



## Differentiation of yeasts and moulds using MMM Beverage Partner's FUN LC RT-PCR Workflow System

### Summary

MMM Beverage Partner's FUN LC RT-PCR Workflow System detects yeasts and moulds in beverage samples. Besides the detection, the melting peak, melting temperature analysis of the detected yeasts or moulds allows the user to make differentiation between the different yeasts or moulds.

The melting peak analysis discovers the differences of the target nucleotide sequences of the different yeasts and moulds. The different target nucleotide sequences result in different melting temperatures while the identical target nucleotide sequences result in the same melting peak. In this study we collected the melting peak data of some relevant yeasts and moulds.

### Background of the melting temperature analysis

The temperature at which a DNA strand separates or melts when heated can vary over a wide range, depending on the sequence, the length of the strand, and the GC content of the strand. Based on these, melting temperature profiles can be used to identify and genotype DNA products. To analyze sample melting temperature profiles, the fluorescence of the samples must be monitored while the LightCycler® temperature is steadily increased. As the temperature increases, sample fluorescence decreases. For HybProbe probes, this is due to the separation of target-probe hybrids resulting in the spatial separation of the dye molecules and a consequent drop in fluorescence. The presence of a mismatch in the sequence of the probe binding site lowers the temperature at which the probe melts off the sequence. The difference in melting temperature depends on the type of mismatch, the mismatch position within the probe sequence, and the base pairs immediately adjacent to the mismatch.

The same melting temperature achieved from different samples indicates that the same yeast or mould can be present and if the melting peaks are different the presence of different yeast and/or moulds are confirmed.

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## Micro-organisms used

*Aspergillus brasiliensis* ATCC 16404  
*Dekkera anomala* DSM 70727  
*Dekkera bruxellensis* DSM 3429  
*Mucor circinelloides* DSM 1175  
*Paecilomyces lilacinus* DSM 846  
*Penicillium chrysogenum* ATCC 10106  
*Penicillium expansum* DSM 1282  
*Rhizopus stolonifer* DSM 2194  
*Talaromyces helicus* DSM 3705  
*Trichoderma flavofuscum* DSM 3500  
*Ulocladium chartarum* DSM 63070  
*Zygosaccharomyces bailii* DSM 70492

*Candida parapsilosis*, isolated from beverage

*Debaryomyces hansenii*, isolated from beverage

*Saccharomyces cerevisiae*, bakery yeast

*Candida pseudointermedia*, artificial sequence

*Metschnikowia pulcherrima*, artificial sequence

*Yarrowia lipolitica*, artificial sequence

## Materials and methods

MMM Beverage Partner's FUN LC RT-PCR Workflow System kit was used according to the kit instruction manual.



The melting peaks of the yeasts and mould are shown in Fig. 1., Fig. 2., and Fig. 3.

