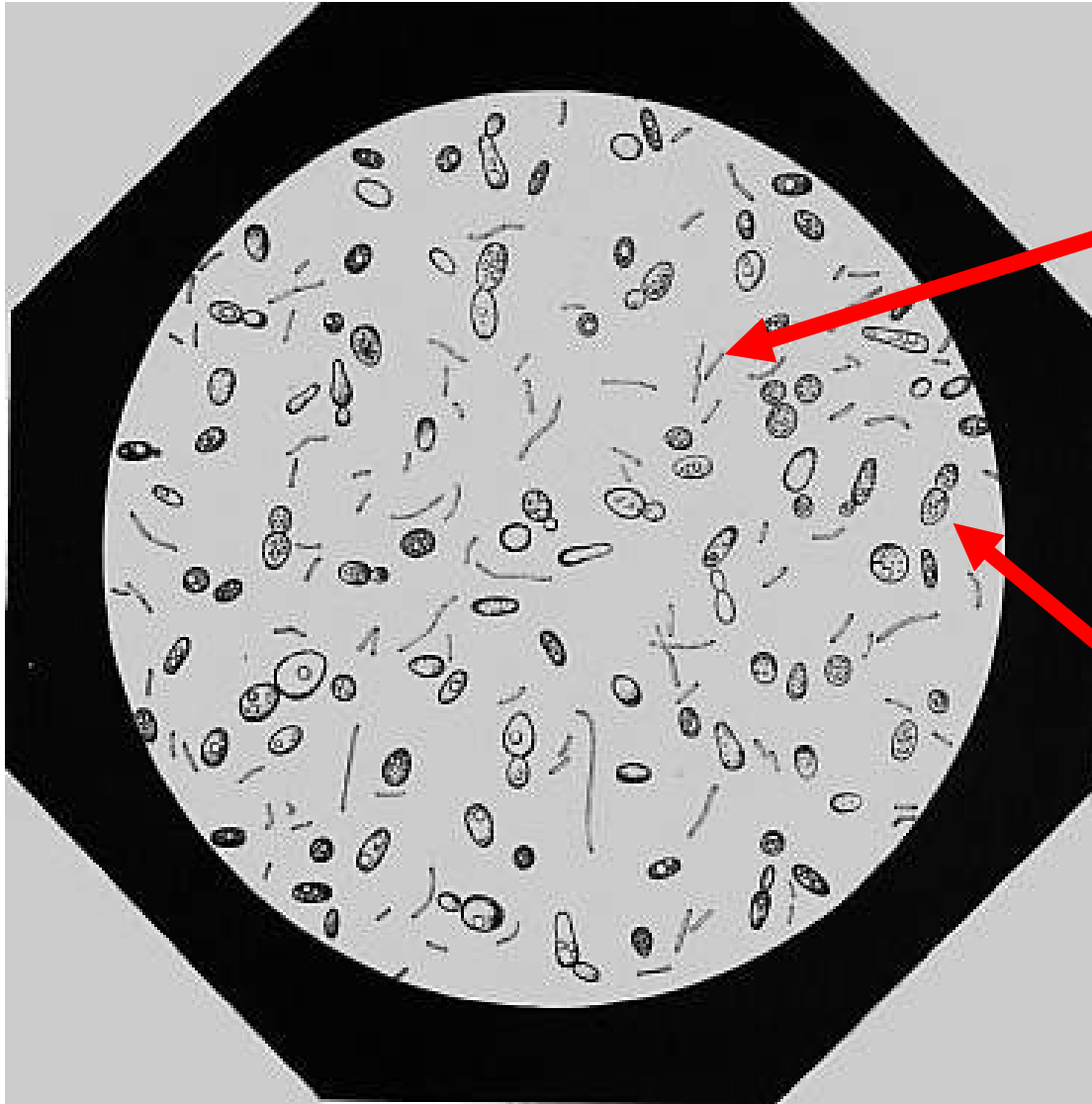


**A drawing of spoiled beer
Etudes sur la bière (1876)**

Pasteurization

**Yeast culture purification
(Emil Hansen 1883)**

Birth of brewery laboratories

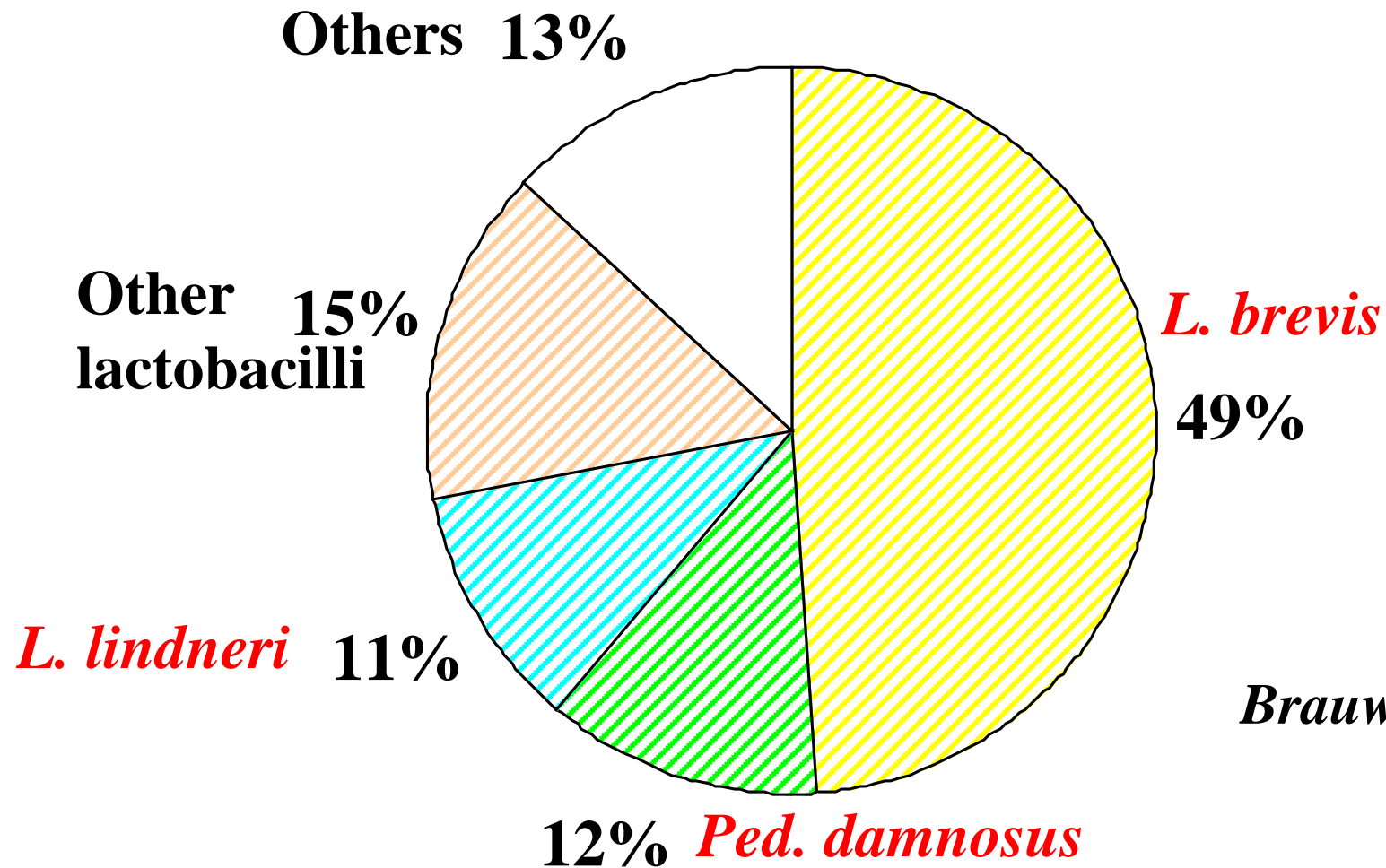


Saccharobacillus pastorianus
(*Lactobacillus pastorianus*
Van Laer 1892)

Saccharomyces pastorianus

A Big Mystery

Almost no descriptions have been found over the past 50 years and *Lactobacillus pastorianus* mysteriously vanished in the brewing history.



