

# High Resolution Melting (HRM) Method for Brettanomyces Yeast Identification

**Authors: Davydenko S. G.<sup>1</sup>, Ivanova V. A.<sup>2</sup>, Meledina T. V.<sup>2</sup>**

**Affiliations:**

**1) Baltika Breweries Part of the Carlsberg Group, Saint Petersburg**

**2) ITMO University, Faculty of Biotechnology (Bio Tech), Saint Petersburg**

## High Resolution Melting (HRM) Method for *Brettanomyces* Yeast Identification

Davydenko S. G.<sup>1</sup>, Ivanova V. A.<sup>2</sup>, Meledina T. V.<sup>2</sup>

1) Baltika Breweries Part of the Carlsberg Group, Saint Petersburg

2) ITMO University, Faculty of Biotechnology (Bio Tech), Saint Petersburg

### Keywords:

*Brettanomyces* yeast, RT-PCR method, HRM

## Relevance

In brewing, *Brettanomyces* are generally considered contaminant. However, some types of beer, such as traditional Belgian ales, lambic, gez, as well as Flanders brown ale and Flanders red ale, owe their unique flavors to this yeast. In addition, with the development of resource-saving technologies, researchers became interested in residual brewer's *Brettanomyces* yeast as a source of biologically active substance – a polysaccharide of the yeast cell walls - beta-glucan.

*Brettanomyces* identification is difficult. Today, molecular biology and microbiology methods are widely used for their identification. The polymerase chain reaction method (PCR) was used in this research.

**High Resolution Melting (HRM) Method for Brettanomyces Yeast Identification**Davydenko S. G.<sup>1</sup>, Ivanova V. A.<sup>2</sup>, Meledina T. V.<sup>2</sup>

1) Baltika Breweries Part of the Carlsberg Group, Saint Petersburg

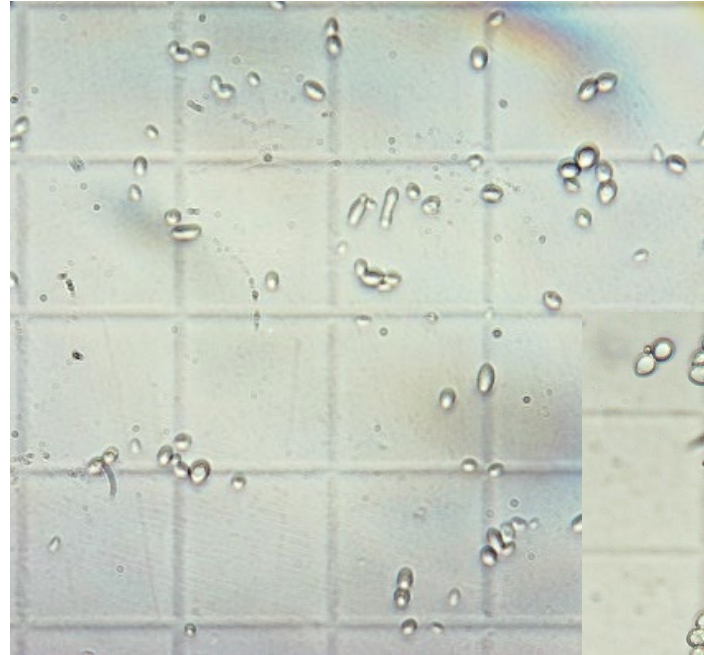
2) ITMO University, Faculty of Biotechnology (Bio Tech), Saint Petersburg

**Keywords:**

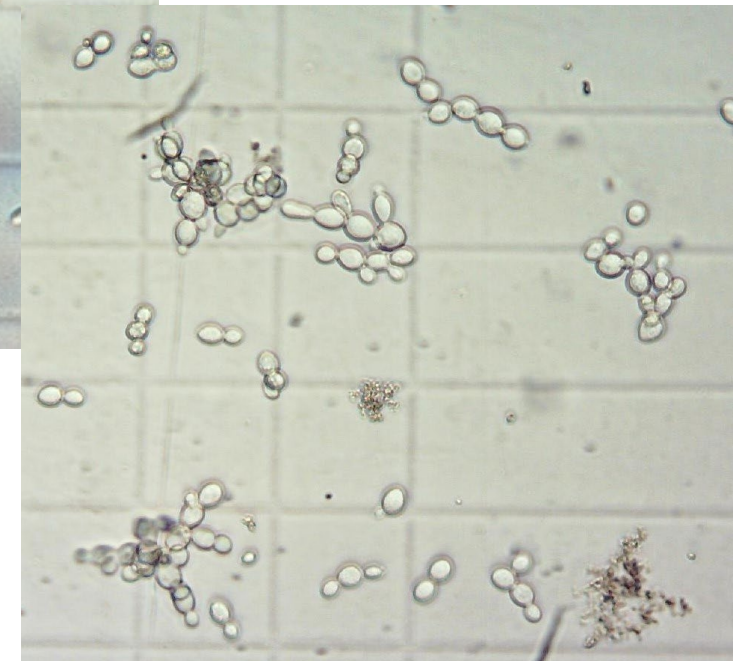
Brettanomyces yeast, RT-PCR method, HRM

**Research Objective:**

the objects of this study were **Brettanomyces** and **Saccharomyces** yeast strains, obtained from the collections of brewing yeast cultures.



