



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

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- *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.

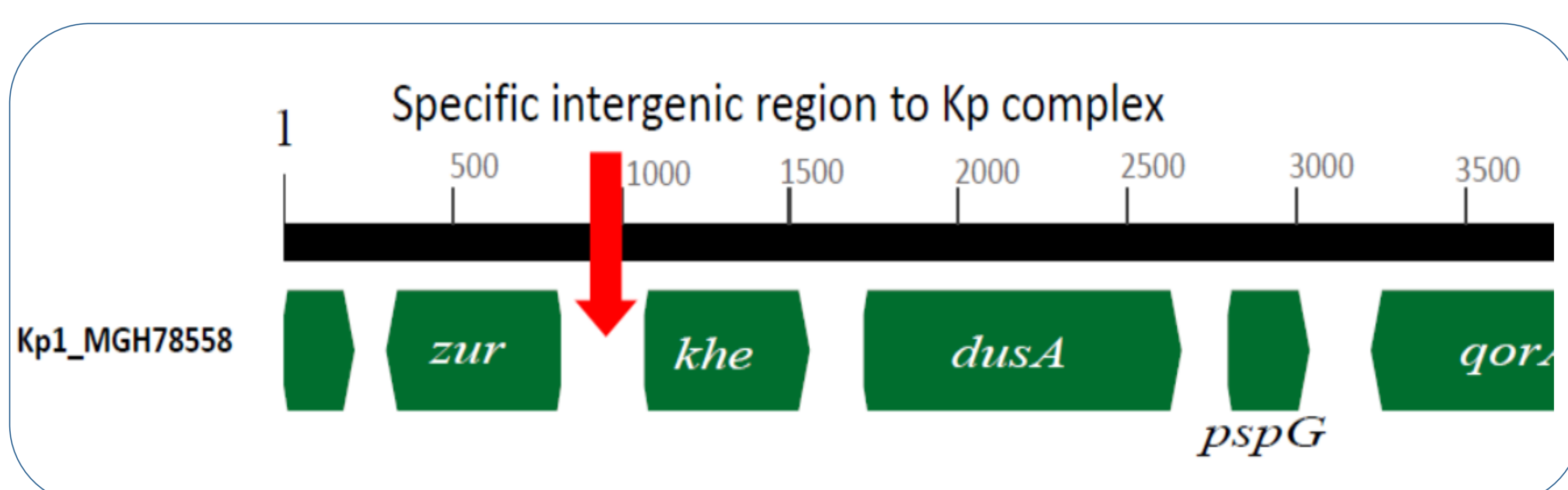


Figure 1. *zkir* (zinc-khe intergenic region) real time PCR assay

• Specificity

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