Brauwelt Back, W. (2003)

A Big Mystery
Where did they go?



Purpose of this study

- 1. To rediscover *L. pastorianus* Van Laer 1892**
- 2. To characterize *L. pastorianus* so that we can detect and identify the species as a beer spoiler**

Part 1

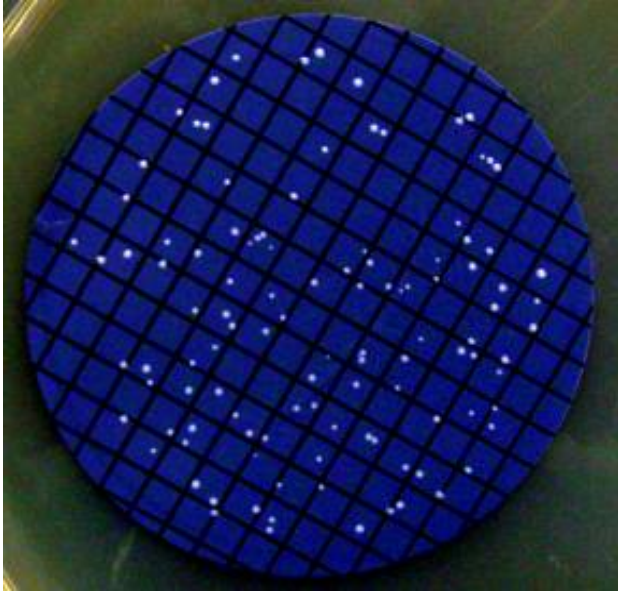
Search for *L. pastorianus* strains and its unique behavior upon primary isolation from brewing environments

According to Van Laer who isolated *L. pastorianus* in 1892

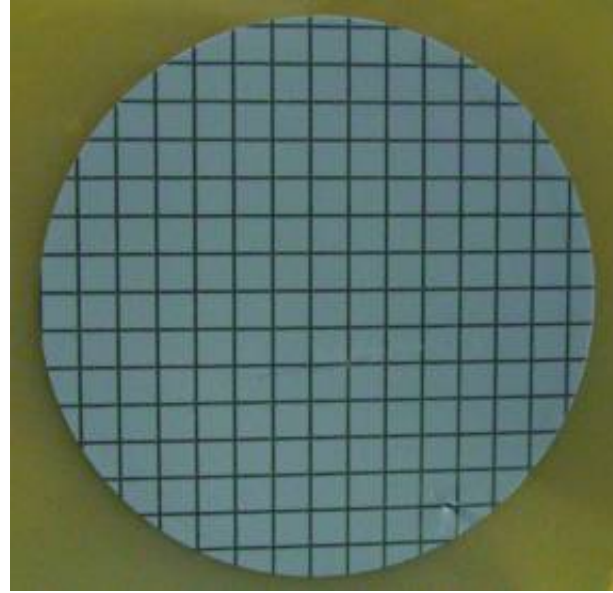
***L. pastorianus* did not grow on normal culture media. However, this species did grow on unhopped beer solidified with gelatin. The colony formation of *L. pastorianus* was extremely slow.**

Later in 1930's, Shimwell found that *L. pastorianus* grew well on normal culture media and could not reproduce its unique culturability.

Lactobacillus paracollinoides, a stealth-like species



Beer agar



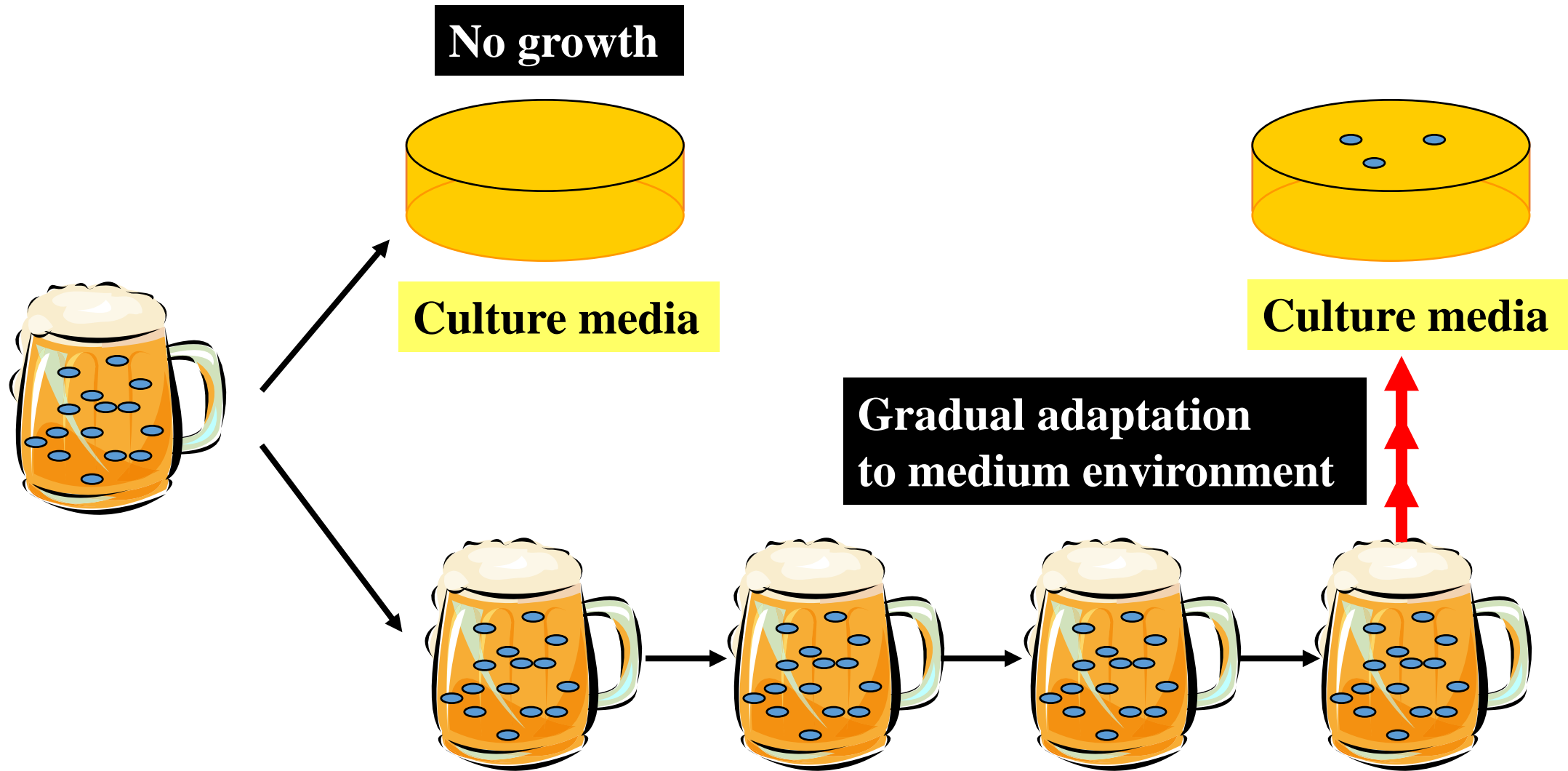
MRS agar

(deMan, Rogosa and Sharpe)