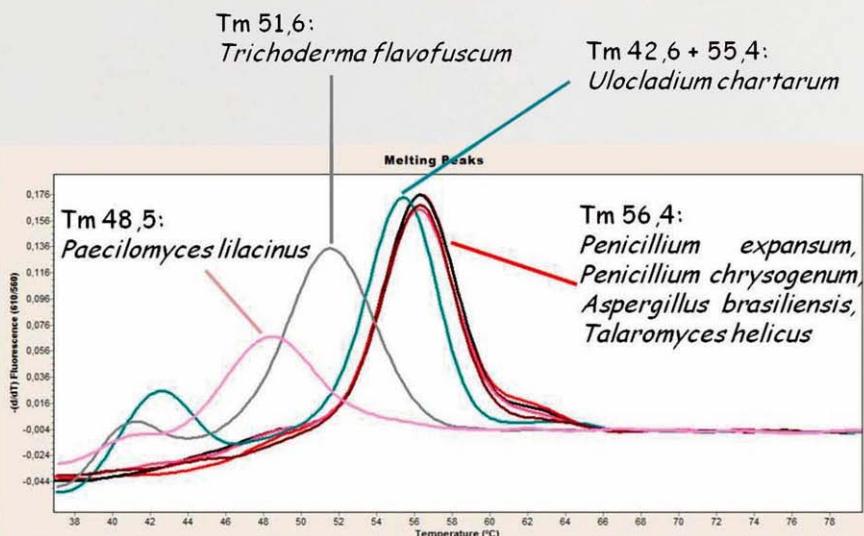


Fig. 1. Melting peaks of different moulds achieved at 610 nm channel

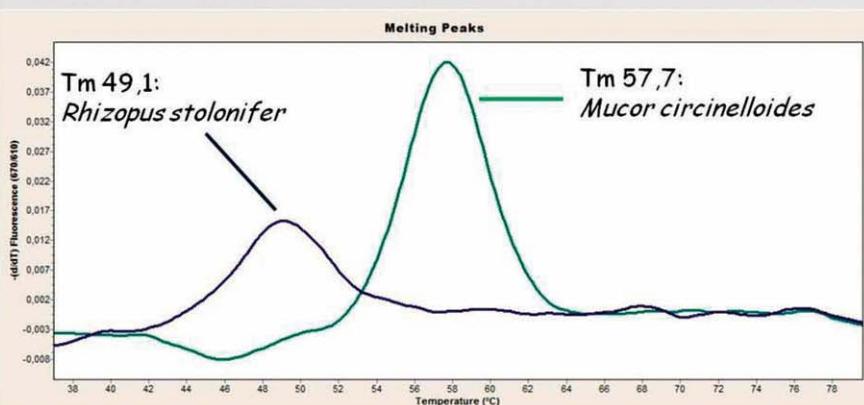


Fig. 2. Melting peaks of different moulds achieved at 670 nm channel

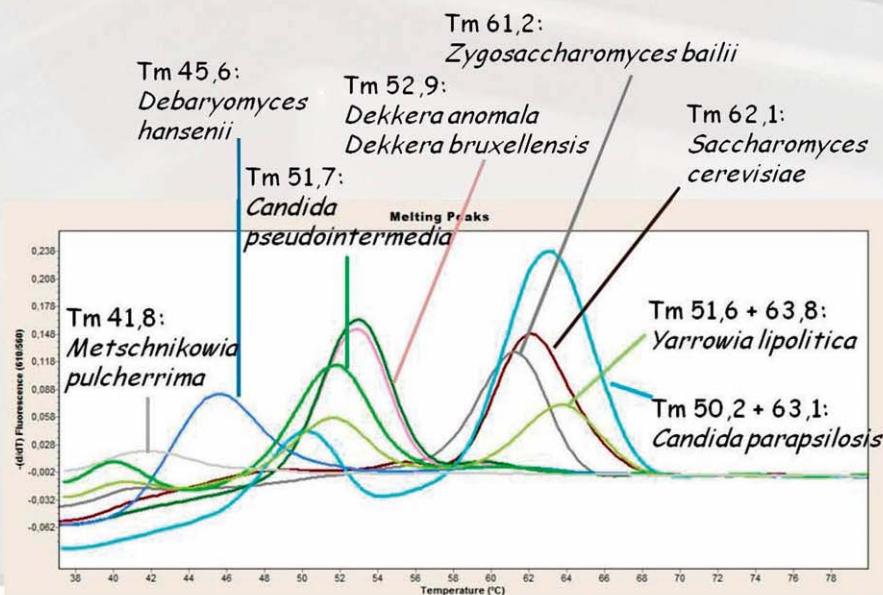


Fig. 3. Melting peaks of different moulds achieved at 610 nm channel

## Results

The melting peaks of the different yeasts and moulds were achieved from the melting peak analysis. The melting peak data are demonstrated in *Table 1*.

Note: It is important to keep the positive control in the valid range in order to use these melting peak data

**Table 1. Melting temperature data of different yeasts and moulds**

Name of micro-organisms	Melting Temperature data, °C
<i>Aspergillus brasiliensis</i>	56,4
<i>Candida parapsilosis</i>	50,2 + 63,1
<i>Candida pseudointermedia</i>	51,7
<i>Debaryomyces hansenii</i>	45,6
<i>Dekkera anomala</i>	52,9
<i>Dekkera bruxellensis</i>	52,9
<i>Metschnikowia pulcherrima</i>	41,8
<i>Mucor circinelloides</i>	57,7*
<i>Paecilomyces lilacinus</i>	48,5
<i>Penicillium chrysogenum</i>	56,4
<i>Penicillium expansum</i>	56,4
<i>Rhizopus stolonifer</i>	49,1*
<i>Saccharomyces cerevisiae</i>	62,1
<i>Talaromyces helicus</i>	56,4
<i>Trichoderma flavofuscum</i>	51,6
<i>Ulocladium chartarum</i>	42,6 + 55,4
<i>Yarrowia lipolitica</i>	51,6 + 63,8
<i>Zygosaccharomyces bailii</i>	61,2

Legends: \* the melting temperature was achieved at the 670 nm channel



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