Quality control begins with yeast culturing

YEAST IN ITS PUREST FORM | Consistency in beer quality is dependent on many factors. In addition to water, malt and hops, brewing yeast is an essential raw material and plays an important role in successfully producing beers of premium quality. The Weihenstephan Research Center for Brewing and Food Quality at Technische Universität München has developed a comprehensive quality control program for the culturing and propagation of brewing yeast, which fulfills the stringent demands placed on their pure yeast cultures by brewers.

THE INSTITUTE OFFERS a broad variety of yeast strains for brewing and distilling, as well as strains for the production of wine and sparkling wine. Bacteria strains are also available for use in creating unique fermented beverages or for biological acidification. An overview of the fermentation microorganisms available, including information on their taxonomy and application is provided in table 1.

Pure yeast cultures are available for delivery in three different forms and can be individually ordered according to the specific needs of the brewery: on agar slants, as a yeast suspension (approximately 10 ml) on sterile cotton or as liquid yeast (approximately 500 ml) in a sterile aluminum bottle. Quality control plays a critical role in each step of yeast propagation. In order to guarantee that a culture is free from beer spoilage microorganisms and other contaminants, various molecular and microbiological methods are utilized. The propagation times required for the different forms of yeast cultures available for delivery are shown in figure 1, including the times when samples are collected for quality control.

Absolute purity

As part of its quality control program, the Weihenstephan Research Center uses selective media to test the pure yeast and bacteria cultures as well as their cultivation media (e.g. wort) and the containers used for shipping, in order to detect any contaminants (bacterial beer spoilers, yeast strains differing from the pure culture, other flora such as wort bacteria). Pure yeast and bacteria cultures should

AVAILABLE FERMENTATION MICROORGANISMS, THEIR TAXONOMY AND APPLICATION

	Fermentation/flocculation characteristics		Type of beer or beverage	Genus/species
Brewing yeast strains	bottom-fermenting	flocculent	pilsener, export, helles, lager, bottom-fermented specialty beer, bottom-fermented alcohol-reduced beer, etc.	*S. pastorianus (ssp. carlsbergensis)*
		non-flocculent		
	top-fermenting	non-flocculent	wheat beer, ale, stout, kölsch, altbier, Belgian specialty beers, etc.	*S. cerevisiae*
	top-fermenting/ bottom-fermenting	non-flocculent	low-alcohol beer	*Saccharomycodes ludwigii*
Distiller's yeast, wine yeast, sparkling wine yeast strains	top-fermenting	non-flocculent	wine, fruit wine, sparkling wine, distiller's mashes, etc.	*S. cerevisiae*
Bacteria strains	–	–	malt-based beverages fermented with alternative microorganisms	*Gluconobacter sp.* *L. amylolyticus* *L. amylovorus*

Table 1

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not contain any other microorganisms. In other words, the starter culture must be absolutely free of contaminants. Within this context, the pure culture strains classified as different species (e.g. top-fermenting *Saccharomyces cerevisiae* brewing yeast strains and bottom-fermenting *Saccharomyces pastorianus ssp. carlsbergensis* brewing yeast strains) are tested using different nutrient media to detect any potentially damaging flora. The same applies to special yeast strains – typically non-*Saccharomyces* strains – and to bacteria starter cultures as well. The goal of the quality control program is to ensure that customers ordering cultures from the Weihenstephan Research Center for Brewing and Food Quality receive starter cultures only of the highest purity.

The methods used to test the nutrient media, the containers used to ship strains of bacteria and special yeast will not be further addressed here, as the primary focus of this article is to describe the quality control measures for top-fermenting and bottom-fermenting strains of brewing yeast.

