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MOLECULAR CHARACTERIZATION OF *Cronobacter sakazakii* ISOLATED FROM DIFFERENT HERBAL TEAS AND MIXTURES IN SERBIA

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Cronobacter sakazakii is an important cause of human infections that can be serious and even fatal among premature neonates and immunocompromised adults or infants. Because of its high tolerance to osmotic stress, *C. sakazakii* is frequently isolated from dried foods, such as powdered infant formula and herbal teas. The aim of investigation was detection, identification and molecular characterization of *Cronobacter sakazakii* isolates from infant formula and various herbal teas collected from Serbian market and tested for import control. *C. sakazakii* was not detected in any of the 360 analysed samples of powdered infant formula. However, 192 out of 520 samples of herbal teas tested were positive for *C. sakazakii* (37.1%). The high prevalence was observed in teas for children (51.6%) and in "baby" teas (44.1%), followed by medicinal teas (38%). The largest one-herb-teas group (221 samples) contained 72 *C. sakazakii*-positive samples (32.6%) and involved *Sennae folium*, *Althaeae radix*, *Menthae piperitae folium*, *Chamomilae flos* and *Urticae folium* teas. Molecular characterization of isolated *C. sakazakii* from different herbal teas by rep-PCR, RAPD and 16S rRNA sequences analysis showed the high similarity to *C. sakazakii* NCTC 8155. Knowing this strain as one of the most pathogenic clinical strains, our results raise concern about the safety risks these foods pose to immunocompromised and healthy consumers, especially for babies and children.

Key words: *Cronobacter sakazakii*, herbal tea, infant formula, RAPD, rep-PCR, 16S rRNA

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INTRODUCTION

Cronobacter sakazakii is a member of a genus *Cronobacter*, formerly *Enterobacter sakazakii*, within the family *Enterobacteriaceae.* A new classification is based on detailed fenotyping and genotyping characterization, and full-length 16S rRNA sequencing (IVERSEN *et al*., 2007; IVERSEN *et al*., 2008). Beside *C. sakazakii*, the genus contains following species: *C. malonaticus, C. turicensis, C. muytjensii, C. dublinensis, C. universalis* and *C. condimenti* (JOSEPH *et al*., 2012). *Cronobacter* are Gram-negative, motile rods with no ability to form spores. They are isolated from various environments as water, soil and foods (FRIEDEMAN, 2007; IVERSEN and FORSYTHE, 2004). *Cronobacter* is highly tolerant to osmotic stress (LIN and BEUCHAT, 2007) hence it is frequently isolated from dried foods, as powdered infant formula, milk powder and herbal teas (BARRON and FORSYTHE, 2007; STOJANOVIC *et al*., 2011; HOCHEL *et al*., 2012). *C. sakazakii* is an emerging opportunistic pathogen. The organism has been isolated from various human clinical specimens such as urine, blood, cerebrospinal fluid, bone marrow, sputum, skin wounds, breast abscess, respiratory secretions and digestive tract samples (CORTI *et al*., 2007; BARRON *et al*., 2007; KUCEROVA *et al*., 2011), as well as from intestinal tracts of various insects and rats (MRAMBA *et al*., 2006; JARADAT *et al*., 2009). It can cause infections ranging from asymptomatic and mild in adults to serious in immunocompromised people and lethal in infants (HEALY *et al*., 2010; YAN and FANNING, 2015). The most risk group is that of neonates, especially those with low birth weight, where infections are causing necrotizing enterocolitis, meningitis, and septicaemiae (MULLANE *et al*., 2007). Virulence of *Cronobacter* varies both between species and within the *C. sakazakii* species (CAUBILLA-BARRON *et al*., 2007; JOSEPH, 2011).

