Design, development and validation of a real-time PCR assay for detection of Klebsiella pneumoniae complex in environmental matrixes

Elodie Barbier, C. Rodrigues, Géraldine Depret, V. Passet, Laurent Gal, Pascal Piveteau, S. Brisse

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E. Barbier[1], C. Rodrigues[2], G. Depret [1], V. Passet [2], L. Gal [1], P. Piveteau [1], S. Brisse [2]

1. INRA, AgroSup Dijon, Univ. Bourgogne Franche-Comté Dijon, Agroecology, France
2. Institut Pasteur, Biodiversity and Epidemiology of Bacterial Pathogens, Paris, France

- ***Klebsiella pneumoniae*** **(Kp) represents a growing public health concern due to the emergence of multidrug resistance and hypervirulence.**

- **Seven closely related phylogroups constitute the Kp species complex: *Klebsiella pneumoniae* (phylogroup Kp1), *K. quasipneumoniae* subsp. *quasipneumoniae* (Kp2) and subsp. *similipneumoniae* (Kp4), *K. variicola* subsp. *variicola* (Kp3) and subsp. *tropicalensis* (Kp5), *K.quasivariicola*' (Kp6) and *K. africanensis* (Kp7).**

- **Kp is described as ubiquitous in nature. Water, sewage, soil and vegetation could be environmental sources of Kp but their exact contribution in its transmission routes is still unknown.**

Objective: To develop a specific and sensitive real time PCR assay for the detection of all phylogroups (Kp1 to Kp7) of the Kp complex from environmental sources.

METHODS

- *In silico* identification of a conserved region in all phylogroups for specific primers design
- **Validation** of a **Sybergreen Real-time PCR assay**
  - **Specificity** was tested on 40 Kp complex members (Kp1 to Kp7) and 90 non-Kp complex bacteria
  - **Sensitivity** was tested on 5g-soil microcosms spiked with Kp1 ATCC13883T after a 24h-enrichment step in LB + ampicillin (10 mg/l) and a short treatment (washing, boiling, dilution)
- **Implementation** on 84 environmental samples (soil, roots, leaves and water)
  - Comparison to culture-based identification by plating enrichments using SCAI medium and MALDI-TOF mass spectrometry

RESULTS

- The assay targets an intergenic region (*zkir*) that we found to be uniquely present in genomes of the Kp complex.

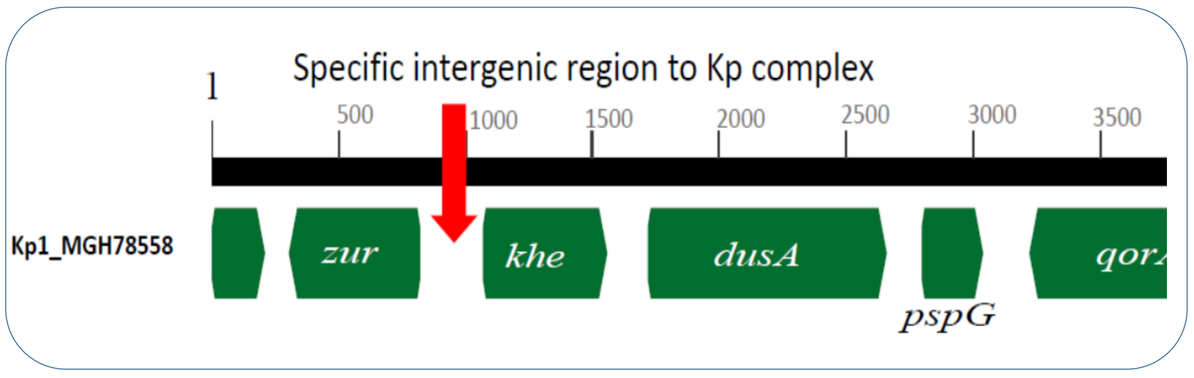

Figure 1. zkir (zinc-khe intergenic region) real time PCR assay

- **Specificity**
  → 100 % positives with Kp complex members (melting T°: 78,5 to 80,1)
  → No false positive with non Kp (Ct>35, melting T°≠).