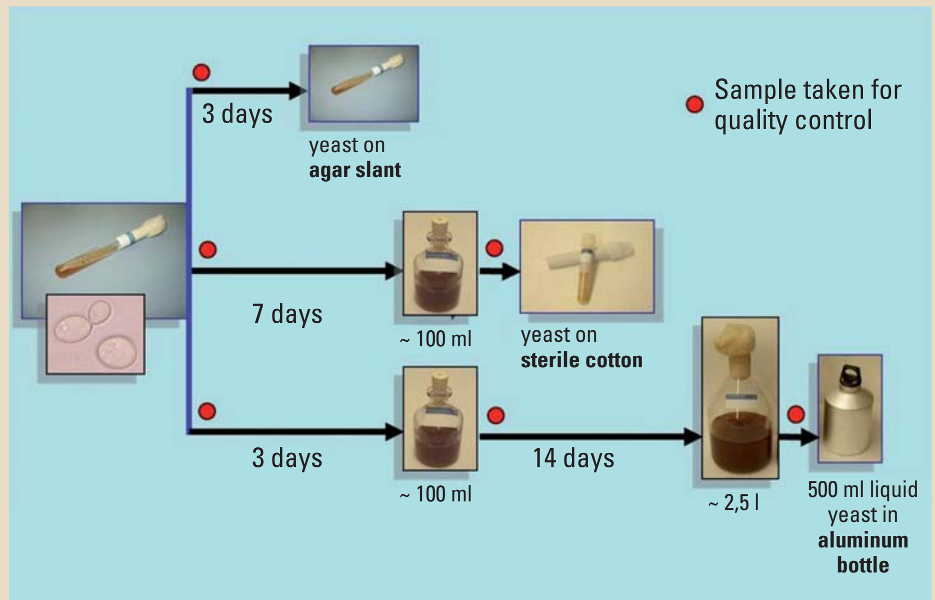

Fig. 1 Propagation times required for the different forms of yeast available for delivery showing the times samples are collected for quality control

The comprehensive procedures used to test the purity of yeast cultures and the corresponding analysis methods are depicted in table 2.

Detection of contaminants

Bottom-fermenting brewing yeast strains are incapable of growth at a temperature of 37 °C. However, the majority of wild yeast

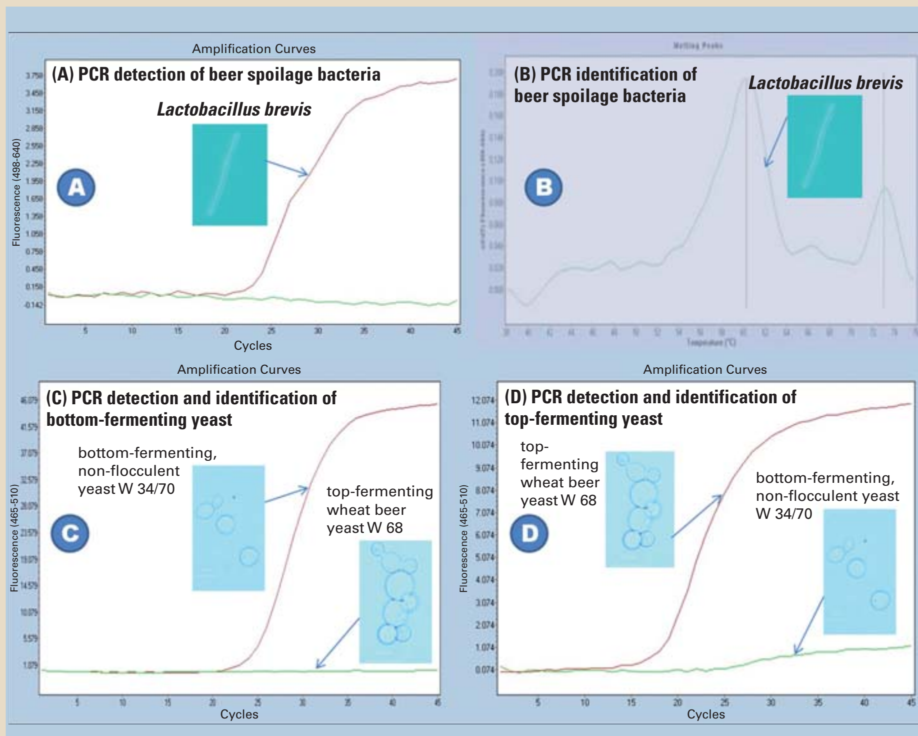

Fig. 2 Detection (A) and identification (B) of beer spoilage bacteria with *Lactobacillus brevis* as an example, and the detection and identification of bottom-fermenting yeast *S. pastorianus ssp. carlsbergensis* (C) and top-fermenting yeast *Saccharomyces cerevisiae* (D) by means of real time PCR analysis