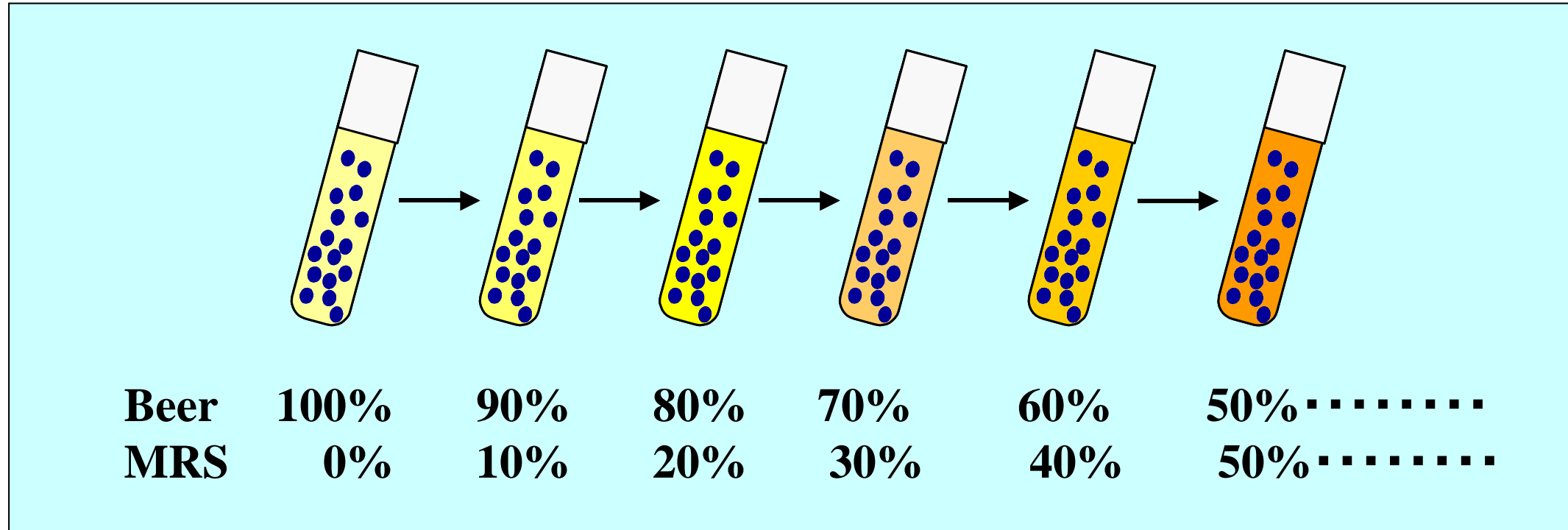


One species fitting the original descriptions of Van Laer and the later studies by Shimwell ?

Peculiar culturability of *L. paracollinoides* (Suzuki *et al.* 2004)



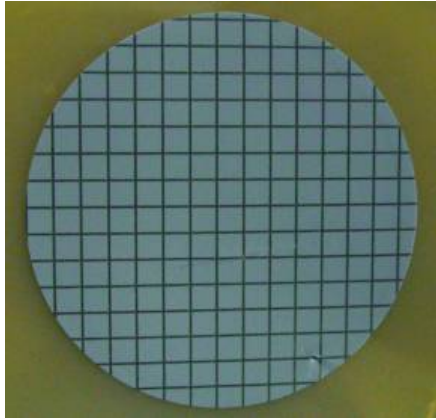
Gradual adaptation process to medium environments



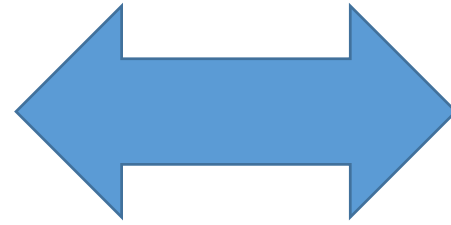
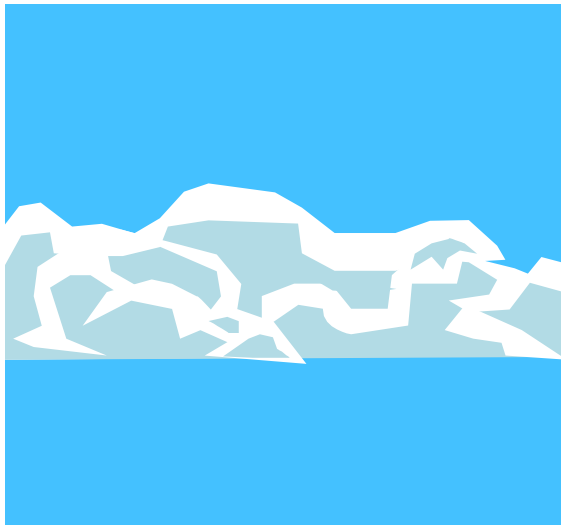
Stepwise adaptation to MRS broth, where degassed beer as a subculture medium was progressively replaced by MRS broth, allowed the cultivation of *L. paracollinoides* on normal culture media.

