DIASTATICUS

WHAT IS S. CEREVISIAE VAR. DIASTATICUS?

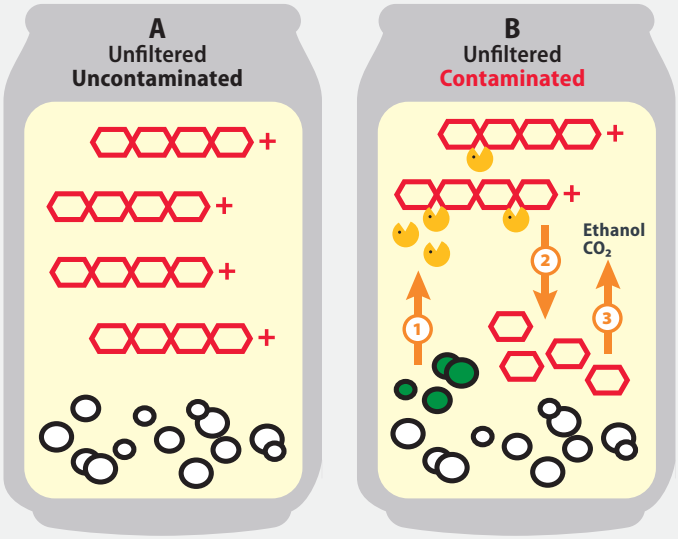
Brewers wort contains different types of sugars (Figure 1), but not all sugars can be metabolized equally by the yeast. Most *S. cerevisiae* strains will metabolize glucose, maltose and maltotriose. For these strains, the unfermented dextrins contribute sweetness and body to the beer. Since dextrins account for 20-30% of the original sugars, this corresponds to an attenuation of 70-80% for most brewing strains. Some natural variants do not metabolize maltotriose (i.e. English strains such as LalBrew Windsor™ and LalBrew London™) and have lower attenuation and more sweetness and body in the finished beer. Some wild yeast strains are able to metabolize dextrins in addition to smaller sugars and achieve attenuation of >90%.

A few *S. cerevisiae* variants are also able to metabolize dextrins to achieve very high attenuation. These strains are referred to as *S. cerevisiae* var. *diastaticus* and possess STA (1, 2 or 3) genes that encode a glucoamylase enzyme that breaks down dextrins into smaller fermentable sugars. Some diastaticus strains have been domesticated for use in Farmhouse/Saison style beers where a higher level of attenuation is desired.

Diastaticus strains are typically alcohol tolerant and able to ferment at high temperatures. Glucoamylase enzymes are thermotolerant and can remain active even after pasteurization. These robust diastaticus strains pose a risk of cross contamination in the brewery if stringent sanitation protocols are not respected. The glucoamylase enzyme is secreted into the fermenting beer, so the simple sugars released by this enzyme can be fermented by any yeast strain. A diastaticus contamination in a beer fermented with standard brewing yeast may result in over-attenuation, higher levels of alcohol, over-carbonation, exploding bottles or cans and possible phenolic off-flavors (Figure 2). But when handled correctly, *S. cerevisiae* var. *diastaticus* strains are magnificent yeast that can produce dry beers with great flavors.

		% of normal wort
	Glucose	10-15
	Maltose	50-60
	Maltotriose	15-20
+	Dextrin	20-30

Figure 1. Typical sugar composition of a normal brewing wort.



In unfiltered, unpasteurized, contaminated beer:

- Over-attenuation
- Higher alcohol (possible regulatory violation)
- Excessive carbonation and gushing
- Exploding packages
- Possible phenolic off-flavors

Figure 2. Contamination by a *S. cerevisiae* var. *diastaticus* strain can result in additional fermentation in packaged beer. (A) In the absence of a diastaticus strain, dextrins are stable in the finished beer. **(B)** (1) A contaminating diastaticus strain (green) secretes a glucoamylase enzyme (orange). (2) Dextrins are broken down into simple fermentable sugars by the glucoamylase enzyme. (3) Simple sugars are fermented resulting in increased ethanol and CO_2 levels. Image credit to Dr. Matt Farber, University of the Sciences. [1]

WHAT ARE COMMON SOURCES OF DIASTATICUS CONTAMINATION?

Diastaticus strains are found in many environments. As a result, cleaning and sanitation are highly important for avoiding diastaticus contamination. We encourage you to speak with your local chemical representatives to establish a cleaning and sanitation regimen conducive to your brewery and specific needs.

Sources

Poor Hygiene:

- Bottling/canning lines (>70% of reported cases) [2]
- Brewhouse
- Fermentation cellar
- Storage cellar

Raw Materials:

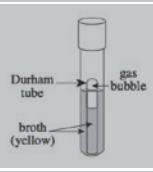
- Contaminated Yeast
- Contaminated Hops (dry hopping)
- Addition of unpasteurized ingredients (fruits, juices, herbs/spices)

* **Check with your raw material suppliers for information about how they detect and control diastaticus contamination.**

DIASTATICUS

HOW CAN I DETECT IT?

