# The Effect of Lactic Acid Bacteria on Congener Composition and Sensory Characteristics of Scotch Malt Whisky

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# ABSTRACT

Lactic acid bacteria (LAB) comprise a major part of the natural microflora of Scotch malt whisky fermentations, due to their tolerance of heat and elevated ethanol concentrations. In this study, their effects on the organoleptic properties of the spirit were investigated. Samples from late (>70 h) fermentations were obtained from whisky distilleries throughout Scotland. Bacteria of varying colony morphologies were isolated, purified, and characterised initially using random amplification of polymorphic DNA – polymerase chain reaction (RAPD-PCR). Isolates with differing RAPD patterns were retained and their ability to produce 10-hydroxystearic acid (10-HSA) from oleic acid was determined qualitatively using high performance thin layer chromatography. 10-HSA is the primary precursor of γ-dodecalactone, which is an important flavour compound in malt whisky responsible for the desirable "sweet and fatty" characteristic of the spirit. Thirty-nine isolates had strong or weak bioconversion activity while 89 isolates displayed negligible or no activity. Forty-two strains, largely from the former category were identified using partial 16S rRNA gene sequences. *Lactobacillus paracasei* was the predominant organism but *L. brevis* and *L. plantarum* were also identified. These 42 strains were assessed for their bioconversion capacity in a semi-quantitative manner using gas chromatography – mass spectrometry (GC-MS) and five isolates, comprising *L. brevis*, two strains of *L. paracasei*, and two strains of *L. plantarum* were selected for further study. These isolates were used in laboratory–scale, simulated whisky fermentations with *Saccharomyces cerevisiae.* Fermentation liquor (wash) was distilled to produce new-make spirit, which was analysed organoleptically by quantitative descriptive analysis. Spirit from fermentations inoculated with *L. brevis* had an enhanced "sweet" character, probably due to the higher γ-lactone levels detected in this whisky, as well as increased "sulfury" and "meaty" notes, most likely due to yeast autolysis. *L. paracasei* enhanced the "green/grassy" notes of new-make spirit, while also adding a "sour" aroma probably resulting from the elevated levels of lactic acid detected in the wash. Like *L. paracasei*, *L. plantarum* increased the "green/grassy" notes of new-make spirit. Further fermentations were carried out in which *L. brevis,* one strain of *L. paracasei*, and one strain of *L. plantarum* were inoculated into fermentations with yeast comprising 90% *S. cerevisiae* and 10% *Torulaspora delbrueckii*, which had been

isolated previously from Scotch whisky fermentations and shown to enhance the concentration of γ-lactones in new-make spirit. Co-fermentation of *L. brevis* with *S. cerevisiae* and *T. delbrueckii* resulted in a spirit with increased "green/grassy", "sweet", and "oily" notes, with decreases in "sulfury" and "meaty" observed when the wild yeast was not present. Spirit derived from co-fermentations of *L. paracasei* and *T. delbrueckii* exhibited increased "soapy", "sour", and "sulfury" notes. Co-fermentation of *L. plantarum* and *T. delbrueckii* caused increases in "green/grassy", "soapy", "sweet", "sour", and "sulfury" notes. Increased concentrations of γ-lactones were detected in new-make spirit distilled from fermentations inoculated with *L. brevis*, presumably contributing to the enhanced sweet character of this spirit. This effect was further amplified by the inclusion of *T. delbrueckii* in the laboratory–scale fermentations.

**For Christine, Neil, and Christopher**

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## ABBREVIATIONS

| | |
|---|---|
| 6-PG/PK | 6-phosphogluconate/phosphoketolase |
| 10-HPA | 10-hydroxypalmitic acid |
| 10-HSA | 10-hydroxystearic acid |
| 10-KSA | 10-ketostearic acid |
| ABV | alcohol by volume |
| ATP | adenosine triphosphate |
| Å | Angstrom(s) |
| BHI | brain-heart infusion |
| BLAST | basic local alignment search tool |
| C | carbon |
| conc. | concentration |
| D | aspartic acid |
| Da | Dalton |
| DHAP | dihydroxyacetonephosphate |
| DNTP(s) | deoxyribonucleoside 5'-triphosphate |
| E | glutamic acid |
| *E.* | *Escherichia* |
| EDTA | ethylenediaminetetraaceticacid |
| f | forward |
| Fe | iron |
| GAP | glyceraldehyde-3-phosphate |
| GC | gas chromatography |
| G+C | guanine and cytosine content |
| h | hour(s) |
| HPAE | high performance anion exchange |
| HPLC | high performance liquid chromatography |
| HPTLC | high performance thin layer chromatography |
| ICBD | International Centre for Brewing and Distilling |
| K | lysine |
| $K_m$ | Michaelis-Menten kinetics |
| *L.* | *Lactobacillus* |

| | |
|---|---|
| LAB | lactic acid bacteria |
| LOX | lipoxygenase |
| min | minute(s) |
| mol% | molar percentage |
| MS | mass spectrometry |
| $NAD^+$ | nicotinamide adenine dinucleotide |
| NADH | reduced nicotinamide adenine dinucleotide |
| O.D. | optical density |
| o/n | overnight |
| *P.* | *Pseudomonas* |
| PCA | principal component analysis |
| PCR | polymerase chain reaction |
| PEP-PTS | phosphoenoylpyruvate dependent-phosphotransferase system |
| $pK_a$ | acid dissociation constant |
| PMF | proton motive force |
| ppb | parts per billion |
| ppm | parts per million |
| r | reverse |
| RAPD | randomly amplified polymorphic DNA |
| rRNA | ribosomal ribonucleic acid |
| *S.* | *Saccharomyces* |
| SD | standard deviation |
| SDS-PAGE | sodium dodecyl sulphate-polyacrylamide gel electrophoresis |
| SG | specific gravity ($g\ l^{-1}$) |
| *Sp.* | *Sporobolomyces* |
| spp. | species |
| SWRI | Scotch Whisky Research Institute |
| *T.* | *Torulaspora* |
| Taq/taq | *Thermus aquaticus* |
| vol. | volume |
| (v/v) | volume:volume ratio |
| (w/v) | weight:volume ratio |
| x g | times gravity |

## PRESENTATIONS

"The Effects of Lactic Acid Bacteria on the Sensory Characteristics of New-Make Scotch Whisky."

- 9th Symposium on Lactic Acid Bacteria: Health, Evolution and Systems Biology; August 31st-September 4th 2008, Egmond aan Zee, The Netherlands.

"Effects of Lactic Acid Bacteria during Malt Whisky Fermentation on Spirit Quality."

- Worldwide Distilled Spirits Conference; September 7th-10th 2008, Edinburgh, Scotland, United Kingdom.