Isolates of *Cronobacter* spp. were characterized by biochemical profiling, genus-specific PCR, 16S ribosomal RNA (rRNA) sequencing and amplified fragment length polymorphism (AFLP) genotyping (TURCOVSKÝ *et al*., 2011), and by serotype, multi-locus sequence typing (MLST) and antibiotic resistance (XU *et al*., 2015). The molecular detection and subtyping methods have significantly improved the identification and characterization of *Cronobacter* isolates. Large number of isolates were characterized using two or more molecular typing - analyses of *rpo*A and 16S rRNA gene sequences (STRYDOM *et al*., 2012), enterobacterial repetitive intergenic consensus (ERIC) and sequence analysis of the *gyrB* gene (CHEN *et al*, 2013), Random Amplified Polymorphic DNA (RAPD) using M13 and ERIC1 primers, 2-step *rpoB*-based PCR and 16S rRNA gene sequence analysis (TSAI *et al*., 2013). Molecular typing enabled detection of virulent strains and comparison of isolates from different hosts and geographic origins.

The aim of this study was to investigate the presence of *C. sakazakii* in infant formulas and herbal teas that are traditionally used as a drink, cure or supplementary in some health problems of babies, children or adults. Using repetitive sequence-based PCR (rep-PCR), RAPD and 16S rRNA sequences, molecular characterization of collected isolates was done and representative isolates were compared to reference strains.

MATERIALS AND METHODS

Isolation of bacteria and phenotyping

In the period of 2007 to 2013, 360 samples of infant formulas were analyzed: samples from market of Serbia (n=203) and samples from Center for Food Examination, Belgrade (n=157). Beside this, the presence of *C. sakazakii* was investigated in 520 samples of herbal teas

from the market of Serbia. For the isolation of bacteria, *Cronobacter sakazakii* standard method ISO / TS 22964:2006 has been used. Identification and phenotyping of bacteria was performed using a commercial test API 20E identification system (API WEB, Biomerieux, France). Control strains were NCTC 8155 *Cronobacter sakazakii* (British Health Agency, England), and ATCC 51329 *Cronobacter muytjensii*, clinical isolate (MicroBioLogics, USA).

Molecular characterization

For PCR amplification, DNA was extracted from bacteria according to method of IBEKWE *et al*. (2000). Prepared DNA was used directly for PCR or stored at -20ºC until use. Genome diversity of *C. sakazakii* isolates was examined using rep-PCR and RAPD. Among rep-PCR, two independent amplifications were used: ERIC (ERIC IR/ERIC 2) and BOX with primer $(GTG)_5$ (JOŠIĆ *et al*., 2012).

RAPD analysis was performed using primers SPH1 (GTGGTGGTGGTGGTG), AP10 (CAGGCCCTTC) as described earlier (ILIČIĆ *et al*., 2016), and AP11 (CAGGCCCTTCA) designed for this study, using the same conditions as for AP10. All primers were synthesized by Metabion International AG, Martinsried, Germany. PCR was carried out in a 50 µl volume with DreamTaqGreen Master Mix (ThermoScientific, Vilnius, Lithuania), 50 ng of prepared DNA template, and 200 nM final concentration of appropriate primer. All PCR amplifications were performed on thermocycler Eppendorf MasterCycler Personal (Eppendorf, Germany).

The PCR products were separated on 1.5% agarose gel (Carl ROTH, GmBh, Karlsruhe, Germany), using electrophoresis system (Apellex, France), stained with ethidium bromide, visualized on a UV transilluminator (Shimadzu 160UV-Vis) and photographed by gel documentation system. Cluster analysis was performed with UPGMA, using Statistica 7 programme.

Molecular phylogenetic analysis was done on the basis of 16S rDNA similarity. For nucleotide sequencing, 16S rDNA was amplified with universal primers fD1/rD1 as already described (JOŠIĆ *et al*., 2012). Amplified products were purified and extracted using GeneJET PCR Purification Kit (Thermo Scientific, Vilnius, Lithuania), according to the manufacturer instructions. The sequence of representative isolates M1, S11, K2 and K5 were obtained using SeqService facility (IMGGI, Belgrade), deposited under accession numbers KU696331-KU696334, respectively, and compared with similar sequences present in the NCBI GenBank database, using the algorithm BLAST. The evolutionary history was inferred using the Neighbor-Joining method (SAITOU and NEI, 1987). There were a total of 1351 positions in the final dataset. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches (FELSENSTEIN, 1985). The evolutionary distances were computed using the Tamura-Nei method using MEGA5 (TAMURA *et al*., 2011).