***Brettanomyces bruxellensis***  
**(WY5112)**



***Saccharomyces cerevisiae***  
**(WLP300)**

50 μm

## High Resolution Melting (HRM) Method for *Brettanomyces* Yeast Identification

Davydenko S. G.<sup>1</sup>, Ivanova V. A.<sup>2</sup>, Meledina T. V.<sup>2</sup>

1) Baltika Breweries Part of the Carlsberg Group, Saint Petersburg

2) ITMO University, Faculty of Biotechnology (Bio Tech), Saint Petersburg

**Keywords:**

*Brettanomyces* yeast, RT-PCR method, HRM

## Results

M 1 2 3 4 5



Results of PCR analysis of 26S RNA fragments obtained using primers DB90F and DB384R specific to *Brettanomyces* yeast.

Figure shows clear bands of a specific PCR product.

Therefore, the nucleotide samples on tracks 4 and 5 belong to *Brettanomyces* yeast.

Figure – bands of a specific PCR product:

M – DNA molecular weight marker;

1-5 - PCR products.

High Resolution Melting (HRM) Method for *Brettanomyces* Yeast IdentificationDavydenko S. G.<sup>1</sup>, Ivanova V. A.<sup>2</sup>, Meledina T. V.<sup>2</sup>

- 1) Baltika Breweries Part of the Carlsberg Group, Saint Petersburg
- 2) ITMO University, Faculty of Biotechnology (Bio Tech), Saint Petersburg

**Keywords:**

Brettanomyces yeast, RT-PCR method, HRM

## Results

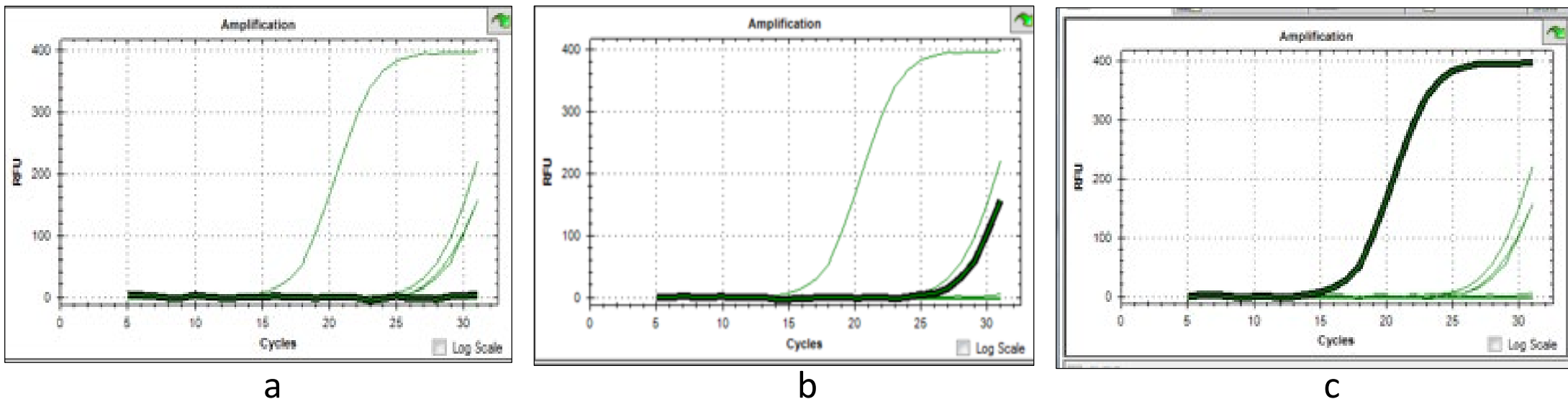


Figure – Results of RT-PCR analysis of 26S RNA fragments obtained using primers DB90F and DB384R, specific for *Brettanomyces*

a – negative control; b – *Saccharomyces cerevisiae*; c – *Brettanomyces bruxellensis*

As can be seen from Figure when analyzing *Brettanomyces bruxellensis* yeast DNA amplification curve started to rise already at the 16th cycle. However, a non-specific rise in the amplification curve was also observed in the case of *Saccharomyces cerevisiae*.