L. paracollinoides fitting the descriptions of *L. pastorianus*

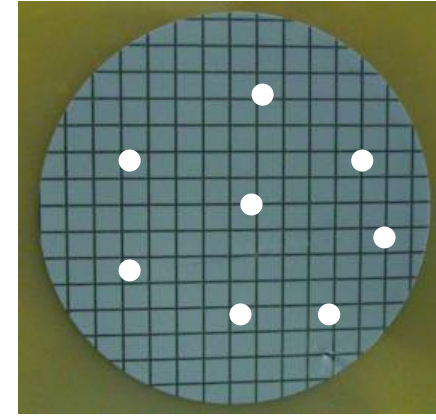
Van Laer



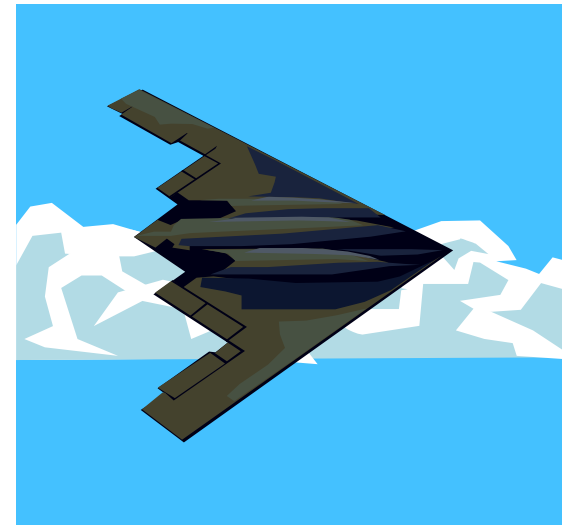
Stealth mode



**Adaptation to
culture media**



Easy-to-culture mode



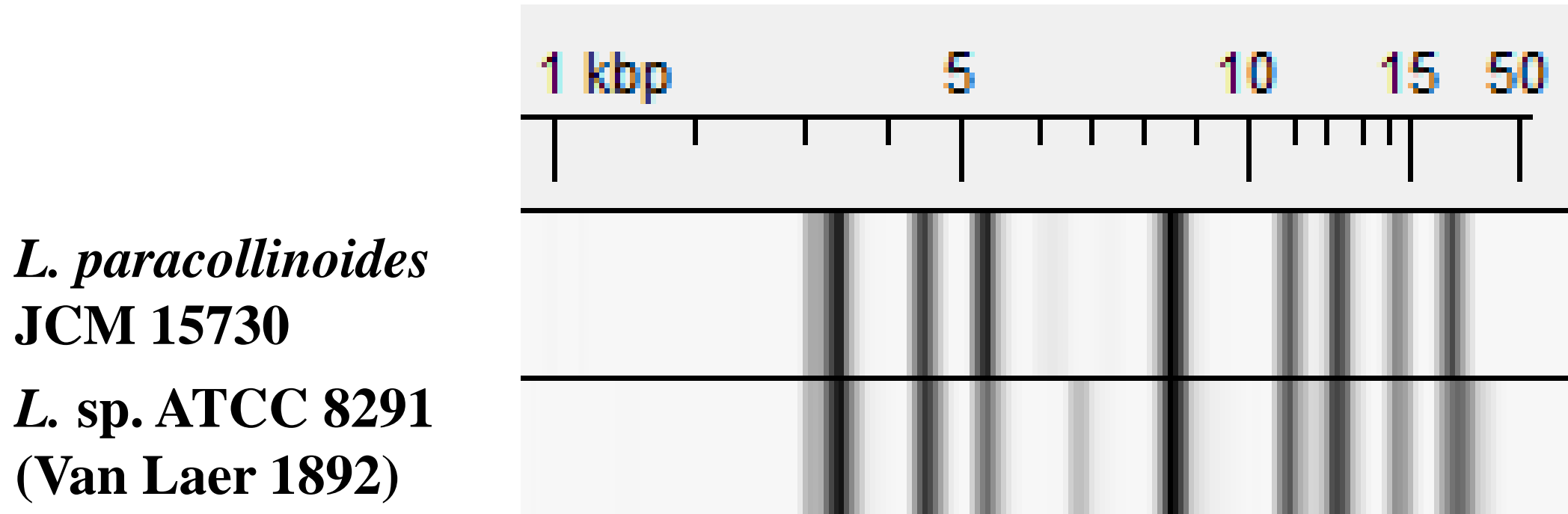
Shimwell

Genetic comparison between *L. paracollinoides* JCM 11969^T and *L.* sp. ATCC 8291 (*L. pastorianus* Van Laer 1892)

16S rDNA sequences	99.8% identical (Criteria: <i>ca</i> 99%)
DNA-DNA hybridization	81.3% homologous (Threshold: 70% or higher)
Isolation	Both from beer

From a genetic standpoint, *L. pastorianus* Van Laer 1892 can be considered as synonymous with *L. paracollinoides*.

Ribotyping of *L. paracollinoides* JCM 15730 and *L. sp.* ATCC 8291 (*L. pastorianus* Van Laer 1892)



***L. pastorianus* Van Laer 1892 also shows an identical ribotyping profile with *L. paracollinoides* JCM 15730.**

Summary 1

1. *L. paracollinoides* was found to be synonymous with *L. pastorianus* Van Laer 1892.

2. *L. pastorianus/paracollinoides* shows unique culturabilities, exhibiting both stealth and easy-to-culture mode .

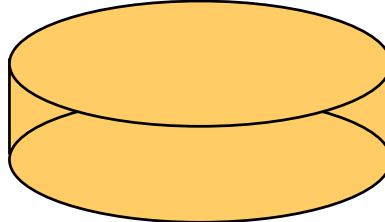
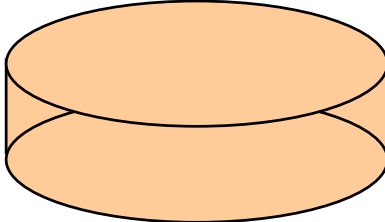
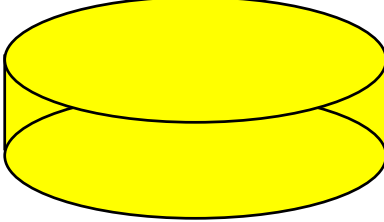
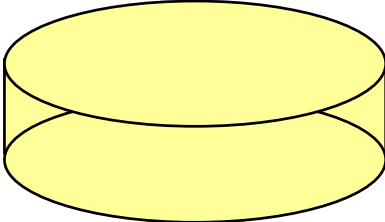
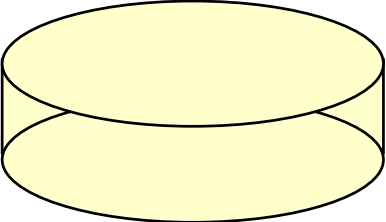
Part 2

Characterization of *L. pastorianus/paracollinoides* focusing on its culturability

Culturability on beer/MRS agar with varying mixing ratios

**Beer
Agar**

**MRS
Agar**



**100% beer
0% MRS**

**75% beer
25% MRS**

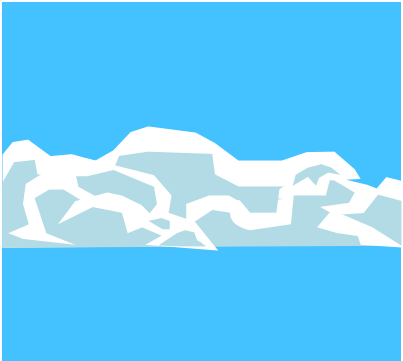
**50% beer
50% MRS**

**25% beer
75% MRS**

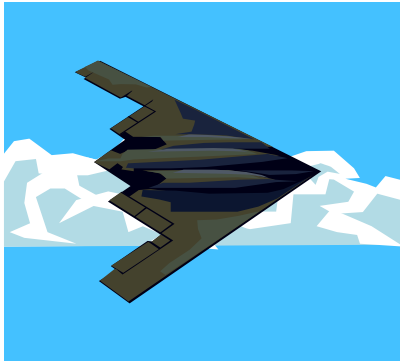
**0% beer
100% MRS**



Culturability?