RESULTS

Detection of bacteria and phenotyping

In the period from 2007 to 2013, 360 samples of infant formulas were collected: 203 samples from the market of Serbia and 157 samples from sanitary inspection analysis. *C. sakazakii* was detected in none of these samples. In the same period, 520 samples of herbal teas were analysed. Most of them were "baby" teas (n=127), medicinal teas (n=81), teas for children (n=62), herbal mixtures (n=29), and other one-herb teas for medicinal or supplementary purpose (Table 1). Phenotyping of *C. sakazakii* was performed using API 20E identification system

collected from 193 positive samples (37.1%). The greatest percent of positive samples were in a group of teas for children, 51.6% (32/62), and "baby" teas, 44.1% (56/127). Molecular characterization of *C. sakazakii* was performed on selected isolates from different sources of herbal tea (Table 2).

Table 1. The prevalence of Cronobacter sakazakii in infant formula and herbal teas

Origin of sample	**Number of samples**	***Cronobacter sakazakii* positive**	
		sample	**percent**
Infant formula	360	0	0
Herbal tea	520	193	37.1
Herbal tea category			
"Baby" tea	127	56	44.1
Tea for children	62	32	51.6
Herbal mixtures	29	2	6.9
Medicinal teas	81	31	38.3
One-herb teas	221	72	32.6

Table 2. Sources of selected isolates used for molecular characterization

Herbal tea	**Isolates**
"Baby" tea	**M1***, K1, S2, S3, S7, S8
Tea for children	S9, S10
Medicinal teas	S4, S5, S6, **S11**
One-herb teas:	
Sennae folium	M2
Althaeae radix	M3, **K2**
Menthae piperitae folium	K3, **K5**
Chamomilae flos	K4
Urticae folium	S1

* isolates used for 16S rDNA sequence analysis are marked as bold

Molecular characterization

Rep-PCR analysis was based on results obtained by ERIC and BOX using $(GTG)_5$ primer (Fig. 1). In cumulative analysis, all isolates of *C. sakazakii* clustered into one cluster with genetic distance of 58% to the *C. muytjensii* ATCC 51329 only (Fig. 2). More than a half of isolates grouped into the same subcluster together with referent strain *C. sakazakii* NCTC 8155, one of the most pathogenic clinical strains. The isolates from "baby" teas - S7 and S8 showed the highest percentage of similarity (78%) to this reference strain. Beside these two strains, together with *C. sakazakii* NCTC 8155 clustered both isolates from tea for children (S9 and S10), and the most of isolates from "baby" teas (K1, S2, S3). Isolates S2 and S3 were the most similar (97%) to each other. Close to them were isolates S10, from tea for children, and S11, from medicinal

tea, with about 76% similarity. The second subcluster was formed of nine isolates divided in two branches. The first included two isolates from medicinal teas (S5 and S6) and M3 from one-herb tea, with 60% similarity to the second branch, which contained five isolates from one-herb teas and only M1 from "baby" tea.

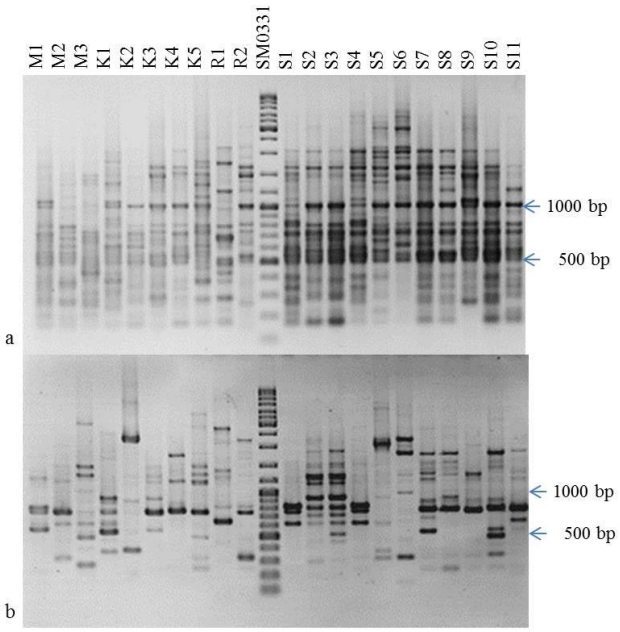

Figure 1. rep-PCR patterns of *C. sakazakii* isolates from herbal teas on the basis of a) BOX elements using $(GTG)_5$ primer and b) ERIC. Reference strains R1- *C. muytjensii* ATCC 51329 and R2- *C. sakazakii* NCTC 8155; *C. sakazakii* isolates M1-M3, K1-K5, S1-S11; SM0331 - GeneRuler DNA Ladder Mix (Thermo Scientific, Vilnius, Lithuania)

