MASTER BREWERS ASSOCIATION OF THE AMERICAS

MBAA Annual Conference
June 5–7, 2014
Palmer House, a Hilton Hotel
Chicago, IL

THE TYPE AND PROPERTIES OF YEASTS AND BACTERIA ISOLATED DURING A MICROBIOLOGICAL SURVEY OF LINDORES ABBEY

Christopher J. Burke, Alex Speers & Annie E. Hill, The International Centre for Brewing & Distilling, Heriot-Watt University, Edinburgh, Scotland, UK.

Introduction

The first recorded transaction involving *aqua vitae* was between the Benedictine monks at Lindores Abbey and the Court of King James IV in 1494.

"To Friar John Cor, by order of the King, to make *aqua vitae* VIII bolls of malt" (Exchequer, 1494)

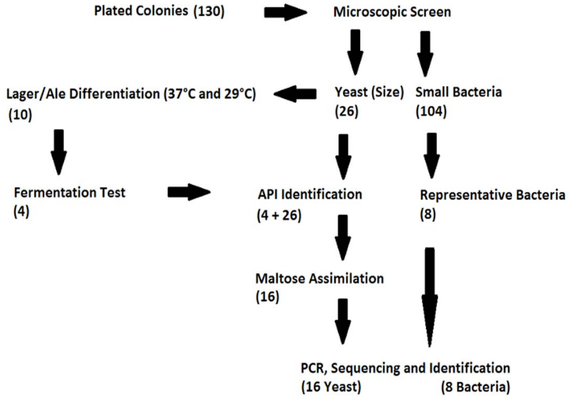
This entry in the Exchequer Rolls of Scotland is the first written record of *aqua vitae* production in Scotland and designates Lindores Abbey as the birthplace of Scotch whisky. Few details of the production methods employed in the creation of the first whiskies are available. Information on whisky fermentation microbiology information is particularly sparse, mainly due to the assumption, until relatively recently, that alcohol production was a chemical process (or an act of God). Traditionally, the wash was fermented in open, wooden wash backs which would have facilitated spontaneous fermentation from yeast and bacteria present in raw materials and equipment. Contact with air also played an important part of fermentation and wild yeast and bacteria would have had a large impact on the final spirit. Originally, any yeast species were considered good enough to start fermentation and it was likely that once one was found it would have been used to inoculate batch to batch.

Comparison of the ruins of Lindores Abbey from 1884 and 2013

With a proposal in place to build a new distillery at Lindores Abbey a survey of the microbiological flora of the Abbey was carried out. Fermentation tests were used to determine the potential of native yeast strains for whisky production.

Methods

The following flow chart was used for the isolation and identification of wild bacteria and yeast:



Air (passive and active sampling) and swab samples were collected from three sites around Lindores Abbey using seven different types of selective media including distillers wort supplemented with ethanol. The yeast and bacterial isolates were recognized by colony and microscopic morphology. Yeast isolates were identified using the API 20C Aux test and by partial 26S ribosomal gene sequencing whereas representative bacterial colonies were identified by partial 16S ribosomal gene sequencing.

Results and Discussion

The results from the microscopic identification of colony isolates are shown in Table 1. Nutrient agar plates had the highest number of bacterial isolates with 30, and 8% ethanol wort plates had the least with 1 (Table 1). WLN plates had the highest number of yeast isolates with 7, and the nutrient and MRS plates had the least with 0. The number of bacterial isolates greatly outnumbered the amount of yeast isolates by a factor of four to one.

Table 1. Colony isolation and identification by microscopy, 400x magnification

Plate Type	Bacterial Colonies	Yeast Colonies	Total Number of Colonies	Percent Bacteria	Percent Yeast
Nutrient	30	0	30	100	0
WLN	15	7	22	68	32
YPD	24	4	28	86	14
MRS	8	0	8	100	0
Wort	12	6	18	67	33
4% Wort	14	6	20	70	30
8% Wort	1	3	4	25	75
Total	104	26	130	80	20

The representative bacterial isolates were identified as: *Brevibacterium frigoritolerans, Nocardioides*, *Exiguobacterium undae*, *Sphingomonas aerolata*, *Enterococcus faecium*, *Enterococcus hirae*, *Pseudomonas* and *Bacillus pumilus*.

The yeast isolates identified by sequencing were: *Aureobasidium pullulans, Cryptococcus wieringae, Parabodo nitrophilus, Cryptococcus tephrensis, Metschnikowia koreensis*, *Debaryomyces hansenii, Metschnikowia fructicola* and *Saccharomyces cerevisiae.*

The majority of bacterial and yeast isolates were determined to have little or no relevance in a distillery fermentation. Therefore, those isolates would also have had very little effect on the production and flavour of the first whiskies. Of the bacteria identified, only *Pseudomonas* sp. would potentially have an effect on new make spirit as it can grow in wort and produce H_2S, which would have imparted a sulphidic aroma.

The *Metchnikowia* species isolated may initiate spontaneous fermentation but the final effect on ethanol and flavour production still need to be investigated. *D. hansenii* may also initiate spontaneous fermentation and can result in a yeasty and ester characteristic to the whisky produced. The strains of *S. cerevisiae* isolated have the potential to be used for the fermentation of *aqua vitae*. A *S. cerevisiae* isolate LCBG-3D6 has previously been isolated from the spontaneous fermentation of *Agave* spp. used to produce Mezcal. It has also been shown to spontaneously ferment alcohol, which is what would have occurred in the first whisky fermentations, and is likely the yeast, or the progeny of the yeast, that fermented the first whisky.

Conclusions

Pseudomonas was the only bacterium identified that may affect the flavour of whisky. Two *Metschnikowia* species of yeast, *M. koreensis* and *M. fructicola,* could initiate fermentation, while *D. hansenii* may impart a yeasty or estery flavour. Three strains of *Saccharomyces cerevisiae* were isolated which could be used in production with strain LCBG-3D6 displaying the most promise.

Acknowledgements
Thanks to Drew Mackenzie-Smith for background information and access to Lindores Abbey. Dr Ash Paradh, Paul Cyphus and Graham McKernan are all thanked for technical assistance.

For further information
Please contact Dr Annie Hill (*a.hill@hw.ac.uk*). More information on this and related projects can be obtained at *www.icbd.hw.ac.uk.*