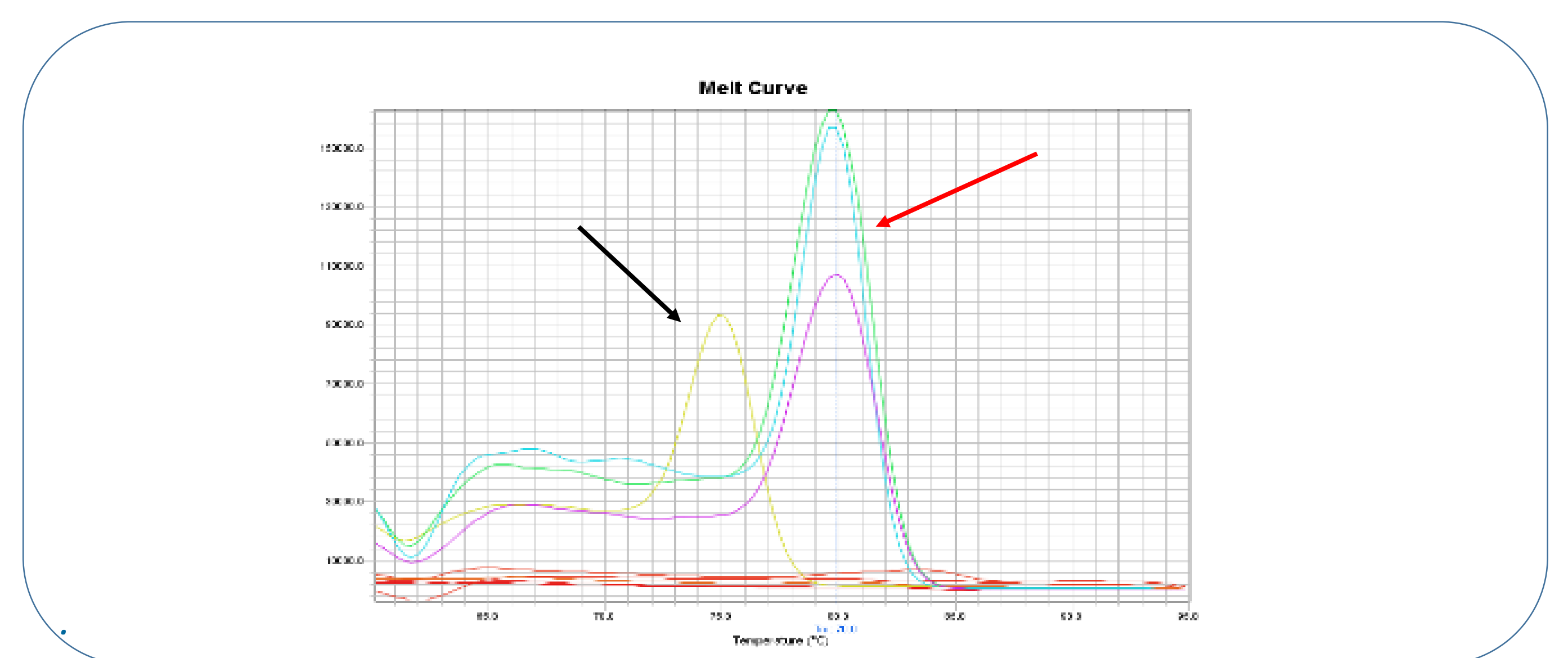


Figure 2 Melting curve peaks of positive (red arrow) and negative (black arrow) sample

• Sensitivity on pure DNA of Kp1 ATCC13883T

- Detection down to 45 pg (15 genome copies)

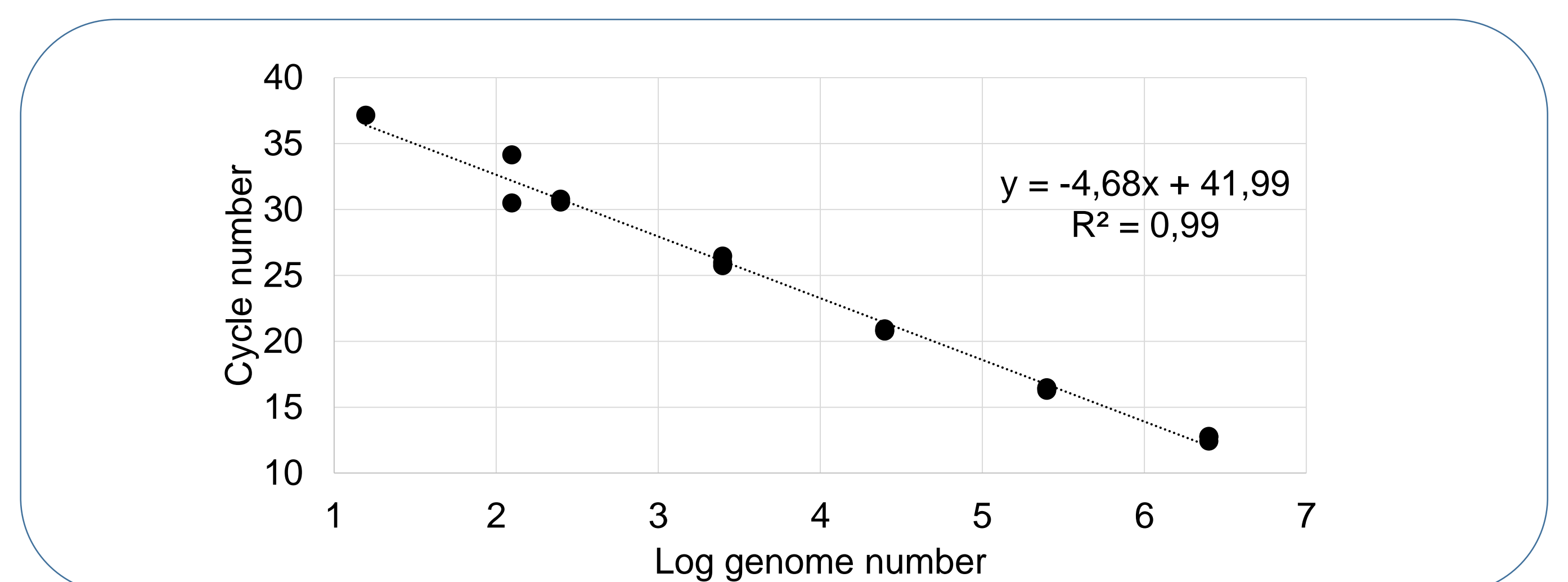


Figure 3 Standard curve for *zkir* assay

• 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.