DEVELOPMENT AND PERFORMANCE EVALUATION OF ISOTHERMAL PCR (LAMP) TO DETECT *KAZACHSTANIA BARNETTII* IN FOOD SAMPLES AND SURFACES OF FOOD PROCESSING ENVIRONMENT

Nicolas Nguyen Van Long[1], Hugo Habert[1], Véronique Huchet[1]

(1)ADRIA Food Technology Institute, Quimper, France

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CONTEXT & PURPOSE

Kazachstania barnettii is a common spoilage yeast able to display remarkable gas production and resistance to preservatives (**Fig. 1**). Despite its prevalence in food industry no specific detection method is easily available to food industry, hindering investigations for contamination sources. The present work describes the development and performance evaluation of a *K. barnettii* detection method that can be directly performed in the facility for surface samples thanks to micro-fluidic DNA extraction and isothermal PCR (LAMP) technology or in microbiology laboratory for food samples.

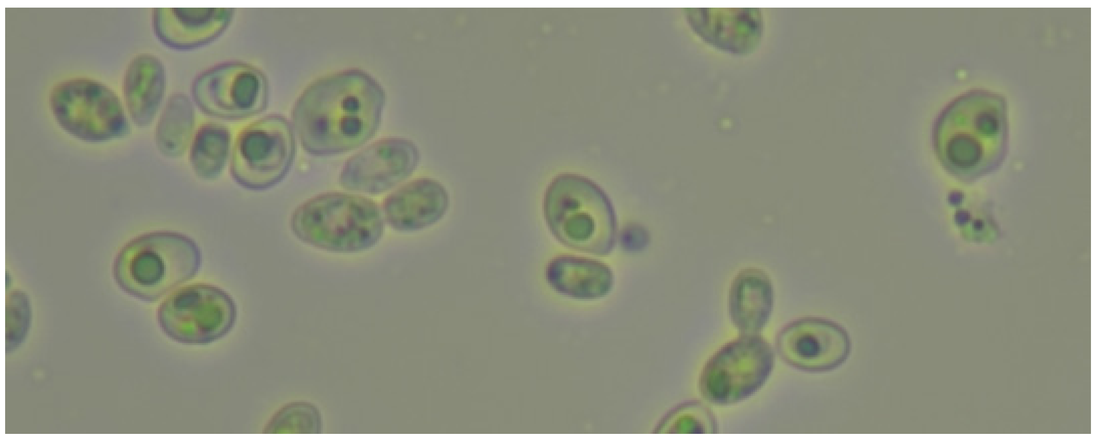


Fig 1 : Microscopic observation of *Kazachstania barnettii* wild strain isolated from the spoiled product (X1000)

MATERIALS & METHODS

The workflow includes an optional enrichment step, DNA extraction, and isothermal PCR-based detection (LAMP). Overall, two different protocols (A and B) were evaluated :

Steps	Protocol A (for surface samples)	Protocol B (for raw materials and finished products)
Sampling	Surface swabbing using viscose swabs	Decimal dilution in Sabouraud broth
Enrichment	No enrichment	24 to 48h at 25°C followed by plating onto DRBC agar and incubation 5 days at 25°C
DNA extraction	Use of microfluidic chips XTRALYZ (Direct Analysis, Grenoble, France) according to manufacturer's recomendation	Pool of colonies transfered into 1.5 mL of sterile water prior to bead beating 10 min at 30 Hz. Centrifugation 3 min at 15,000 RCF. 10-fold dilution prior to PCR
LAMP PCR reaction	4 µL of DNA extract mixed with 5 µL of WarmStart Colorimetric LAMP 2X Master Mix (New England Biolabs), 1 µL of primer mix (10X). Incubation 1 hour at 65 °C. Visual evaluation of the results (**Fig. 3**). Primers were the following: Kbarn F3 : AACTCCATCTCAGAGCGG, Kbarn B3 : ATAAAATACTCCACAGTGTGTAG, Kbarn FIP : CACCTACGGAAACCTTGTTACGA, GAATTTGTACAAACTTGGTCA, Kbarn BIP : TAAAGAAATATGGATTCTGGCG, GGCAGCGTGAAATGGAAACG	

LAMP performance was assessed by means of protocols based on ISO 16140-2:2016 approach:

- **Inclusivity/exclusivity** were evaluated by testing 2 wild strains of *K. barnettii* (target) isolated from spoiled vegetable-tartare and 25 non-target yeasts and molds. Final DNA concentration was 4 ng/µL.
- **Robustness** of the protocol B was also performed by mixing 1 colony of *K. barnettii* among 20 colonies of non-target species (*Saccharomyces cerevisiae, Candida sake, and Kazachstania aerobia*) and testing in parallel the same cocktails without the target yeast.
- Enrichment optional step was evaluated by testing 72 non-inoculated (natural) food samples (vegetable-tartare and oinions) with protocol B after 0, 24h and 48h incubation time
- Only **LOD50** for protocol A is currently available. *K. barnettii* cells were suspendend in TS solution. Concentration was determined using malassez cell and verified by classical enumeration on Potatoe Dextrose Agar. For pure culture LOD50, target concentration levels were 2, 5 and 10 CFU per mL and analysis were performed according to protocol A. For surface LOD50, 1 mL of inoculum was poured on 15x15cm stainless metal coupons. Coupons were dried overnight, analyzed according to protocol A. The probability of detection was modelled as a function of concentration by means of the spreadsheet freely available in ISO 16140 maintenance portal (https://standards.iso.org/iso/16140/-2/ed-1/en/amd/1/PODLOD_ver11.xls)

RESULTS

On pure cultures samples the protocol A showed 50% detection probability (LOD50) for concentrations of 3,3 ± 0,8 CFU per sample (**fig 3**). On surfaces, the level of detection is 39,0 ± 40,0 CFU per 225cm², which can be converted into 0.17 CFU/cm² . The LOD50 values obtained in the present study are consistent with low contamination levels that can occur in the food manufacturing facility and could be helpful to determine contamination sources among surfaces samples.