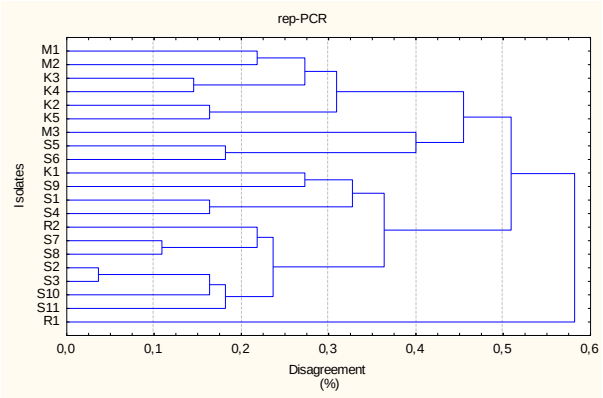

Figure 2. Dendrogram of similarity *C. sakazakii* isolates from herbal teas on the basis of rep-PCR analysis

RAPD analysis was performed using SPH1, AP10 and AP11 primers (Fig. 3). Summary dendrogram revealed that all isolates were grouped into one major cluster divides in two subclusters, except S10 isolate. This isolate, showing genetic distance 52%, originated from tea devoted for children (Fig. 4). Twelve isolates formed one subclusters with genetic distance of 43% to the other containing six isolates and reference strains. Isolates S2 and S3 were the most similar (90%). Similarity of isolates S1 from *Urticae folium* and S4 from medicinal tea was 86%, while S5 and S11 both from medicinal teas, showed 76% similarity. Two isolates from "baby" teas (S7, S8), one from tea for children (S9), isolates from *Menthae piperitae folium* and from *Chamomilae flos* belonged to the same branch. In subcluster 2, isolates M3 (*Althaeae radix*) and K1 ("baby" tea) grouped with referent strain *C. muytjensii* ATCC 51329 with genetic distance of 41%. The separate branch formed isolates from different origins- medicinal tea (S6), one-herb tea (K2, M2) and "baby" tea (M1), with 42% differences from the first branch. Isolates M1, from "baby" tea and M2, from *Sennae folium* were the closest to *C. sakazakii* NCTC 8155, with similarity of 79%.

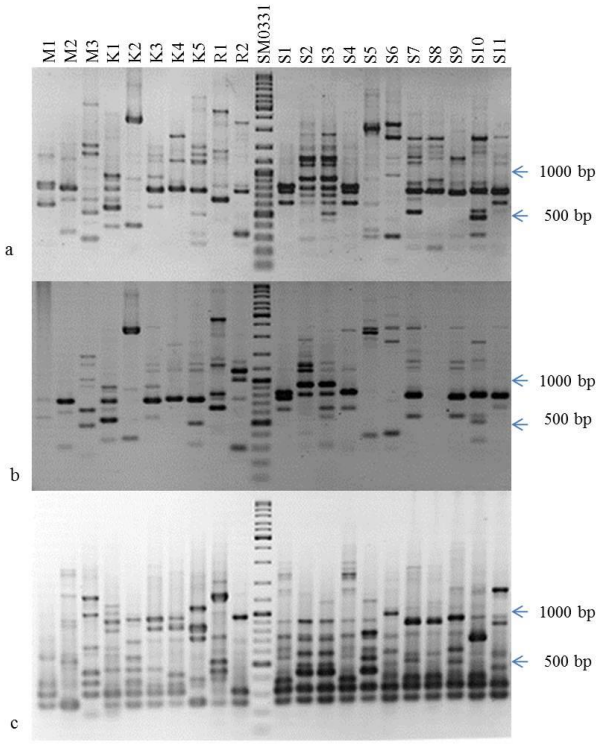

Figure 3. RAPD patterns of *C. sakazakii* isolates using a) AP10, b) AP11 and c) SPH1 primers. Reference strains R1- *Enterobacter* (*Cronobacter*) *muytjensii* ATCC 51329 and R2- *C. sakazakii* NCTC 8155; *C. sakazakii* isolates M1-M3, K1-K5, S1-S11; SM0331 - GeneRuler DNA Ladder Mix (Thermo Scientific, Vilnius, Lithuania)

