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Antimicrobial Activity of *Enterococcus faecalis* Against Selected Isolates and Some Pathogenic Microorganisms

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Abstract: Ten isolates of *Enterococcus faecalis* were tested for antimicrobial activity against closely related and less closely related pathogenic gram-positive and gram-negative indicator strains. Using the agar spot test *Enterococcus faecalis* MI2, MI6, MI126 and MI128 showed strong activity against selected enterococcal isolates. *E. faecalis* MI6 and MI128 showed strong antimicrobial activity against *Listeria innocua* NCTC. 11288 but enterococcal isolates did not show activity against selected pathogenic strains of some other species. A clear inhibition zone around the spotted test culture observed antimicrobial activity of *E. faecalis* isolates. The diameters of zones of inhibition ranged from 2-8 mm.

Key words: Antimicrobial activity, microorganisms, *E. faecalis*

Introduction

Enterococci are gram-positive, facultative anaerobic and lactic acid producing bacteria (Laukova *et al.*, 1993). The inhibitory substances produced by enterococci active against pathogens have become of great interest in recent years (Villani *et al.*, 1993). The antimicrobial activity of *E. faecalis* against *Listeria monocytogenes* has also been investigated (Arihara *et al.*, 1991) Lactic acid bacteria are known to produce bacteriocins (Siragusa, 1992). Bacteriocins are proteins or protein complexes (Koniskey, 1982) not active against producer strains but which can inhibit the growth of other closely related bacteria. It was recently reported that there are some bacteriocins, which can also inhibit the growth of less closely, related pathogenic microorganisms (Laukova *et al.*, 1993). Bacteriocins produced by lactic acid bacteria can inhibit the growth of a variety of food-borne pathogens including *Staph aureus; clostridium botulinum* and *L. monocytogenes* (Lewus *et al.*, 1991; Kaiser and Montville, 1993; Kato *et al.*, 1993). Bacteriocins or bacteriocin like substances produced by gram-positive bacteria have a broad spectrum of antimicrobial activity (Tagg *et al.*, 1976). It has been shown that lactic acid bacteria can inhibit the growth of undesirable microorganisms such as *L. monocytogens* in foods through bacteriocins (Daba *et al.*, 1991). Due to their proteinaceous nature bacteriocins would be digested into amino acids in the human gastrointestinal tract. This would be an advantage for bacteriocins as food-additives as compared with the other chemical antimicrobial agent (Piard *et al.*, 1992). The activity of bacteriocins against Gram-positive food borne pathogens has increased interest towards their potential application in foods (Garver and Muriana, 1993).

In this study the antimicrobial activity of *E. faecalis* strains against selected gram-positive and gram-negative bacteria was investigated. Four strains of *E. faecalis* were found to have antimicrobial activity.

Materials and Methods

Bacterial isolates and culture conditions: The present study was conducted in the Department of Biology, University College, London in 1998. *E. faecalis* MI1, MI2, MI3, MI4, MI5, MI6, MI7, MI126, MI127 and MI128 were obtained from University College Hospital U.K. The indicator strains namely *Staphylococcus aureus, Escherichia coli* 1/770, *E. coli* 3/904, *Salmonella enteritidis, Salmonella typhimurium, Bacillus magaterium, Bacillus cereus* and *Listeria innocua* NCTC 11288 were provided by Dr. David G. Smith Department of Biology (University College London). All isolates were grown in Brain Heart Infusion broth (BH1, Oxoid CM 225) at 37 °C. These cultures were stored in refrigerator and transferred weekly. Fresh overnight cultures were used in the experiments.

Detection of antimicrobial activity: Antimicrobial activity of *E. faecalis* isolates against other selected bacteria was detected by the agar sport test (Fleming *et al.*, 1975). *E. faecalis* strains were inoculated into test tubes containing 5 ml of sterilized BHI broth and incubated at 37 °C for overnight, using a separate test tube for each strain. Five µl of overnight culture of each strain was spotted on Brain Heart Infusion agar (BHI, oxoid 375) plates. All plates were incubated at 37°C for overnight. The indicator strains were also inoculated into 5 ml sterilized BHI broth and incubated overnight at 37 °C. Fresh overnight culture (0.1 ml) of each indicator strain was mixed with 5 ml of BHI soft agar (1.5%), cooled to 45 °C and overlaid on the overnight grown spotted cultures of *Enterococcus*, the soft agar was allowed to solidify. All the plates were incubated at 37 °C overnight. After overnight incubation, antimicrobial activity was seen by a clear zone around the spotted culture. Zones of inhibition were measured in millimeters.