## High Resolution Melting (HRM) Method for *Brettanomyces* Yeast Identification

Davydenko S. G.<sup>1</sup>, Ivanova V. A.<sup>2</sup>, Meledina T. V.<sup>2</sup>

1) Baltika Breweries Part of the Carlsberg Group, Saint Petersburg

2) ITMO University, Faculty of Biotechnology (Bio Tech), Saint Petersburg

**Keywords:**

*Brettanomyces* yeast, RT-PCR method, HRM

## Results

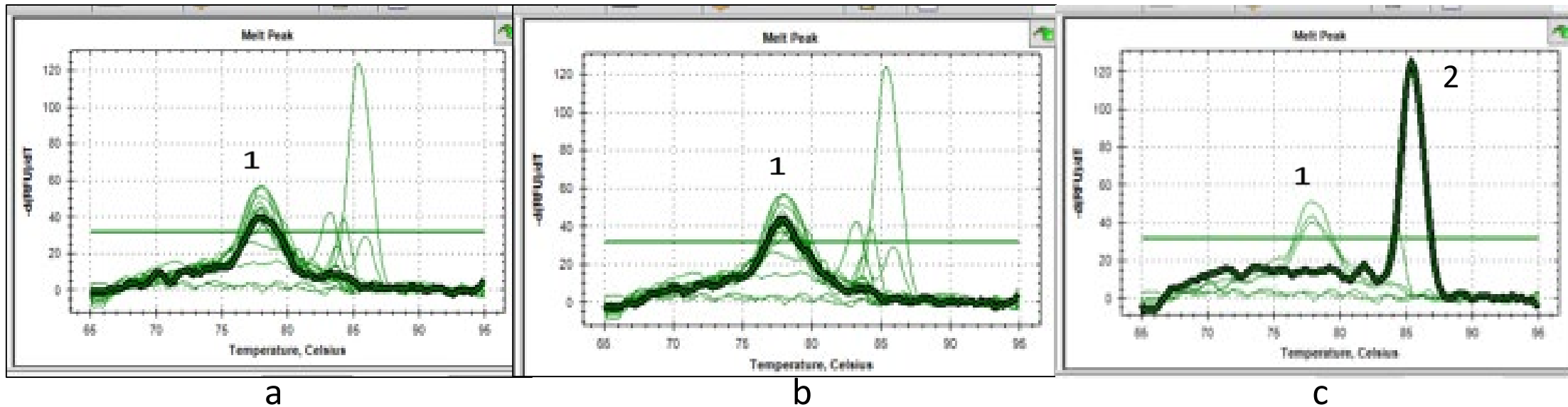


Figure – Results of HRM analysis of 26S RNA fragments obtained using primers DB90F and DB384R, specific for *Brettanomyces*

a – negative control; b – *Saccharomyces cerevisiae*; c – *Brettanomyces bruxellensis*

Figure shows non-specific peak №1 in all samples, and only the graph "c" clearly shows the peak №2 of fine melting of the amplified *Brettanomyces* DNA fragment.

## High Resolution Melting (HRM) Method for Brettanomyces Yeast Identification

Davydenko S. G.<sup>1</sup>, Ivanova V. A.<sup>2</sup>, Meledina T. V.<sup>2</sup>

1) Baltika Breweries Part of the Carlsberg Group, Saint Petersburg

2) ITMO University, Faculty of Biotechnology (Bio Tech), Saint Petersburg

### Keywords:

Brettanomyces yeast, RT-PCR method, HRM

## Conclusions

As can be seen from the presented data, the use of HRM analysis allows to identify peaks of amplified specific DNA fragments and to ignore non-specific peaks. So, modern methods of molecular biology allow to identify microorganisms most accurately and in the shortest possible time. This undoubtedly distinguishes them from the traditionally used microbiological and biochemical methods of detection and identification. Moreover, the approaches of molecular biology and molecular genetic methods in particular, help researchers not only in identification issues, but also for the effective management of directed biosynthetic processes.

## References

1. Meledina, T.V. Prospects of the Yeast Genus Brettanomyces Using in Brewing / T.V. Meledina, V.A. Ivanova, S.G. Davydenko // Almanac of Young Scientists' Scientific Works of ITMO University. - 2017. - Vol. 1. - Pp. 193-196.

### Acknowledgement

Authors show their appreciation to the Baltika Breweries Part of the Carlsberg Group for the equipment provided for the research.

**High Resolution Melting (HRM) Method for Brettanomyces Yeast Identification**

Davydenko S. G.<sup>1</sup>, Ivanova V. A.<sup>2</sup>, Meledina T. V.<sup>2</sup>

1) Baltika Breweries Part of the Carlsberg Group, Saint Petersburg

2) ITMO University, Faculty of Biotechnology (Bio Tech), Saint Petersburg

**Keywords:**

Brettanomyces yeast, RT-PCR method,  
HRM

# Thank you for your attention!

Authors: Davydenko S. G.<sup>1</sup>, Ivanova V. A.<sup>2</sup>, Meledina T. V.<sup>2</sup>

**Affiliations:**

1) Baltika Breweries Part of the Carlsberg Group, Saint Petersburg

2) ITMO University, Faculty of Biotechnology (Bio Tech), Saint Petersburg

Contact details: [davydenko@baltika.com](mailto:davydenko@baltika.com); [vaivanova@itmo.ru](mailto:vaivanova@itmo.ru); [tymeledina@itmo.ru](mailto:tymeledina@itmo.ru)