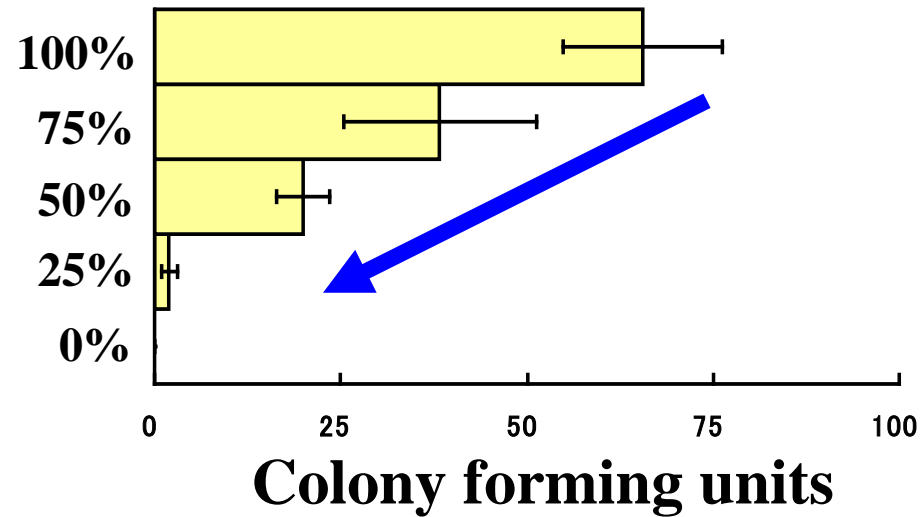


Stealth mode

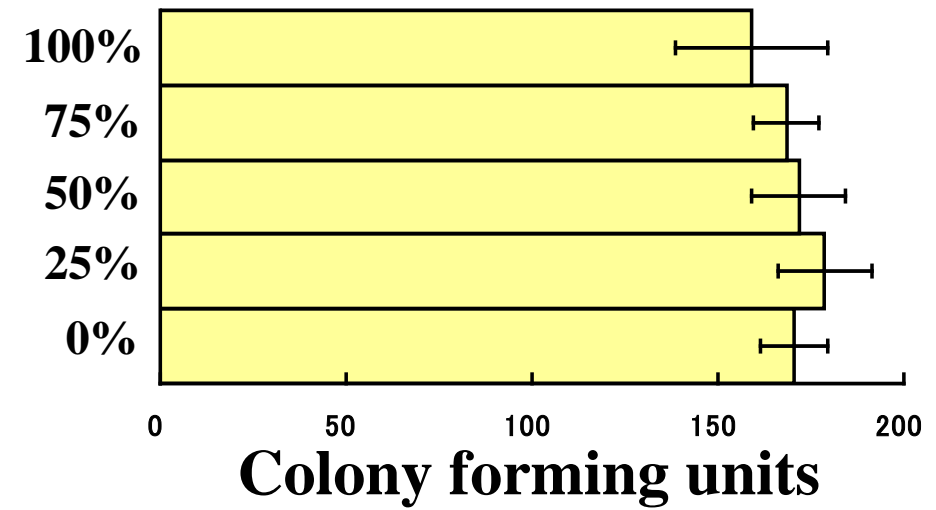


Easy-to-culture mode

Culturability on beer/MRS agar with varying mixing ratios



Stealth-mode
L. paracollinoides
JCM 11969^T



Easy-to-culture mode
L. paracollinoides
JCM 11969^T

MRS contains inhibitors affecting negatively the growth of *L. pastorianus/paracollinoides*.

**Identification of negative factors for the growth of
*L. pastorianus/paracollinoides***

Selection of advanced beer-spoiler detection (ABD) agar as a base medium

<Medium compositions>

MRS broth (powder)	2.61g
Sodium acetate	0.5g
Cycloheximide	10mg
Agar	15g
Beer (pilsner-type)	1000ml
pH 5.0	

The medium contains 0.26% MRS broth in beer and its pH is adjusted to 5.0 to facilitate the growth of hard-to-culture beer spoilers (Suzuki *et al.*, *J. Appl. Microbiol.*, 2008)

Basic experimental protocol

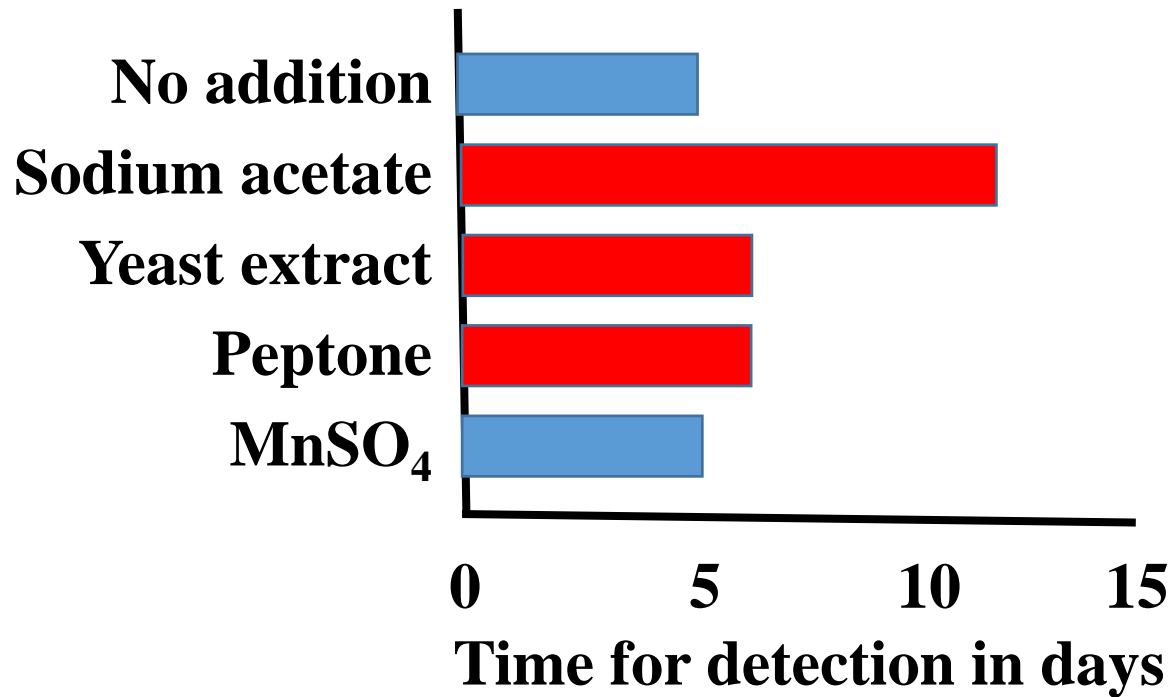
<Medium compositions of ABD>

MRS broth (powder)	2.61g
Sodium acetate	0.5g
Cycloheximide	10mg
Agar	15g
Beer (pilsner-type)	1000ml
pH 5.0	