Results and Discussion

The antimicrobial activity of *E. faecalis* strains against enterococci was determined by using the same strains as test strains and as indicator strains. It was found that 4 (40%) out of 10 *E. faecalis* strains showed antimicrobial activity against *Enterococcus* strains (Table 1). The antimicrobial activity of *E. faecalis* strains against selected pathogenic microorganisms (indicator strains) such as *Staph aureus, E. coli* 1/770, *E. coli* 3/904, *S. typhimurium S. enteritidis, B. magaterium, B. cereus* and *Listeria innocua* NCTC 11288 was measured. Antimicrobial activity was only found toward *L. innocua* NCTC 11288 and not against other strains (Table 2).

Four strains of *E. faecalis* produced inhibitory agent, which was active against Enterococcus strains and two *Enterococcus faecalis* strains produced inhibitory agent against L. innocua NCTC 11288 (Table 1, 2). On the other hand none of the selected gram-negative bacteria tested were inhibited, among gram-positives bacteria tested, only *L. innocua* NCTC 11288 was inhibited by *E. faecalis* strains.

Due to proteinaceous nature and bactericidal mode of action the inhibitory molecule produced by enterococci as concluded to be a bacteriocin by Kato *et al.* (1993). In this study the inhibitory

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Table 1: Antimicrobial activity of *E. faecalis* by using the same strains as tested strains and as indicator strains by the agar spot test. Inhibition zone (mm)

Test strains	Indicator stains (*E. faecalis*)									
E. faecalis	MI1	MI2	MI3	MI4	MI5	MI6	MI7	MI126	MI127	MI28
MI1	0	0	0	0	0	0	0	0	0	0
MI2	8	0	6	8	5	7	5	4	5	5
MI3	0	0	0	0	0	0	0	0	0	0
MI4	0	0	0	0	0	0	0	0	0	0
MI5	0	0	0	0	0	0	0	0	0	0
MI6	3	0	2	4	3	0	0	3	2	2
MI7	0	0	0	0	0	0	0	0	0	0
MI126	5	0	5	5	5	4	4	0	4	3
MI127	0	0	0	0	0	0	0	0	0	0
MI128	0	0	3	2	2	2	2	1	0	0

Table 2: Antimicrobial activity of *E. faecalis* stains against selected gram-positive and gram-negative pathogenic strains. Inhibition zone (mm)

Test strains		Indicator strains						
E. faecalis	*Staph. Aureus*	*E. coli* 1/770	*E. coli* 3/904	*S. enteritidis*	*S. typhimurium*	*B. cereus*	*B. megaterium*	*B. innocua* NCTC-11288
MI1	0	0	0	0	0	0	0	0
MI2	0	0	0	0	0	0	0	0
MI3	0	0	0	0	0	0	0	0
MI4	0	0	0	0	0	0	0	0
MI5	0	0	0	0	0	0	0	0
MI6	0	0	0	0	0	0	0	5
MI7	0	0	0	0	0	0	0	0
MI126	0	0	0	0	0	0	0	0
MI127	0	0	0	0	0	0	0	0
MI128	0	0	0	0	0	0	0	3

activity was found towards closely related bacteria as reported by Lankuva *et al.* (1993) and not against less closely related pathogenic gram-negative bacteria and some gram-positive strains. Koinisky (1982) defined a bacteriocin as a protein or protein complex synthesized by a bacterium which is inhibitory to a narrow range of other susceptible bacteria but is not self inhibitory. It seems likely that the inhibitory molecules produced by enteroccal isolates in this study were bacteriocins. McKay (1990) demonstrated the antimicrobial activity of *E. faecium* against a wide range of *Listeria* spp. *E. faecium* strain CCM4231 produced a bacteriocin which showed inhibitory effect against *Clostridium perfringens* (Laukova *et al.*, 1993). In another study three strains of *E. faecalis* were reported to inhibit *L. monocytogenes* (Arihara *et al.*, 1991).

