Identification of the recently described *Cronobacter condimenti* by an *rpoB*-gene-based PCR system

*Enterobacter sakazakii* is an important foodborne pathogen, which can cause meningitis, necrotizing enterocolitis and bacteremia in neonates. Updating the original taxonomy of *E. sakazakii* by using a polyphasic approach has resulted in the definition of six novel species (*C. sakazakii, C. malonaticus, C. turicensis, C. muytjensii, C. dublinensis* and *Cronobacter* sp. genomospecies 1) and the transfer of these species to a novel genus, *Cronobacter* (Iversen et al., 2007, 2008), in order to facilitate their continued inclusion in schemata for diagnosis, with the novel genus being contaxic with *E. sakazakii*. In a study by Stoop et al. (2009), an *rpoB*-gene-targeted PCR assay was developed, which enables the identification of members of the genus *Cronobacter* to the species level.

In a recent study by Joseph et al. (2012) an additional species, *C. condimenti*, was described within the genus *Cronobacter*, and a novel species designation, *C. universalis*, was proposed for the formerly named *Cronobacter* sp.genomospecies 1. However, as of yet, no species-specific identification systems are available for *C. condimenti*. Here, we report an extension of the *rpoB*-gene-based PCR system (Stoop et al., 2009) for identification of this species. The *rpoB* gene encodes the β-subunit of the bacterial ribosomal polymerase, which is a well-recognized polymorphic marker and is useful for the identification of bacteria. The *rpoB* gene sequence of *C. condimenti* 1330T (= CECT 7863T = LMG 26250T) was deposited in GenBank (http://www.ncbi.nlm.nih.gov/genbank/) under the accession number JQ316670.

A primer pair (Ccon-f: 5’-AACGCCAAGCCAATCTCG-3’; and Ccon-r: 5’-GTACCCGCCATTTTGTCT-3’) was designed by multiple alignment analysis of the *rpoB* gene sequences of members of all species of *Cronobacter* described thus far using CLUSTAL W (Thompson et al., 1994). Specificity of the PCR assay was evaluated using *C. condimenti* 1330T as positive control and 27 non-target strains, comprising isolates from human, food and environmental origins, including: four *C. sakazakii* strains (E601, E602T = ATCC 29544T, E604 and E828), four *C. malonaticus* strains (E265, E621, E825 and E829T = LMG 23826T), five *C. turicensis* strains (E3032T = LMG 23827T, E626, E627, E681 and E688), two *C. universalis* (formerly *C. genomospecies* 1) strains (E797T = NCTC 9529T and E680), five *C. muytjensii* strains (E603T = ATCC 51329T, E456, E488, E616 and E769), and seven *C. dublinensis* strains (*C. dublinensis* subsp. *dublinensis* DES187T = LMG 23823T, *C. dublinensis* subsp. *laussannensis* E515T = LMG 23824T and *C. dublinensis* subsp. *lactaridi* strains E464T = LMG 23825T, E465, E791, E798 and E799). These strains were part of a taxonomic study performed by Iversen et al. (2007, 2008). The use of these previously characterized strains provided a valid basis for the assessment of the specificity data.

For PCR amplification, mixtures were prepared (total volume 50 μl) containing GoTaq Green Master Mix (Promega) with 1.5 mM MgCl₂, 200 μM dNTPs each and 10 pmol primers (final concentrations).

Thermal cycling was carried out using an initial denaturation step of 95°C for 2 min, followed by 30 cycles of denaturation at 95°C for 1 min, annealing at 58°C for 30 s and elongation at 72°C for 60 s. The amplification product was resolved on a 1% agarose gel followed by ethidium bromide staining and examination under UV light. The PCR assay was performed using both extracted DNA (10 ng per reaction) (DNAeasy Blood & Tissue kit, Qiagen) as well as boiled colony material. The latter method included the resuspension of one colony (grown on tryptic soy agar) in 100 μl distilled water, followed by lysis of cells by heating the suspension at 100°C for 10 min and final separation of the DNA from cellular debris by centrifugation for 2 min at 10,000 g. Five microlitre volumes of the resulting supernatants were used for the PCR.

With the primer pair Ccon-f/Ccon-r, the target strain yielded the expected amplification product size (689 bp), whereas no amplification product was obtained for all the non-target strains (100% specificity).

In conclusion, we propose that this extended *rpoB*-gene-based PCR assay, in combination with a *Cronobacter* genus specific PCR (Lehner et al., 2006), is a reliable and time saving method to detect all members of the genus *Cronobacter* described so far. We would like to stress the fact that the PCR primers previously described for the identification of *C. genomospecies* 1 (Stoop et al., 2009) may also be used for the identification of *C. universalis*.

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