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Taxonomic study of a novel beer-spoilage Lactobacillus species closely related to *Lactobacillus rossiae*

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Summary

✓ Although beer has high microbiological stability, few species can spoil beer and produce turbidity, off-flavor, and/or undesirable taste.
✓ To control the microbiological quality, brewers must understand the characteristics of each beer-spoilage microorganisms.
✓ In recent years, novel beer-spoilage microorganisms have been reported.
✓ This study reports beer-spoilage ability and taxonomy of VTT E-94560 isolated from beer.

Origin and current taxon of VTT E-94560

Isolated From Finland beer → **deposited** to VTT culture collection as *Leuconostoc lactis* → **reclassified** as *Lactobacillus rossiae**

*The potential beer-spoiler studied in the present report [ref.(3)].

Physiological characterization

The distinction between VTT E-94560 and *L. rossiae* JCM 16176^T as determined via typical physiological tests, is summed up (Table 1).

✓Cells of VTT E-94560 were shorter than those of *L. rossiae* JCM 16176^T.
✓VTT E-94560 can ferment many more kinds of carbohydrates than *L. rossiae* JCM 16176^T ; however only JCM 16176^T can ferment L-Arabinose

Table 1
Physiological defferences between VTT E-94560 and *L. rossiae* JCM16176^T
+, positive reaction
-, negative reaction
w, weak reaction

	Lactobacillus sp. VTT E-94560	*L. rossiae* JCM 16176^T
cell width x length(μm)	0.5 x 0.5-1.0	0.5 x 1.0-1.5
Acid production from:		
L-Arabinose	-	+
D-Mannose	+	w
Melibiose	+	-
D-Arabitol	+	-
Gluconate	+	w
2-ketogluconate	+	-

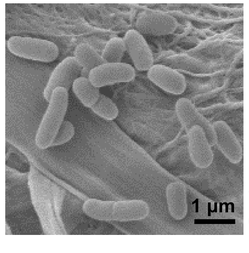


Fig. 1 SEM observation of cultured cells
VTT E-94560 cells were cultured at 25℃ in degassed pilsner-type beer adjusted to pH5.0.

Beer-spoilage ability

(1) Bacterial inoculation tests
Inoculated and incubated at 25° C, examined for visible growth up to 90 days (Fig. 2, Table 2).

Strain:
VTT E-94560 or *L. rossiae* strains
Sample:
bottled commercial pilsner beers (pH 4.0–4.6, 10–20 BU, alc. 4.8–7.1%)
Concentration: 1,000 cfu/ml

(2) Hop resistance genes (*horA* and *horC*)-specific PCR test
A relationship between beer-spoilage ability and the presence of hop resistance genes were reported in some lactic acid bacteria .
Presence of hop resistance genes, *horA* and *horC,* were examined by PCR test using *horA or horC*-specific primers [ref.(1),(2)] (Table 2).

Fig.2 Example of spoiled beers caused by inoculating of VTT E-94560
Using commercial bottled pilsner beer (pH4.85, 20BU, alc.7%).
Left, uninoculated (negative control)
Right, inoculated

Table 2
Results of inoculation tests and hop resistance genes-specific PCR test
(1) inoculation tests, -, no growth; numbers (1-90), days to cause visible growth
(2) PCR tests, +, positive; - , negative

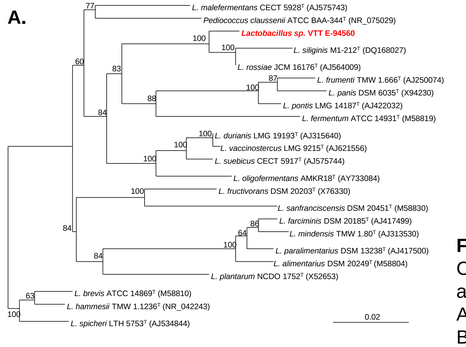
strains	(1) inoculation test 10BU pH4.0 alc.4.8%	17BU pH4.4 alc.5.0%	20BU pH4.6 alc.7.1%	(2) PCR test *horA*	*horC*
Lactobacillus sp. VTT E-94560	**30**	**80**	**23**	**+**	**+**
L. rossiae JCM 16176^T	N.T.	-	-	-	-
L. rossiae ABBC 636-639*	N.T.	-	N.T.	-	-

*ABBC 636-639 are our original *L. rossiae* strains isolated from brewing environment

✓ VTT E-94560 carried both *horA* and *horC* and showed beer-spoilage ability.
✓ Under these testing conditions, including beer-type and strain selection, both the beer-spoilage ability and the presence of hop-resistance genes of VTT E-94560 differ from those of other *L. rossiae* strains.

Taxonomic study

To clarify the classification of VTT E-94560, sequence analysis and DNA-DNA hybridization were performed（Fig.3）.



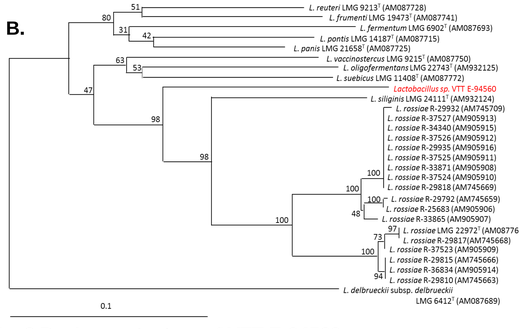


Fig.3 Phylogenetic tree of VTT E-94560
Constructed using the neighbor-joining method. Percentages at nodes were obtained from 1,000 bootstrap replications.
A.Based on the complete 16S rRNA gene sequence
B.Based on the partial *pheS* * gene sequence
*reported to allow the analysis intraspecific heterogeneity.

✓ The gene sequence similarity between the complete 16S rRNA of VTT E-94560 and that of *L. rossiae*^{T}, the most closely related species to VTT E-94560, was **97%**, which is lower than the minimum value (98.7%) required for the species to be identified as the same.
✓ The phylogenetic tree based on the partial *pheS* gene sequence suggested that VTT E-94560 differed from other *L. rossiae* strains over the intraspecific heterogeneity level.
✓ DNA-DNA relatedness between VTT E-94560 and *L. rossiae* JCM 16176^T was **4%**, which is too low for the species to be identified as the same.

conclusion

✓ **VTT E-94560 belongs to a novel Lactobacillus species.**
✓ **VTT E-94560 showed beer-spoilage ability when used to inoculate low bitterness beers.**

reference

(1) A new and rapid method for determination of beer-spoilage ability of lactobacilli. Sami *et al*., 2007.
(2) Isolation of hop-sensitive variant from *Lactobacillus lindneri* and identification of genetic marker for beer spoilage ability of lactic acid bacteria. Suzuki *et al*., 2005.
(3) Beer spoiling microorganisms – a current over view. Hutzler *et al*., 2013.
(4) VTT web site (http://culturecollection.vtt.fi/m/html?p=m)
(5) Reclassification of *Lactobacillus rossiae* VTT E-94560 as *Lactobacillus curtus* sp. nov. Asakawa *et al*., in submission

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