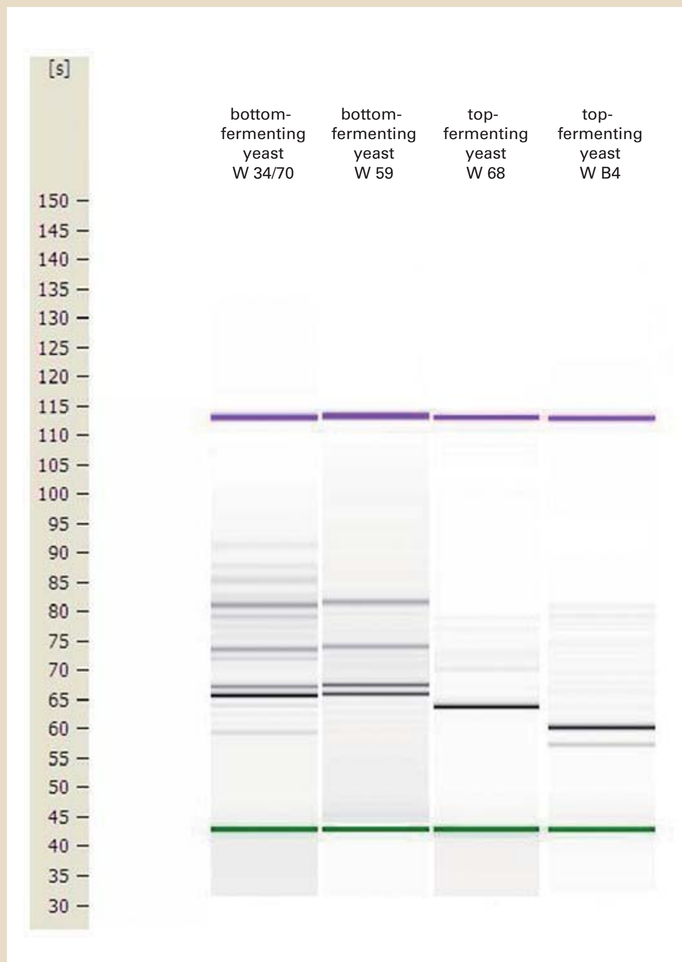

Fig. 3 Differentiation of yeasts at the strain level using PCR capillary electrophoresis with *Saccharomyces pastorianus ssp. carlsbergensis* W 34/70, W 59 and *Saccharomyces cerevisiae* W 68, W B4 as examples

species and top-fermenting yeast strains can grow at this temperature [1, 3]. If a universal growth medium for yeast, such as wort, YPG, YGC or YM is inoculated with bottom-fermenting yeast and incubated at 37 °C, any contamination with wild yeast or top-fermenting yeast can be detected (table 2.1) [3].

Brewing yeast cannot grow in a nutrient medium if the concentration of copper sulfate ($CuSO_4$) exceeds 200 ppm. The growth of most wild yeasts is not inhibited at this concentration. Therefore, the addition of $CuSO_4$ at 200 ppm to a universal growth medium for yeast (e.g. YM) can serve as a means for detecting wild yeast contaminations in top- and bottom-fermenting brewing yeast samples (table 2.2) [2, 3, 7].

Active pure yeast cultures are transferred to fresh medium every 4 - 6 weeks. In addition to the YM 37 °C and YM + $CuSO_4$ tests for contamination with wild yeast, the fresh cultures are also tested using selective media: crystal violet agar (*Saccharomyces*-type contaminants), lysine agar (non-*Saccharomyces*-type contaminants) and completely fermented beer (for the detection of over-attenuating yeasts) (table 2.3) [8, 9].

Pediococcus sp., *Lactobacillus sp.* and gram-negative beer spoilage bacteria can be detected using NBB nutrient broth under anaerobic conditions [1]. For this test, a drop or a swab sample of the yeast is placed in a tube containing NBB broth (table 2.4).

Pure yeast cultures should not harbor any other microorganisms (e.g. wort bacteria, indicator flora). Yeast infusion broth is suitable as a medium for the detection of bacteria in flora typically found with brewing yeast. Samples of brewing yeast placed in yeast infusion broth should exhibit no bacterial growth. This test is performed as part of the quality control for pure cultures, so that they are certain to be absolutely free from contaminants (table 2.5).

Although visual examination of yeast cultures using a microscope is considered imprecise (low limit of detection) and subjective (dependent on the level of expertise of the person operating the microscope), it remains indispensible as it affords a rapid overview of the purity of the yeast culture. Obvious production problems (wort with a high concentration of particulates, microbial flora as contaminants) can be at least initially identified and quickly evaluated with a microscope. If contaminants are readily evident during the initial examina-

tion, the yeast must be discarded immediately (table 2.6).

Fresh propagation yeast should contain the highest number of living cells, or conversely, the number of dead cells in the production yeast should be as low as possible. Dead yeast cells can be identified and quantified by staining with a solution of methylene blue; the number of cells is expressed as a percentage. The viability of the yeast should be no lower than 95 percent (table 2.7).