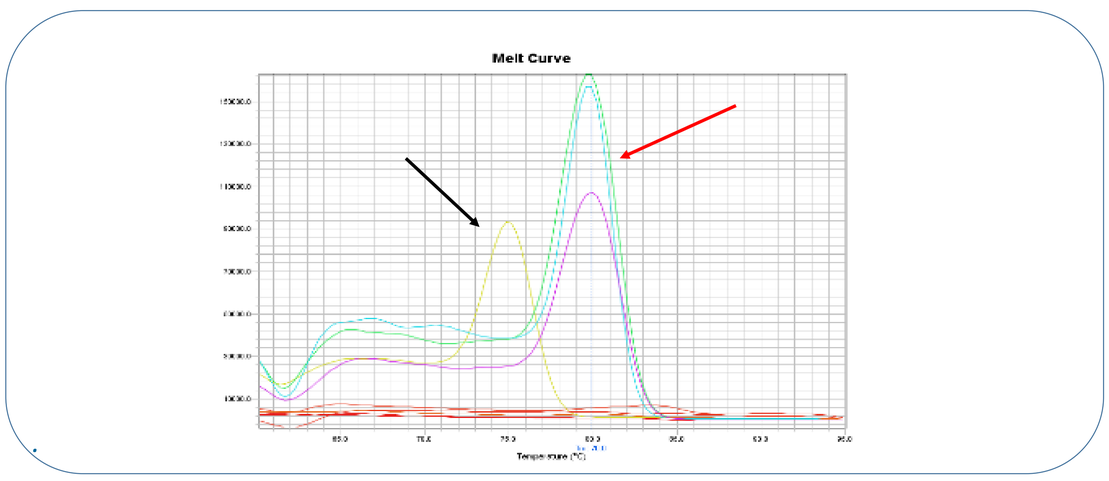

Figure 2 Melting curve peaks of positive (red arrow) and negative (black arrow) sample

- **Sensitivity on pure DNA of Kp1 ATCC13883T**
  → Detection down to 45 pg (15 genome copies)

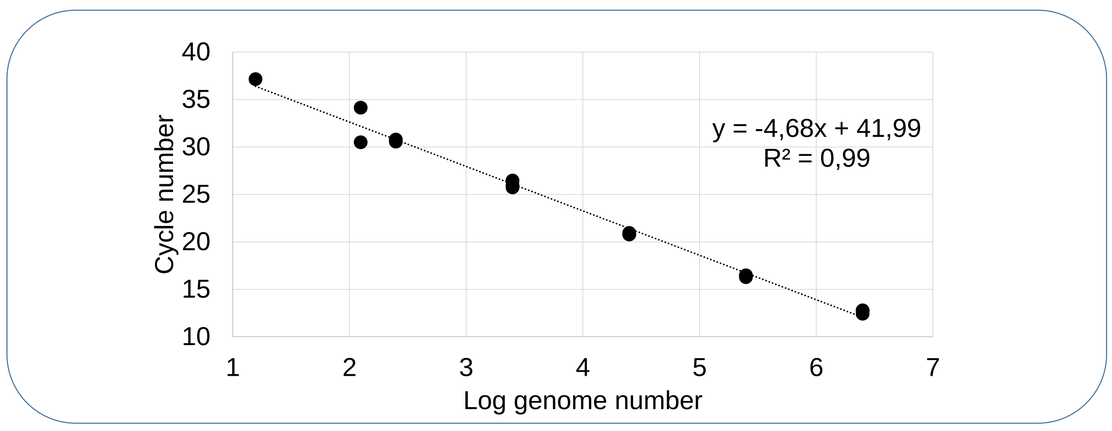

Figure 3 Standard curve for zkir assay

- **Sensitivity on soil microcosms spiked with Kp1 ATCC13883T**
  →Detection down to 7.0 × 10^{-1} cfu per g after a 24h-enrichment step

- **Higher detection of Kp from environmental samples**
  → 28/84 (33.3 %) environmental samples positive with the zkir assay (Ct: 18 to 36, melt curve peak: 78,5 to 80,1°C)
  → 24/84 (28.5 %) positive with culture-based method and MALDI-TOF MS identification.

CONCLUSION AND PERSPECTIVES

- **We developed a specific and sensitive real time PCR assay for the rapid detection of all phylogroups of the Kp complex.**
- **This system can detect as low as 1 bacteria in a 5 g sample after a 24h-enrichment step, with higher sensitivity than culture-based methods.**
- **Inter-laboratory validation on food samples and on stools of animals and intensive care unit patients is ongoing. It is also used for broad screening of potential environmental sources of Kp.**

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