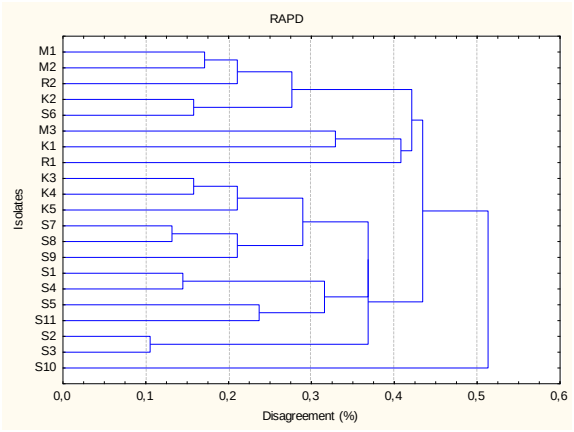

Figure 4. Dendrogram of similarity *C. sakazakii* isolates from herbal teas on the basis of cumulative RAPD analysis

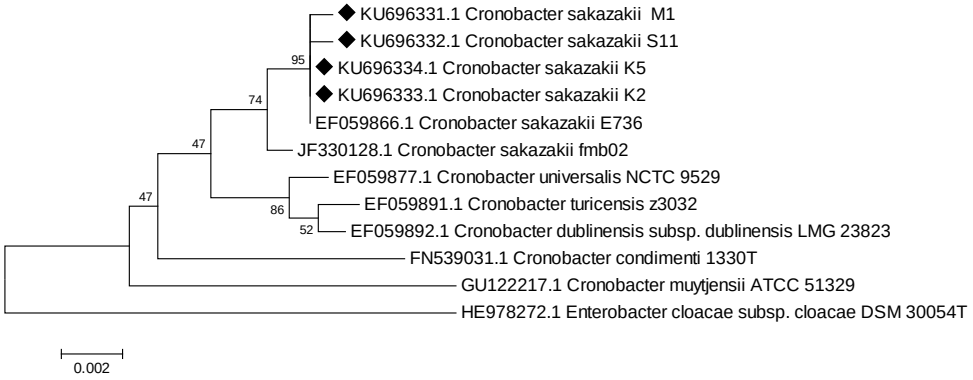

Figure 5. Phylogenetic analysis based on 16S rRNA gene partial sequences (1351 bp) of *C. sakazakii* strains isolated from herbal teas. The evolutionary history was inferred using the Neighbor-Joining method and evolutionary distances were computed using the Tamura-Nei method using MEGA5. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches. T-type strain; *C. sakazakii* strain E736 (NCTC 8155); ♦- strains tested in this study.

Nucleotide sequencing of a region of 16S rDNA, amplified with universal primers fD1/rD1, enabled precise identification and confirmation of *C. sakazakii*. The nearly full-length 16S rDNA (1.5 kb) sequences were amplified from "baby" tea (M1), medicinal tea (S11) and two isolates from one-herb teas (K2, K5) and submitted to GenBank with the accession numbers KU696331- KU696334, respectively. Based on BLAST analysis, comparison with a large number of deposited sequences of 16S rDNA gene proved all isolates *C. sakazakii*. Sequence

alignment indicated that the 16S rDNA sequences of the 4 isolates were 99%-100% identical to those of type strain *C. sakazakii* NCTC 8155 (gb|CP012253.1|) (E736), as well as to *C. sakazakii* strain SP291 (gb|CP004091.1|) and *C. sakazakii* strain fmb09 (gb|JF330135.1|). Phylogenetic analysis of 12 strains based on 16S rRNA gene partial sequences including 1351 bp in final data set showed that all tested strains belong to *C. sakazakii* (Fig. 5).