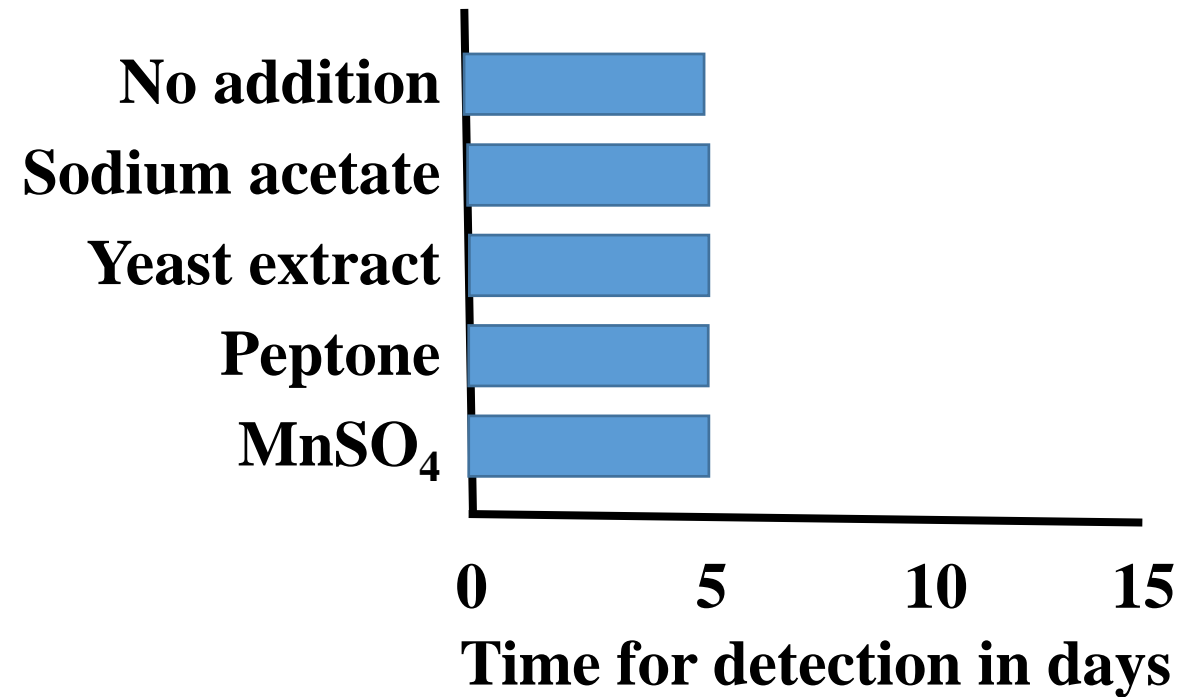
- 1. Nutrient:** Each ingredient was added to ABD at the level of normal medium (MRS)
- 2. pH:** pH of ABD was adjusted between 4.7 and 5.9
- 3. Agar:** Normal microbiological grade v. s. highly purified agar (agarose grade)

Effects of nutrients on the growth of JCM 11969^T

Stealth mode

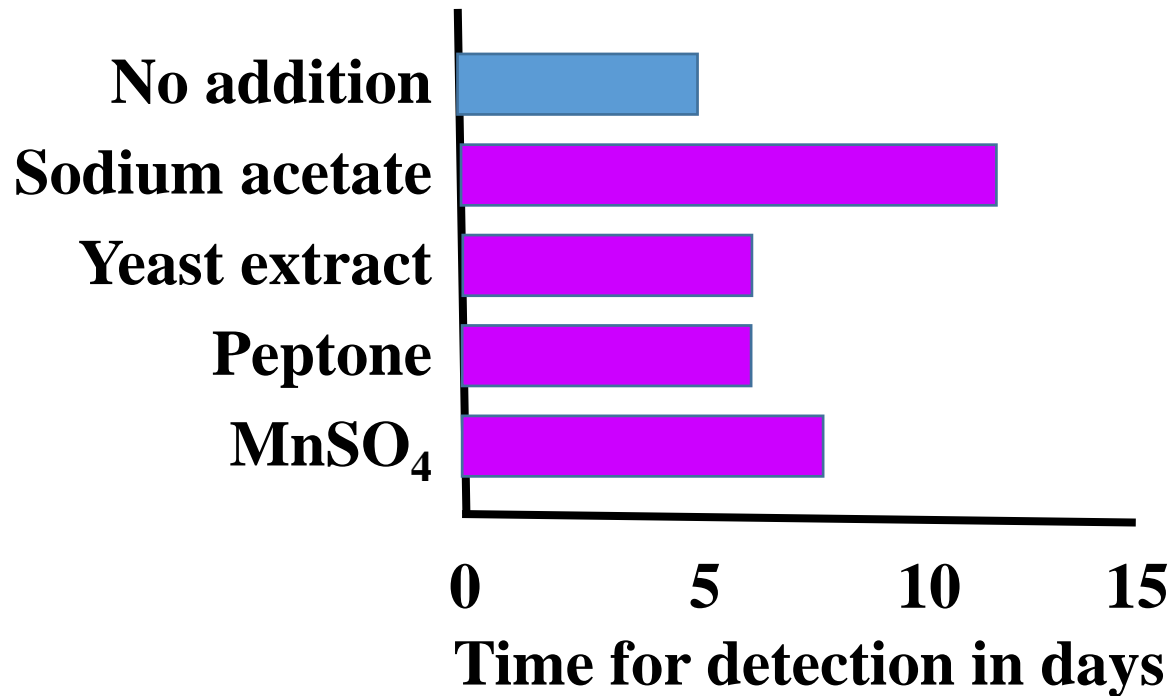


Easy-to-culture mode

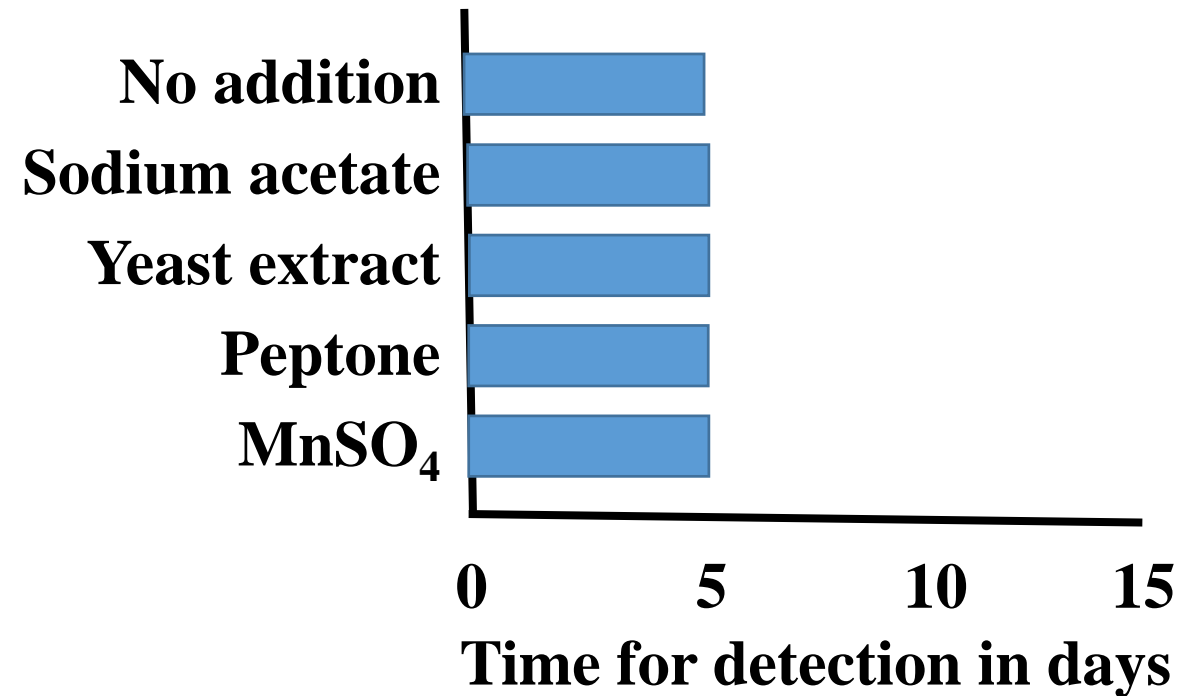


Effects of nutrients on the growth of JCM 15729

Stealth mode



Easy-to-culture mode



The growth of *L. pastorianus/paracollinoides* was inhibited by many of the nutrients supposed to foster the growth of lactic acid bacteria.