It was shown that bacteriocin or bacteriocin like substances produced by gram-positive bacteria have a broad spectrum of antimicrobial activity (Tagg *et al.*, 1976). It was reported that bacteriocins produced by lactic acid bacteria are active against a variety of food-borne pathogens including *B. cereus, C. perfringens, Listeria* spp. and *S. aureus* (Lewus *et al.*, 1991). *E. faecalis* 226 produced the inhibitor substance known as enterocin 226NWC. Enterocin 226NWC has a narrow range of activity; it inhibited only strains of enterococci, *L. innocua* and *L. monocytogenes* (Villani *et al.*, 1993). It was reported that the production of enterocin 226NWC is encoded by a 5.2 kb conjugative plasmid. Enterocin 226NWC, due to its action against *Listeria*, may play an important role in food protection and preservation (Villani *et al.*, 1993). *Leuc. mesenteroides* ssp. *Mesenteroides* FR52 produced a bacteriocin named mesenterocin 52 which showed antimicrobial activity against *Leuconostoc, Enterococcus* and *Listeria* strains (Mathieu *et al.*, 1993). Mesenterocin 52 differed from mesentocin 5, a bacteriocin produced by *Leuc. mesenteriodes* UL5, which was active against *L. monocytogenes* but had no action on *Leuconostoc* strains (Daba *et al.*, 1991). *E. faecium* isolated from Nigerian dairy product produced bacteriocins named enterocin 01 was reported active against some strains of *Lactobacillus, Enterococcus* and *Listeria*. The antimicrobial activity of enterocin 01 was reported to be stable at 100°C for 5 minutes and at pH 2.0 and 6.0 (Olasupo *et al.*, 1994).

E. faecalis ssp. *Liquefaciens* S48 produced the bacteriocin Bc-48 which inhibited the related strains of *E. faecalis* only. This strain produced the peptide antibiotic AS-48 (mutant), a low molecular weight antibiotic which can inhibited gram-positive and gram-negative bacteria. Bacteriocin Bc-48 was stable in a pH range 5.5 to 9.0 and was highly sensitive to temperature and inactivated at 50 °C after 60 minutes (Lopez-Lara *et al.*, 1991).

Many lactic acid bacteria have been shown to inhibit the growth of a wide variety of food spoilage organisms (Jack *et al.*, 1995). Some *Lactococcus lactis* isolates produce an inhibitory agent called nisin and nisin producing strains has been used in food preservation especially dairy products (Jack *et al.*, 1995). Some bacteriocins produced by lactococci and lactobacilli have been reported active against wide range of gram-positive bacteria and some have also inhibited gram-negative species (Jack *et al.*, 1995). The antimicrobial activity of some strains of enterococci was shown to be plasmid encoded (Arihara *et al.*, 1991). Plasmid-borne genes encode many bacteriocins produced by gram-positive bacteria but some like nisin have been reported to be transposon associated (Jack *et al.*, 1995).

Bacteriocin production is influenced by cultural conditions. For example some strains required rich media for the production of bacteriocins. It was shown that BHI broth is the best medium for the production of bacteriocins (Lopez-Lara *et al.*, 1991). The pH of the medium also has a large impact on bacteriocin production. *Lactobacillus bravaricus* MN produces a bacteriocin bavaricin MN. The levels of this bacteriocin increase about 12-fold from pH 6.5 to pH 6.0 in pH controlled fermenters (Kaiser and Montville, 1993). Bacteriocins can be sued in food industry as potential natural food preservatives against several spoilage bacteria and pathogens (Daba *et al.*, 1991). Bacteriocin typing has been used in studies of Gram-negative and Gram-positive bacteria. Enterococci can be typed according to the production of or the sensitivity to bacteriocins (Tagg *et al.*, 1976). In this study 10 isolates of E. faecalis were tested for antimicrobial activity. Four isolates showed strong antimicrobial activity against different tested

strains. The use of inhibitory agent producing microorganisms in food may provide a natural means of preservation because these organisms have the ability to inhibit the pathogens.

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