Diastaticus strains can cause over-attenuation due to metabolism of dextrins when present even in very low levels (<10 cells per ml). Therefore, detection of diastaticus contamination is an important part of any brewery QC program, especially for breweries who use known diastaticus strains in their beers. There are several methods for detecting diastaticus contamination, each with their own advantages and disadvantages.

NAME	DESCRIPTION	PROS	CONS
LCSM Plates	Lin's Cupric Sulfate Medium – used for the detection and quantitative determination of wild yeast populations in a yeast or beer sample.	• Low cost	• Growth of non –diastaticus wild yeast strains on this media results in false positives • Low sample volume
Starch Plates (i.e. Farber Pham Media)	Plates with starch as the carbon source will allow growth of *S. cerevisiae* var. *diastaticus*, but not other *S.cerevisiae* brewing strains	• Low cost	• Low sample volume • Only detects presence or absence (non-specific)
PCR Genetic Test	PCR is a common laboratory technique that can detect the presence or absence of specific DNA fragments.	• Fast and specific results • Greater sensitivity can be achieved by propagating a sample in enrichment media • Allows for identification of specific strains	• Initial investment required (approx. $10k) •Non-quantitative • Low sample volume (greater volume possible with enrichment)
Real time PCR (qPCR)	Real Time PCR is a more sensitive PCR technique that quantifies the amount of the targeted DNA that is present in the sample.	• More sensitive than regular PCR • Faster than regular PCR • Allows for identification of specific strains • Quantitative results • Greater sensitivity can be achieved by propagating a sample in enrichment media (no longer quantitative)	• Higher initial investment required (approx. $50-80K) • Low sample volume (greater volume possible with enrichment)
Modified Durham Test 	This is a simple test that is run by adding 1g of sample to a 2% solution of maltodextrin (or fully attenuated beer) and monitoring possible gas production over 2 weeks.	• Low cost • Can detect small amount of diastaticus cells (<10 cells of *S. cerevisiae* var. *diastaticus* per sample) [3]	• Long waiting time (up to 2 weeks) • Sample must be free of other fermentable sugars. Yeast must be starved and washed to avoid false positives. • Low sample volume (greater volume possible with enrichment)
Ankom Test	A shake flask test, similar to Modified Durham test, which measures gas production from a 25g sample.	• Shorter wait time • Larger sample volume (25g vs 1g for Modified Durham Test)	• Sample must be free of other fermentable sugars. Yeast must be starved and washed to avoid false positives.
Sensory and Monitoring	Keep packaged samples at room temperature for ongoing monitoring for a long period of time (at least the listed shelf life) for changes in sensory profile and increases in alcohol and CO_2 levels.	• Does not require any special equipment	• Very long waiting time – problems may not be detected until the product is already sold to customers. • Requires storage space for a library of packaged beer samples from each batch. • Record keeping and regular sensory evaluation is laborious and time consuming

For more information on detection methods for diastaticus yeast contact our support team at ***brewing@lallemand.com***

DIASTATICUS CONTROL IN LALLEMAND DRY BREWING YEAST

Lallemand Brewing dry yeast samples are assessed for possible contamination with diastaticus yeast after every production and again after packaging. We use four tests: LCSM plates, real time PCR (qPCR) with enrichment, modified Durham test and fermentation test followed by sensory evaluation. **A positive diastaticus test at any level results in a rejected lot**. Additionally, known diastaticus strains are produced at a separate facility. Lallemand dry brewing yeast is traceable by lot number, which allows us to better support brewers by re-testing our retention samples for a specific lot in the event of an over-attenuation issue in the brewery.

NON-DIASTATICUS METHODS FOR FARMHOUSE/SAISON STYLES

Some brewers choose to avoid known diastaticus strains to reduce the risk of cross contamination. It is possible to produce a beer similar to a traditional Farmhouse/Saison style without using a traditional diastaticus Saison yeast. To do this, select a yeast strain that produces fruity esters and spicy phenols (i.e. LalBrew Abbaye™ or LalBrew Farmhouse™) and modify the brewing process to achieve higher attenuation. There are several approaches to achieve greater attenuation with a non-diastaticus yeast including lower mash temperature, use of adjuncts, or the pre-boil addition of an exogenous glucoamylase enzyme to the mash or kettle.

[1] Farber, M., 2018, Development of a selective medium for detection of Saccharomyces cerevisiae var. diastaticus in the brewery, Brewing Summit, August 12–14, 2018, San Diego
[2] Meier-Dörnberg, T., Jacob, F., Michel, M., & Hutzler, M. (2017). Incidence of Saccharomyces cerevisiae var. diastaticus in the Beverage Industry: Cases of Contamination , 2008 – 2017, 54(4), 140–148.
[3] Fischborn, T., 2018, Detection of low concentrations of Saccharomyces cerevisiae var. diastaticus in a high population of S. cerevisiae, Brewing Summit, August 12-14, 2018, San Diego