Novel Serotype Specific Rapid Detection Method for *Cronobacter sakazakii*

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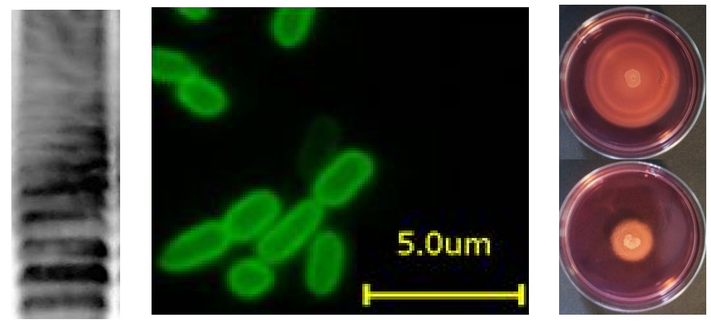
Cronobacter sakazakii is an opportunistic, ubiquitous, Gram-negative bacterium, belonging to the family of *Enterobacteriaceae*. This species is of special interest, because its occurrence in powdered infant formula (PIF) has been associated with causing life threatening infections in premature neonates. Severe conditions such as necrotizing enterocolitis (NEC), sepsis and meningitis result in a mortality rate as high as 80 %. Up to date, the occurrence of *C. sakazakii* in PIF is being monitored according to standardized microbiological methods, requiring up to 6 days for a positive test result. Here we present novel and highly specific sandwich enzyme immunoassays as a rapid detection and serotyping method for *C. sakazakii*.

Generated monoclonal antibodies were characterized using immunoblot analysis of lipopolysaccharide (LPS) extracts, immunofluorescence and motility assays.



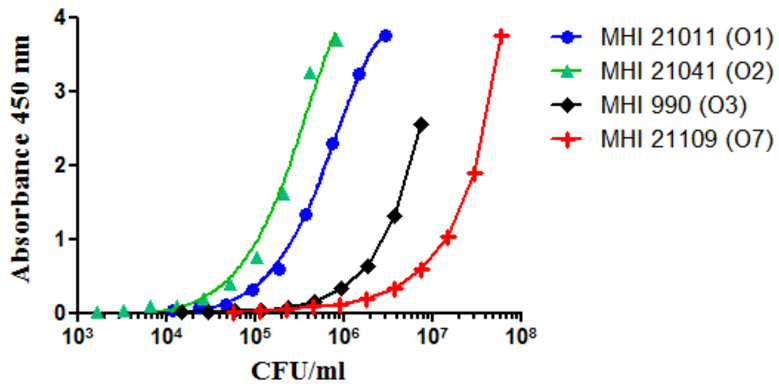
C. sakazakii serotype O2-strains tested with mAb 2F8 (O2).

Characteristics and reactivity of the produced serotype-specific mAbs

mAb	IgG-Subtype	Specific for		Target	Inclusivity
		Species	Serotype		
1C4	IgG	*C. sakazakii*	O1	EPS	13/14
2F8	IgG	*C. sakazakii*	O2	LPS	20/20
1A11	IgG	*C. sakazakii*	O3	LPS	13/15
2B7	IgG	*C. sakazakii*	O7	Flagella	8/11

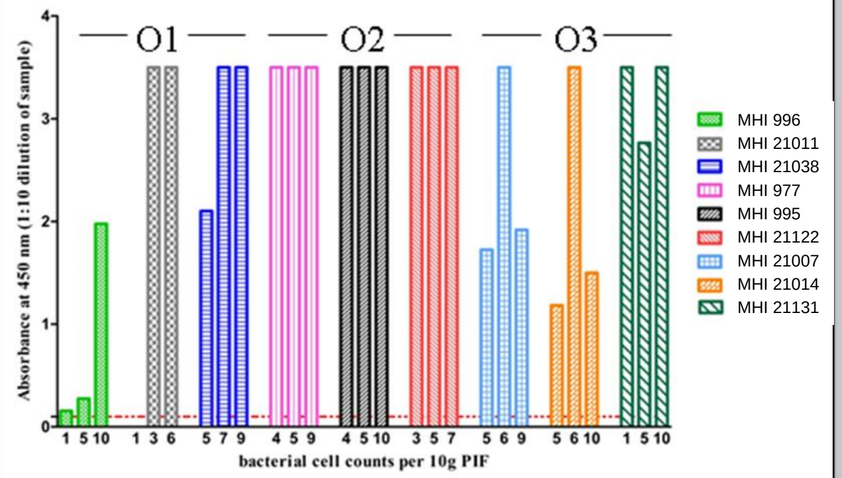
Inclusivity of the mAbs was determined by analyzing strains of the same serotype in indirect EIAs. Additionally, the reactivity of the mAbs with strains other than *C. sakazakii,* including closely related strains and more distantly related genera, was determined. All mAbs reliably recognized the respective *C. sakazakii* serotype and showed no cross-reactivity with other *Cronobacter spp.* or other members of the *Enterobacteriaceae* family. Only mAb 1A11 (O3) exhibited considerable cross-reactivity with *C. muytjensii* serotype O1-strains because both pathogens have an identical LPS biosynthetic operon (Jarvis *et al*.,2011).

Establishment of optimized mAb-based sandwich EIAs



In order to establish mAb-based sandwich EIAs for the detection of the different serotypes, each of the mAbs was used both as coating and - after labeling with HRP - as detection antibody in sandwich EIAs. Strain specific detection limits between 2 x 10^3 and 1 x 10^5 CFU/ml could be realized.

Application of the sandwich EIA for the detection of *C. sakazakii* of the serotypes O1, O2 and O3 in powdered infant formula



PIF-samples (10 g) were artificially contaminated with bacterial cell counts ranging from 1 – 10 CFU. After 15 h of enrichment, samples were directly analyzed in the sandwich EIAs. The cut-off value (absorbance ≤ 0.1) of the EIAs is indicated by the dashed red line. The LOD for the sandwich EIA was found to be as low as 1 CFU/10 g of PIF.

Conclusion

- → **Production of monoclonal IgG antibodies that specifically recognize *C. sakazakii* serotypes O1-O3 and O7**
- → **Development of sandwich EIA capture systems**
- → **Accurate and reliable detection of *C. sakazakii* serotypes O1-O3 in pure culture and powdered infant formula within 15 hours**
- → **First approach to simultaneously detect and serotype *C. sakazakii* based on mAbs**

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