Rapid detection with PCR

Detection and evaluation using the selective media 2.1 to 2.5 can require anywhere from three to seven days. The Weihenstephan Research Center utilizes modern polymerase chain reaction (PCR) analysis methods in conjunction with conventional tests with culture media, so that conclusive information about the purity of a sample is available just 2 - 4 hours after it is collected. Analysis methods designed to detect obligate and potential beer spoilage bacteria are listed in tables 2.8 - 2.10, as well as those for top and bottom-fermenting yeast strains [3 - 6]. These methods offer rapid and reliable analysis not only for determining the correct identity of yeast samples, but are also used to test for trace contaminations with beer spoilage bacteria or cross-contaminations with the "wrong" type of brewing yeast (e.g. bottom-fermenting yeast with top-fermenting yeast or vice-versa) [3-5]. With PCR, false identification of top-fermenting and bottom-fermenting yeast as well as inoculation mistakes can be monitored and eliminated. Figure 2 shows the analysis results of real time PCR used in the quality control of pure yeast cultures, as it is used at the Weihenstephan Research Center. Graph A in figure 2 shows the detection of beer spoilage bacteria, graph B is the identification curve, while graphs C and D show the analysis of bottom and top-fermenting yeast, respectively. For example, if a bottom-fermenting yeast *S. pastorianus ssp. carlsbergensis* W 34/70 is tested and contains no contaminants, curves A, B and D will be negative, while C will be positive. With PCR, a rapid purity test can be completed within only four hours. The Weihenstephan Research Center also employs additional PCR tests for wild yeast strains, acetic acid bacteria, lactic acid bacteria and *Enterobacteriaceae* in cases of suspected contamination and for batch quality control.

Taxonomic differentiation of yeast strains

In addition to the methods described above, differentiation to the strain level is also possible using a combination of PCR and capillary electrophoresis methods (see figure 3).

An example of the banding patterns resulting from capillary electrophoresis which allow *Saccharomyces pastorianus ssp. carlsbergensis* W 34/70, W 59 and *Saccharomyces cerevisiae* W 68, W B4 to be distinguished from one another is shown in figure 3. W 34/70 and W 59 are two types of flocculent, bottom-fermenting yeast; W 68 is a top-fermenting wheat beer yeast and W B4 is a distiller's yeast.

This method is suitable for routine monitoring of strain identity. If the banding pattern varies from the original pattern that is specific for the species, this is an indication that the strain is either contaminated with another species or that the strain has changed genetically.

The Weihenstephan Research Center is working on expanding this system and creating a reference data bank of capillary electropherograms.

ANALYSIS METHODS FOR QUALITY CONTROL OF PURE YEAST CULTURES

	Culturing method				
Detection of	**wild yeast strains and top-fermenting Saccharomyces yeast strains**	**wild yeast strains**	**foreign yeast strains**	**bacteria**	
Method	**1. 37 °C test = hopped wort (bottom-fermenting)**	**2. YM + $CuSO_4$ (top-fermenting)**	**3. crystal violet, lysine, fully attenuated beer (batch quality control)**	**4. NBB broth**	**5. Yeast infusion broth**
Saccharomyces pastorianus ssp. carlsbergensis bottom-fermenting yeast	X		X	X	X
Saccharomyces cerevisiae top-fermenting yeast		X	X	X	X
	microscopy methods		**molecular biology tests**		
detection of	**foreign microbes**	**dead yeast cells**	**bacteria**	**identification of yeast strains**	
Method	**6. Visual examination with microscope Foreign microbes Foreign particles**	**7. Viability Examination for dead cells in %**	**8. PCR screening beer spoilage bacteria**	**9. PCR *Saccharomyces pastorianus***	**10. PCR *Saccharomyces cerevisiae***
Saccharomyces pastorianus ssp. carlsbergensis bottom-fermenting yeast	X	X	X	X	X
Saccharomyces cerevisiae top-fermenting yeast	X	X	X	X	X

Table 2

Summary

An overview of selected quality control measures typically used with top and bottom-fermenting brewing yeast cultures is provided above. Equivalent quality control measures are employed for all other microorganisms available from the Weihenstephan Research Center.

All microscopy, microbiology and molecular biology methods are performed in the laboratory of the Microbiology department at the Weihenstephan Research Center, which is accredited according to DIN EN ISO/IEC 17025:2005. ■

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