Effect of pH

pH	4.7	5.0	5.3	5.6	5.9
Stealth mode JCM 11969 ^T	5	5	11	No growth	No growth
Easy-to-culture JCM 11969 ^T	5	5	5	5	14
Stealth mode JCM 15729	5	6	8	9	12
Easy-to-culture JCM 15729	5	5	5	5	6

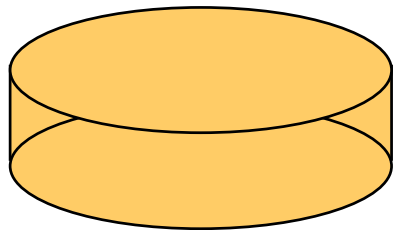
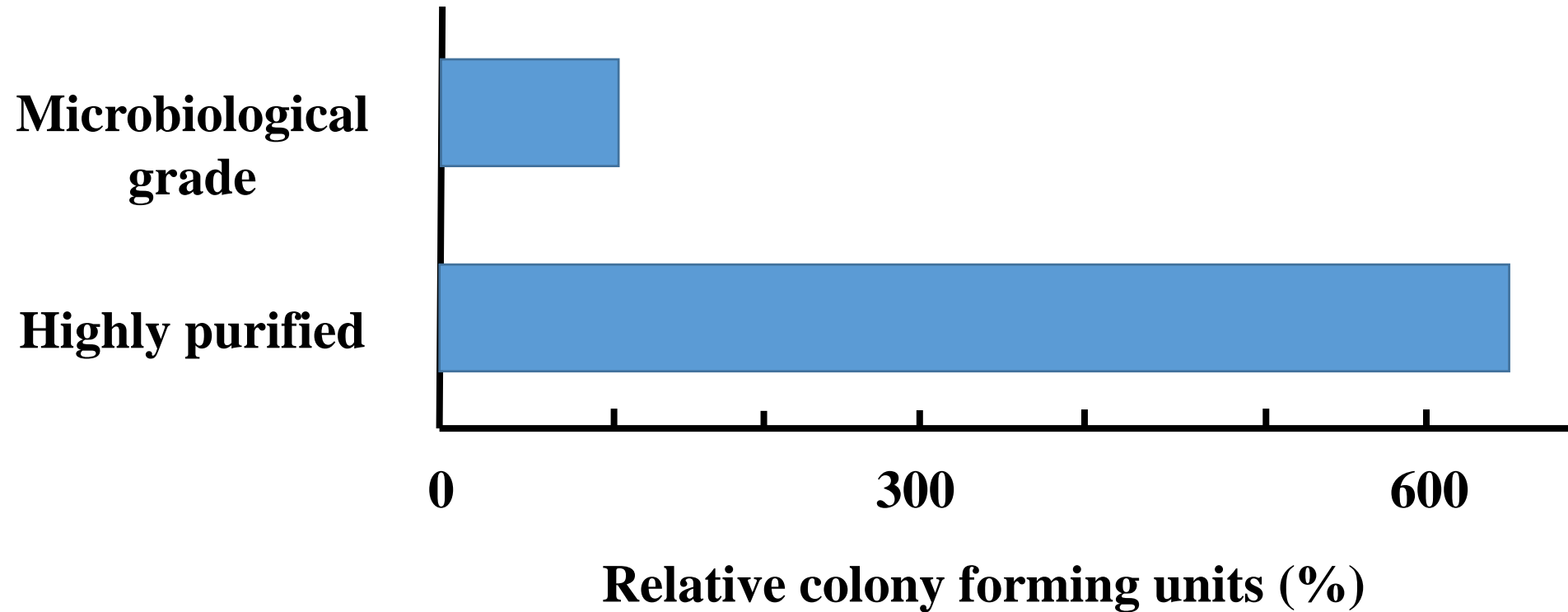
Time for detection is shown in days.

Anaerobic incubation at 25 degree Celsius (up to 14 days)

Experiments were conducted in triplicates.

Stealth mode *L. pastorianus/paracollinoides* grows optimally below pH 5.0 but its growth was considerably inhibited in the pH ranges over 5.3.

The effects of agar on the growth of JCM 11969^T



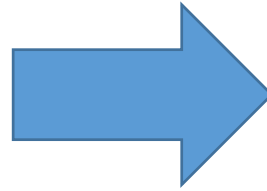
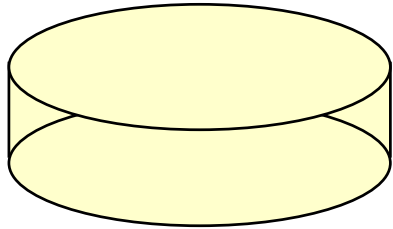
Most of the media used in the brewing industry are solidified with agar.

Summary 2

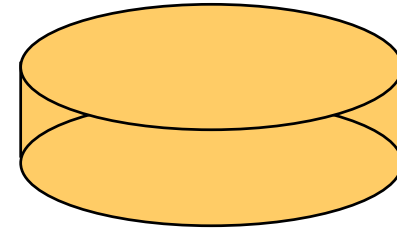
- 1. *L. pastorianus/paracollinoides* was sensitive to several nutrient sources supposed to facilitate the growth of lactic acid bacteria.**
- 2. Its optimal growth pH range lies below 5.0, reflecting the pH value of beer.**
- 3. *L. pastorianus/paracollinoides* exhibited sensitivities to agar components.**

Progress of microbiological culture media

Days of Pasteur and Van Laer



Present days



**Unhopped beer gelatin plate
(Home-made)**

Gelatin for solidification

Beer-based nutrient-poor medium

pH below 5.0

**MRS agar, Raka Ray etc.
(Commercially mass-produced)**

Agar for solidification

Nutrient rich medium

pH greater than 5.4

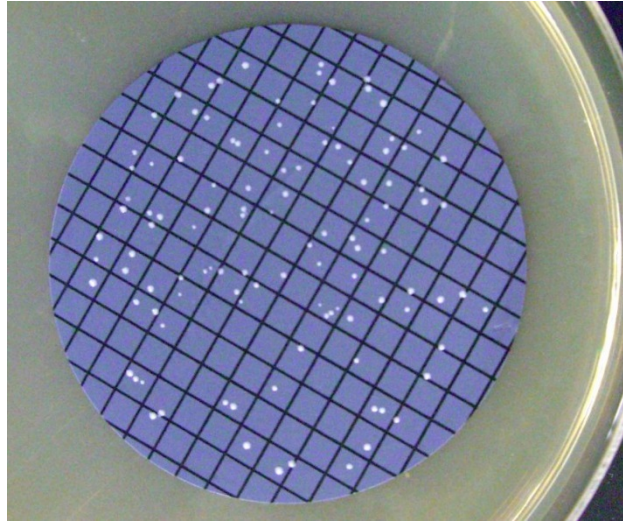
The progress of culture media may be one of the main reasons why *L. pastorianus* mysteriously vanished after 1950s.

