Enterocins in relation to *Cronobacter* spp. By A. Lauková[1] and M. Marounek[2]. [1]*Institute of Animal Physiology Slovak Academy of Sciences, Šoltésovej 4-6, 04001 Košice, Slovakia.* [2]*Czech University of Life Sceinces, Department of Microbiology, Nutrition and Dietetics, 165 21 Prague 6-Suchdol, Czech Republic*

Introduction

At present, the genus *Cronobacter* includes 7 species; however, mostly isolated species as opportunistic human pathogens are *Cronobacter sakazakii* (previously classified as *Enterobacter sakazakii)*, *Cronobacter malonaticus, C. turicensis*[(1).] Most of the *Cronobacter* spp. strains described in the literature have been isolated from clinical sources and food[(2)] . To know the reservoirs of Cronobacters is important to prevent their spreading. Mozrová et al.[(3)] presented characterization of Cronobacters even from the dust of the vacuum cleaners in housholds. Because e.g. Cronobacters from retail food were with variable reaction to different antibiotics or they were resistant e.g. to erythromycin, we would like to check susceptibility or resistance of environmental strains of Cronobacters (kindly supplied by prof. Marounek) to bacteriocins (enterocins). Enterocins (Ents) represent ribosomally synthetized proteinaceous antimicrobial substances with inhibitory activity against related and/or more or less related bacteria which are produced mostly by the species *Enterococcus faecium*[(4)]. Our Laboratory of Animal Microbiology (LAM) have been focused on enterocins study for years. Enterocins have been successfully applied to reduce spoilage bacteria in different animal and/or food[(5-7)]. Therefore, the aim of this study was to test sensitivity of *Cronobacter* spp. as causative human agents to Ents produced by the strains *E. faecium* of different origin (our isolates). This aim was conducted dominantly from the basic research point of view to test the antimicrobial spectrum of our enterocins.

Material and Methods

Eighty-five samples of the dust from vacuum cleaners of households were provided. Twenty-four strains were isolated and characterized as presented previously by Mozrová et al.[(3)].

Semi-purified enterocins (Ent M, Ent 55, Ent 2019, Ent M3a, Ent A,P and durancin-like) were used in the study. They are produced by the *E. faecium* strains of different origin, isolated at our LAM (Slovak Academy of Sciences, Košice, Slovakia) and *E. durans* ED26E/7. Ent M and Ent A (P) are produced by environmental *E. faecium* strains AL41 and CCM7419=EK13. *E. faecium* EF2019-CCM 7420 from rabbit faeces produces Ent 2019. *E. faecium* M3a from rabbit meat produces Ent M3a; Ent 55 is produced by avian *E. faecium* EF 55 and durancin-like 26E/7 is produced by the strain isolated from traditional Slovak „Bryndza“ cheese. Enterocins were semi-purified according to previously reported protocols[(8-10)]. Ents were with initial activity as follows: Ent 55, Ent A (P), Ent 2019 and durancin-like substance 12 800-25 600 AU/ml; Ent M, Ent M3a 6 400AU/ml against the principal isolate *E. avium* EA 5 strain (our isolate from piglet). Sensitivity of Cronobacters to Ents and durancin was tested by the quantitative agar spot test[(11)]using Trypticase soy agar (Becton & Dickinson, Cockeysville, USA). Inhibitory activity was expressed in Arbitrary units per mililiter (AU/ml); it responses to the highest dilution of Ent causing the inhibition of the growth of the indicator strain.

Results and Discussion

Among 24 isolates allotted to the species *Cronobacter sakazakii* (16), *C. malonaticus* (8) and *C. turicensis* (1), 12 strains (50%) showed sensitivity to 1 Ent and 12 (50%) were resistant to Ents used. From 16 strains of *C. sakazakii*, 7 strains (43.75%) were sensitive to durancin-like substance 26E/7 (Table 1). *C. sakazakii* DBM3371was however sensitive to 5 of 6 Ents used; it was the most sensitive strain to Ents. Its growth was not inhibited only by Ent 2019. The growth of 4 strains of *C. malonaticus* was inhibited by Ent-durancin 26E/7 (Table1). Inhibitory activity was 100 AU/ml. *C. turicensis* was resistant to Ents used. Bacteriocins and Ents involving here are nowdays known to have activity not only towards closely related Gram-positive but also against Gram-negative bacteria[12] . In our previous *in vivo* results with application of e.g. Ent M, Ent 2019 in rabbits or ostriches even tendency to reduce Camplylobacters was noted[13] and the counts of coliforms in animals were diminished repeatedly in more experiments[14-16]. The question is, why Cronobacters were sensitive even to up to now not completely purified substance as durancin-like is; that is, almost half strains were sensitive to durancin. We dont know mode of action, but we have inhibition. The results achieved will be repeated again and more detaily provided e.g. by competitive activity of durancin with the most sensitive DBM3371 strain. In general, these preliminary results are very promising for the further study.

Conclusion

Twelve Cronobacterss showed sensitivity to durancin-like substance 26E/7. *C. sakazakii* DBM3371was however sensitive to 5 of 6 Ents tested with activity 100 AU/ml. Although preliminary testing, it indicated promising direction in this study.

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Table 1 Sensitivity of *Cronobacter* spp. to enterocins

Cronobacters	Ent M	Ent A (P)	Ent 55	Ent 2019	Ent M3a	Ent-dur 26E/7
Cronobacter sakazakii DBM3290	-	-	-	-	-	100
DBM3292	-	-	-	-	-	100

DBM3293	-	-	-	-	-	100
DBM3371	100	100	100	-	100	100
DBM3372	-	-	-	-	-	100
DBM3374	-	-	-	-	-	100
DBM3429	-	-	-	-	-	100
DBM3433	-	-	-	-	-	100
Cronobacter malonaticus DBM3375	-	-	-	-	-	100
DBM3426	-	-	-	-	-	100
DBM3376	-	-	-	-	-	100
DBM3432	-	-	-	-	-	100

Initial activity of enterocins*:* Ent 55, Ent A (P), Ent 2019 12 800- 25 600 AU/ml; Ent M, Ent M3a 6400AU/ml. Activity is expressed in AU/ml.