PhD Thesis Abstract

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"Characterization of Enterococcus faecalis isolated from intensive care units and food samples, and testing their ability for bacteriocins production"

Enterococcus faecalis belongs to lactic acid bacteria which are commonly known to produce antimicrobial peptides called bacteriocins. In this study, 38 *E. faecalis* isolates were isolated from food samples (n=11) and clinical samples (n=27).

These Enterococcus isolates were identified to the genus level depending on microscopical examination, culture characters of colonies on bile esculin agar, testing for lactic acid production and PYR test. Identification to the species level was based on sequencing of 16s rRNA gene.

Phenotypic characterization of some virulence factors belonging to Enterococcus isolates revealed that 21 *E. faecalis* isolates (55.3%) were able to hydrolyse gelatin as a result of proteolytic gelatinase activity. A number of 12 *E. faecalis* isolates (31.5%) were able to make complete blood hemolysis (β hemolysis) as a result of cytolysin activity. A number of 22 *E. faecalis* isolates (57.9%) were able to hydrolyse bile salts as a result of bile salt hydrolase activity.

Three strains isolated from food samples and nine strains isolated from clinical samples showed an ability to produce bacteriocins.

Continuing phenotypic characterization for bacteriocinogenic isolates was done by detection of fermentation profile using (API 50 CH) system and antibiotic resistant pattern. All bacteriocinogenic *E. faecalis* isolates were sensitive to

Ampicillin and Vancomycin while all of them were resistant to Polymyxin B, Clindamycin and Fusidic acid.

Genotypic characterization for bacteriocinogenic isolates was done by investigating the epidemiology and population structure by a mean of multi locus sequence typing (MLST) of 7 housekeeping genes and that resulted in presence of these isolates in between sequence types 116, 141 and 6.

The presence of 17 common bacteriocins encoding genes were analyzed and revealed absence of any structural genes corresponding to bacteriocins production except Enterolysin A and Cytolysin genes which were only detected in one bacteriocinogenic strain OS6.

The *E. faecalis* OS13 isolate was shown to produce a large amount of narrow spectrum and highly potent bacteriocin named enterocin OS13. Enterocin OS13 was purified and is comprised of two novel bacteriocin peptides that inhibited the growth of antibiotic resistant nosocomial *E. faecalis* and *E. faecium* isotates. The two peptides designated enterocin OS13α and enterocin OS13β were purified to homogeneity from the culture supernatants by ammonium sulphate precipitation, cation-exchange chromatography and reverse phase chromatography. The molecular weight of enterocin OS13α and enterocin OS13β was determined to be 8079 Da and 7859 Da, respectively. Both bacteriocins are heat labile. Enterocin OS13α is sensitive to proteinase K enzyme while enterocin OS13β is resistant.

E. faecalis OS13 belongs to the sequence type 116 (ST116) and is being γ-haemolytic, bile salt hydrolase negative, and gelatinase positive phenotypes. Antibiotic sensitivity test showed that the OS13 isolate is sensitive to ampicillin, penicillin, vancomycin, erythromycin, kanamycin and gentamicin.

In conclusion, nosocomial infections caused by multiresistant Enterococci are an ever increasing problem. Antibiotic resistant clones colonize the hospitals and acquire and transmit antibiotic resistance by horizontal gene transfer. Enterococcal infections refractive to vancomycin, methicillin and daptomycin occur. Thus novel therapeutic agents for treatment of such infections are needed. Bacteriocins producing strains or just their bacteriocins have been predicted to be useful in the control of gastrointestinal microflora, and in the protection against pathogens.

To the best of our knowledge, this is the first time to report and identify Enterolysin A in between pathogenic *E. faecalis* isolated from clinical specimen collected from human in Egypt.

From our knowledge, this is the first time for *E. faecalis* with ST116 to be isolated from food sample**.**

Bacteriocin peptides like the enterocinOS13 α & β represent novel antimicrobial agents perfectly designed to combat multiresistant nosocomial Enterococcal infections, without disturbing commensal microflora.

However, several safety criteria should be taken into consideration for bacteriocinogenic *E. faecalis* before being used as a probiotic culture to improve microbiological quality of fermented foods or medically used to fight antibiotic resistant bacteria.

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