Part 3

Survey of the brewing environments

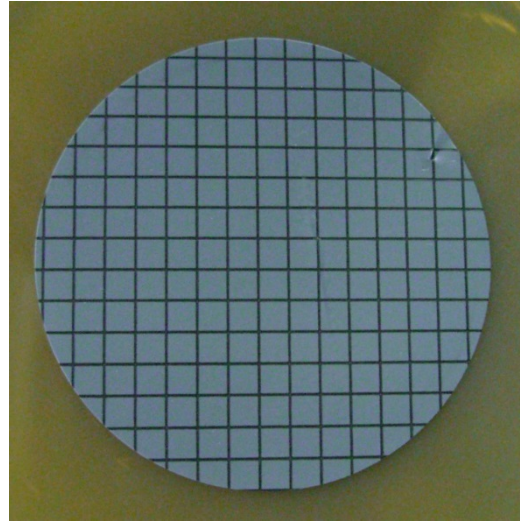
Survey of brewing environments

ABD



112CFU

MRS



0CFU

Swab sample
L. paracollinoides

ABD



2408CFU

MRS

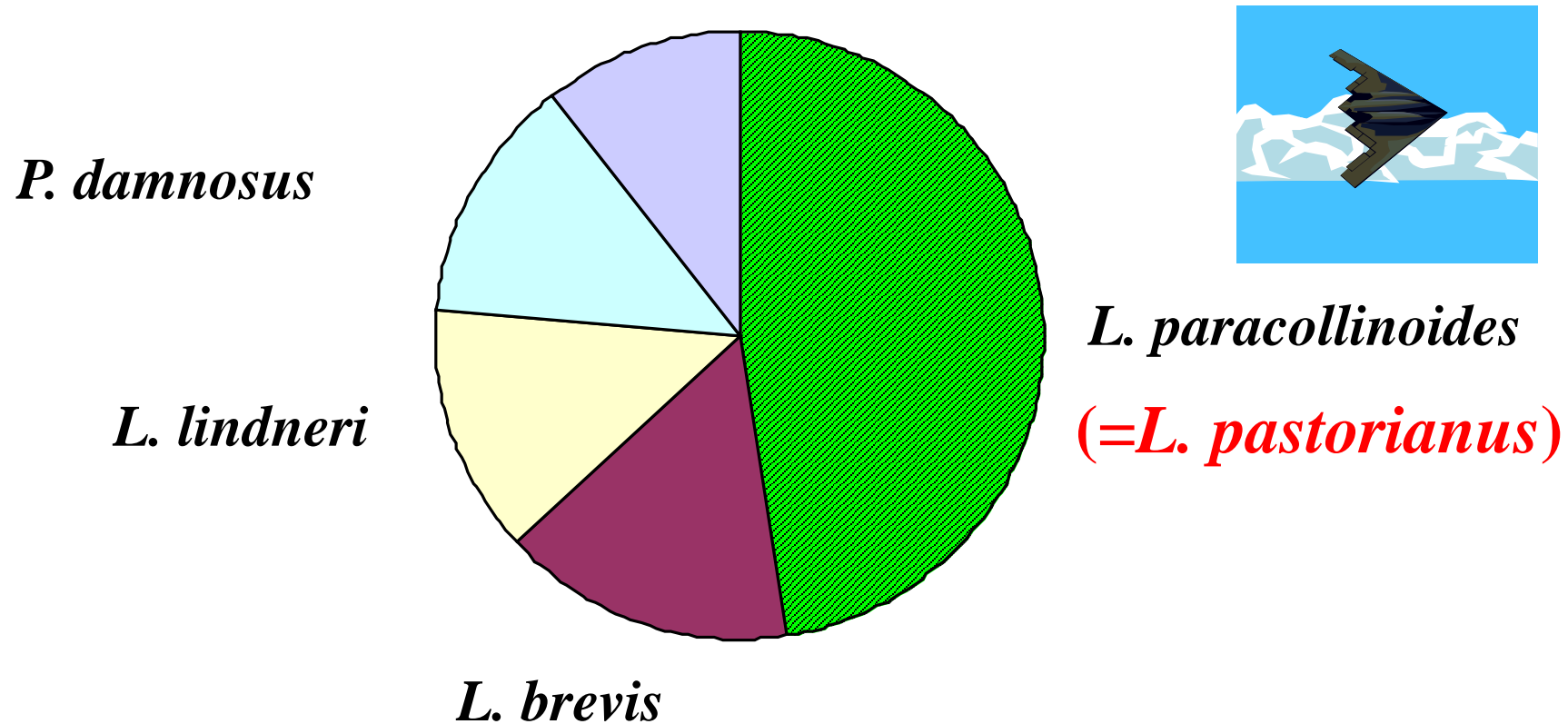


0CFU

Recycled container (Keg beer)
L. paracollinoides

The samples were inoculated in degassed beer and incubated anaerobically at 25 degree Celsius for up to 14 days. Only the visibly turbid beers were diluted and tested for culturability. A total of 38 beer spoilage strains were obtained.

Ubiquitous presence in the brewing environments



All the 18 *L. pastorianus/paracollinoides* strains exhibited stealth mode and did not grow on conventional media.

What does this study mean ?



***L. pastorianus* has been ubiquitous in brewing environments but escaped the detection by culture media for so many decades.**

**All the *L. pastorianus/paracollinoides* strains possess
strong beer spoilage ability**

Strains	Growth in degassed beer (days)
JCM 11969^T	+ (6)
JCM 15728	+ (4)
JCM 15729	+ (4)
JCM 15730	+ (4)
JCM 15731	+ (6)
Strain Lp9	+ (5)
Strain Lp10	+ (4)
Strain Lp11	+ (4)
Strain Lp12	+ (4)
Strain Lp13	+ (4)
Strain Lp14	+ (4)
Strain Lp15	+ (4)
<i>L. brevis</i> ABBC45	+ (4)
<i>L. lindneri</i> DSM 20692	+ (5)

**Degassed pilsner beer
(pH4.2) 25 degree Celsius
Inoculation: 3 x 10³ cells/ml**

***L. pastorianus/
paracollinoides*
strains**

Conclusions

- 1. *L. pastorianus/paracollinoides* exhibits unique culturabilities and requires a special culture medium for detection.**
- 2. *L. pastorianus/paracollinoides* vanished after 1950s due largely to the progress of microbiological culture media.**
- 3. *L. pastorianus/paracollinoides* is widely distributed in the brewing environments and this study helps us develop a better culture medium for the detection of this potent beer spoilage species.**

*Thank you for
your kind attention !*