DISCUSSION

C. sakazakii was investigated in our study as known foodborne pathogen that causes life-threatening diseases in neonates and infants, as well as disease in adults. Although the predisposing conditions in adults are not clearly known, several cases of confirmed *C. sakazakii* infection in adults were correlated with other symptoms: bacteraemia with multiple splenic abscesses in a 75-year-old institutionalised woman (SEE *et al*., 2007); urinary tract infection in a 63-year-old lady with chronic renal failure, with end stage renal damage on maintenance hemodialysis (BHAT *et al*., 2009); primary bacteremia, pneumonia and acute cholecystitis in 3 adult patients had solid organ malignancy and received immunosuppressive drugs, and one had cardiac arrest (TSAI *et al*., 2013); bacteremia secondary to a suspected cyst infection in a heart-and-kidney transplant patient with polycystic kidney disease (TAMIGNIAU *et al*., 2015) etc. *C. sakazakii* infection was confirmed in non-immunocompromised individual as postsurgical osteomyelitis of the femur in a young, otherwise healthy man (CORTI *et al*., 2007).

All cases highlighted investigation of appearance and prevalence of *C. sakazakii* in different food from the Serbian markets. The most important is the presence in powdered infant formula, as the most frequent source of the pathogen. *C. sakazakii* infections among neonates include very serious consequences such as necrotizing enterocolitis, meningitis and bacteremia, with case fatality rates up to 80% (HEALY, 2010). Because of that, it is very promising that no one of 360 analysed powdered infant formula from the market of Serbia was contaminated with *C. sakazakii.* No *Cronobacter* was detected in commercial powdered infant formula and infant food formula in China (LI *et al*., 2014). The low contamination rate in infant formula reported also JARADAT *et al*. (2009), with only one *C. sakazakii* positive sample of infant food among the 76 samples of infant, milk and non-milk dairy products tested. Several investigations suggested that contaminations of milk powders are originating most likely from the environment then raw milk (LEHNER *et al*., 2010). CASALINUOVO *et al*. (2014*)* confirmed *C. sakazakii* in only one sample of artisan mozzarella cheese made from cow's milk in Italy and suggested that it could be related to external contamination during the phases of production.

C. sakazakii has been isolated from a wide range of environmental sources other than infant formula and milk powder. It was detected in raw and processed food, in dry as well as in fresh and in frozen products, in fermented and cooked food products, meat, fish, in beverages and water suitable for the preparation of food, in ready-to-eat foods (FRIEDEMANN, 2007; JARADAT *et al*., 2009; XU *et al*., 2015). Among plants species, *C. sakazakii* is found in wheat, rice, herbs, spices, cereal, fruit, legume, lettuce and other vegetables (FRIEDEMANN, 2007; OSAILI and FORSYTHE, 2009; JARADAT *et al*., 2009; LI *et al*., 2014).

Among 520 samples of herbal teas tested during our investigation, *C. sakazakii* was detected in 37.1%. The highest prevalence was observed in teas for children - 51.6% (32/62) and then in "baby" teas - 44.1% (56/127). *C. sakazakii* -positive samples from the one-herb-teas group (72/221) represented 32.6% and involved *Sennae folium*, *Althaeae radix*, *Menthae piperitae folium*, *Chamomilae flos* and *Urticae folium*. All of these medicinal plants are

consumed as teas for medicinal or supplementary purpose, while chamomile, mint and nettle leaves are in common usage as a drink in healthy customers.

Similarly, JARADAT *et al*. (2009) reported that herbs and spices harboured the high number of *Cronobacter* isolates, indicating plants as a possible reservoir of this pathogen. They confirmed *C. sakazakii* in different medicinal plants – liquorice, thyme, anise, chamomile, fennel, sage**,** as well as in environmental samples (vacuum dust). Herbal mixtures tested in our study showed 6.9% appearances of *C. sakazakii* (2/29), which is lower than 73.3% confirmed in mixed spices (11/15) reported by JARADAT *et al*. (2009).