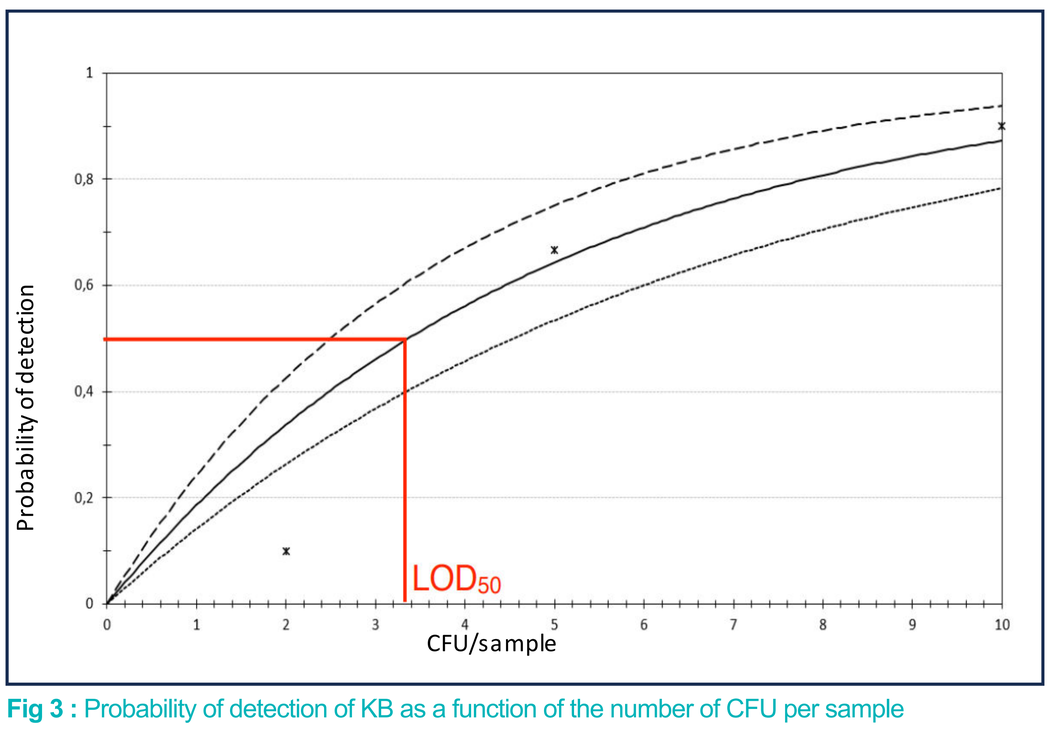


Fig 3 : Probability of detection of KB as a function of the number of CFU per sample

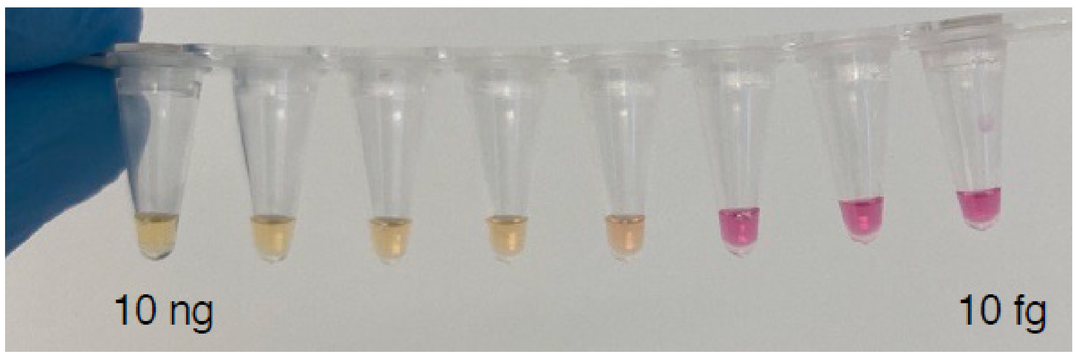


Fig 2 : Visual examination of the LAMP reaction. The picture displayes 10-fold dilutions of pure target DNA from 10 ng to 10 fg. Yellow color indicates a positive result and pink color indicates a negative result

For inclusivity and exclusitivy evalyation, all species tested for exclusivity yielded negative results, whereas both strains of *Kazachstania barnettii* tested yielded positive results. For robustness evualuation, all samples containing a *Kazachstania barnetti* colony yielded a positive response, whereas those without showed a negative response.

Over the 72 non-inoculated samples tested with protocol B, 49 were naturally contaminated by *K. barnettii*. Yet the obtention of positive results was significantly improved by the optional enrichment step as only 39 samples were positive without enrichment (0h), 48 samples were positive after 24h enrichment and 49 samples were positive after 48h enrichment. The positive samples obtained after 0h and 24h enrichment were still positive after 48h enrichment.

Significance The present methodology can detect low contamination levels of *K. barnettii* in food products and surface samples and can help food manufacturers to identify contamination sources inside the facility or to screen raw materials. Interestingly, surface samples can be analyzed in less than one hour with the microfluidic DNA extraction and the devices used could be easily transported to the facility for point-of-care analysis.