Our results are in concordance with the numerous studies. BAUMGARTNER *et al*. (2009) confirmed *Cronobacter* spp. in ready-to-eat foods, with 61% of sprout-fresh herbs and 27% of dried herbs-spices sample positive. The presence of *Cronobacter* spp. was detected in 16.7% of the 60 analysed spices and herbs available on the Polish market, mainly samples of herbs (basil, tarragon, parsley) and one sample of a seasoning blend (Provence herbs) (GARBOWSKA *et al*., 2015). SINGH *et al*. (2015) also indicated plants as a reservoir of *Cronobacter* spp., according to higher prevalence of this pathogen in herbs and spices (34%) than environmental samples (23%), and emphasized the screening of plant materials before their incorporation in food matrices. In Serbia, STOJANOVIC *et al*. (2011) identified *C. sakazakii* biochemically in 48 out of 150 teas samples; to the best of our knowledge no molecular characterization of isolates from Serbia was reported.

Molecular characterization of selected *C. sakazakii* isolates in this study was performed in order to estimate their diversity and comparison with reference strains *C. sakazakii* NCTC 8155 and *C. muytjensii* ATCC 51329. Both, rep-PCR and RAPD revealed informative patterns of *C. sakazakii* isolates from herbal teas. Based on cumulative rep-PCR analysis using ERIC and $(GTG)_5$ primers, *C. muytjensii* ATCC 51329 reference strain was separated (58%) from tested isolates and *C. sakazakii* NCTC 8155. The most isolates from teas for babies and children grouped in the same subcluster with reference strain NCTC 8155, while isolates from medicinal teas, one-herb tea and one isolate from "baby" tea formed separate subcluster. Results of cumulative ERIC and BOX analyses obtained in this study are similar to those of other authors. Comparing *C. sakazakii* from infant formula milk, YE *et al*. (2009) used ERIC analysis and clustered 24 strains in two groups, while ATCC 51329 formed a separate branch. In addition to ERIC, YE *et al*. (2010) used RAPD and antibiotic resistance patterns to determine the phenotypic and genotypic characterization of *C. sakazakii* strains isolated from infant formula milk. They reported 16 ERIC and 18 RAPD fingerprint types, as well as 6 antibiotic resistance patterns after analyses of 22 isolates. ERIC patterns showed a closer correlation to antibiotic resistance patterns than RAPD.

RAPD analysis by AP10, AP11 and SPH1 primers used in this work clustered isolates from different sources. High similarity of two isolates from teas for babies (S2 and S3) revealed by rep-PCR was confirmed by RAPD. S10 isolate from tea for children showed the highest genetic distance to all isolates using this method. The reference strain *C. muytjensii* ATCC 51329 clustered with isolates from *Althaeae radix* and "baby" tea showing genetic distance of 41%. Isolate from "baby" tea and *Sennae folium* were the closest to *C. sakazakii* NCTC 8155. Our results confirmed RAPD as useful in comparison of *C. sakazakii* from different isolation sources, as already reported. DRUDY *et al*. (2006) used this method to characterize a collection of 56 *C. sakazakii* isolates from environmental and food sources. The presence of *C. sakazakii* in sunsik flours (sea tangle, brown rice, non-glutinous rice, dried anchovy) were confirmed by

sequences analyses of tDNA, ITS (internal transcribed spacer sequences) and 16S rRNA, while RAPD was carried out to compare isolates to ATCC strains, as well as a monitoring tool to determine the contamination route of pathogen during processing (CHOI *et al*., 2008). To analyse main reservoirs and contaminated sources of *C. sakazakii*, KIM *et al*. (2011) compared isolates from *sunshik* products, its ingredients, and root vegetable farm's soils by RAPD, 16S rRNA and pulsed-field gel electrophoresis (PFGE). The high similarity levels of RAPD patterns were obtained between clinical strains and isolates from *sunshik* products and root vegetables (yam, carrot), indicating that root vegetables can be an important contamination source of *Cronobacter* spp. in *sunshik* products.

Using 16S rRNA-based identification, all tested strains were identified as *C. sakazakii* despite the variety of teas as isolation sources. The highest similarity was obtained to *C. sakazakii* NCTC 8155 reference strain, *C. sakazakii* strain SP291 isolated from a factory producing powdered infant formula (POWER *et al*., 2013) and *C. sakazakii* strain fmb09 - one of the 13 isolates from cereal, cereal products and spices (LI *et al*., 2014). According to JARADAT *et al*. (2009), 16S rRNA sequencing is pivotal to confirm the identity of the isolates and none of the biochemical, chromogenic or PCR primers proved to be a reliable method for confirmation of the identity of the isolates *C. sakazakii*. They tested 233 samples of food, infant formula and environment from different countries using initial screening with API 20E and isolated 42 *Cronobacter* spp. After characterization using 3 chromogenic media (α- MUG, DFI and EsPM) and 8 sets of PCR primers detecting ITS, 16S rRNA, zpx, gluA, gluB, OmpA genes, *Cronobacter* spp. strains were not confirmed because the methods showed false positives or false negatives. The final confirmation step was done by 16S rRNA sequence analysis identifying only 29 of the 42 isolates as *Cronobacter* spp. Identification and confirmation of *Cronobacter* spp. by 16S rRNA sequence analysis was used for isolates from different origin (infant formula, medicinal plants, various foods and environmental samples) and for their phylogenetic analysis (TURCOVSKÝ *et al*., 2011; STRYDOM *et al*., 2012; TSAI *et al*., 2013; SINGH *et al*., 2015).

Characterization of *C. sakazakii* isolated from different herbal teas in Serbia, performed using phenotyping by API 20E and genotyping by rep-PCR, RAPD and 16S rRNA sequences, revealed high similarity to *C. sakazakii* NCTC 8155 reference strain. RAPD and rep-PCR enabled genotyping and comparison of isolates from different herbal teas.

CONCLUSION

Frequent findings of *C. sakazakii* in herbal teas, especially in baby and children teas, and their genetic similarity to one of the most pathogenic clinical strain- reference strain *C. sakazakii* NCTC 8155, indicate this bacterium as a great threat in causing fatal infections.

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MOLEKULARNA KARAKTERIZACIJA *Cronobacter sakazakii* IZOLOVANIH IZ RAZLIČITIH BILJNIH ČAJEVA I NJIHOVIH MEŠAVINA U SRBIJI

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Izvod

Cronobacter sakazakii je uzročnik humanih infekcija koje mogu prouzrokovati ozbiljne i čak fatalne posledice kod odojčadi i imunokompromitovanih odraslih osoba i dece. Zbog visoke tolerancije na osmotski stres, *C. sakazakii* je često izolovan iz dehidratisanih proizvoda, kao što su formule za bebe u prahu i biljni čajevi. Cilj ovih istraživanja je detekcija, identifikacija i molekularna karakterizacija *Cronobacter sakazakii* izolata iz formula za bebe i različitih biljnih čajeva uzorkovanih u prodavnicama u Srbiji ili testiranih pri kontroli izvoza. U svih 360 analiziranih uzoraka mleka u prahu za bebe nije detektovana kontaminacija bkterijom *C. sakazakii*. Medjutim, u 192 od 520 testiranih uzoraka čajeva utvrdjeno je prisustvo *C. Sakazakii* (37.1%). Visoka zastupljenost je detektovana u čajevima za decu - 51.6% i u "bebi" čajevima - 44.1%, a zatim u čajevima za medicinsku upotrebu -38%. Najveća grupa čajeva koji sadrže 1 biljnu vrstu (221) sadržala je 72 *C. sakazakii* - pozitivnih uzoraka (32.6%) i uključivala je čajeve od *Sennae folium*, *Althaeae radix*, *Menthae piperitae folium*, *Chamomilae flos* i *Urticae folium*. Molekularna karakterizacija izolovanih *C. sakazakii* iz različitih biljnih čajeva rep-PCR, RAPD i 16S rRNA analizom sekvenci ukazala je na visok stepen sličnosti soju *C. sakazakii* NCTC 8155. S obzirom da je ovo jedan od najpatogenijih kliničkih sojeva, ovi rezultati ukazuju na potrebu za povećanom kontrolom zbog mogućeg rizika koju ove namirnice predstavljaju za imunokompromitovane i zdrave korisnike, a posebno za bebe i decu.

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