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A Multidimensional Approach to *Enterococcus faecalis*

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Enterococcus faecalis **formally known as** ***Streptococcus faecalis*** **is the most active bacteria of genus** ***Enterococcus.*** **Due to its' role as a pathogen and its' contribution as a Lactic acid bacteria make it the topic of debate.** ***Enterococcus faecalis*** **is naturally present in normal microflora of gastrointestinal tract and in oral cavity of human in non fatal form. But its' involvement in endodontic, endocarditic, pelvic and urinary tract infections make it more lethal; this is happened due to the presence of virulence factors (aggregation substance, gelatinase, cytolysin, pheromones etc.) and antibiotic resistant gene etc. It is found that in most hospital acquired infections the strains of** ***Enterococcus faecalis*** **are resistant to many antibiotics e.g. vancomycin, gentamycin, streptomycin, ampicillin and tetracycline but the strains resistant to Vancomycin and Ampicillin make it worse pathogens. Due to the production of biogenic amines from them, they contaminated the poultry, beef and pork products. Despite of its negative features some of its properties make it mandatory in dairy products specially in cheese e.g. Mozzarella, Cebriro, Venaco etc. due to proteolysis, lipolytic activity and citrate metabolism it can produce acetaldehyde, ethanol, diacetyl, acetone, and acetoin which increase the unique texture, flavor and aroma of cheese. The bacteriocin excreted by** ***Enterococcus faecalis*** **can prevent the growth of other unsafe bacteria and used as a food additives. The selection of** ***Enterococcus faecalis*** **should be strain depended and** ***Enterococcus faecalis*** **transfer their virulence train via conjugative transfer of plasmid within strains. So this review comprises the feature of** ***Enterococcus faecalis*** **as friend and foe.**

Key words: *Enterococcus faecalis*, virulence factors, enterocin, starter culture, cheese.

Streptococcus faecalis was first used by Andrews and Horder in 1906 to identify an organism of faecal origin; they isolated this organism from a patient with endocarditis which was able to ferment mannitol and lactose but not raffinose. *Enterococci* are part of the dominant microbiota of several dairy products. They are used in the dairy produsts as starter cultures. However, they are also caught up in severe multi-resistant nosocomial human infections (Dardir *et al.,* 2011). However, although fermented foods containing *Enterococci* have a long history of safe use, the presence of *Enterococci* in food is of considerable concern for the food industry and consumers. Indeed, *Enterococci* are considered as emerging pathogens of humans and are often associated with hospital acquired infections (Franz *et al.,* 1999).

Particularly, strains of *E. faecalis* as a predominant species and to, a lesser extent *E. faecium* have been reported to be epidemiological relationship and involved in human pathogenesis (Giraffa, 2003 and Franz *et al.,* 1999). *E. faecalis* is easily cultured in the laboratory, and pathogenicity is not of great concern under normal laboratory conditions.

From past few years *Enterococcus faecalis* has been emerged with regard to teeth in post treatment disease, in which it noticed in microflora as well as in monocultures. It was found that presence of *E. faecalis* was encouraged by conventional endodontic techniques. *E. faecalis* are the part of normal microflora of

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gastrointestinal part and oral cavity (Portenier *et al.*, 2003).

E. faecalis is a persisted due to its ability to survive under harsh environmental conditions. It grows in high salt concentrations, with in a high temperature and bears a broad pH range and starves until an adequate nutritional supply becomes available. *E. faecalis* ferments mannitol, sucrose, sorbitol and aesculin and grows on tellurite bood agar producing black colonies (Mathew and Bhoopathy).

Total of 91% of the *E. faecalis* strains were resistant to phages. LAB when used as a starter culture in the presence of bacteriophages maintains the homogeneity of products (Jarvis and Meyer, 1986; Jensen *et al*., 1973 and Oberg *et al.*, 1998). *E. faecalis* has been reported as essential constituent of starters for the manufacture of yogurt (El-Samragy *et al.*, 1988 and Fayed *et al.*, 1989) fermented products in India (Huggins, 1984), and cheese such as palmita (Cabrera *et al*., 1994, 1998 and 1999), manchego (Ordonez *et al.*, 1978) and cheddar (Jensen *et. al.*, 1973) based on their ability to produce acid and flavor related compounds (Fayed *et al.*, 1989).

As adjunct starter cultures, *Enterococci* release natural antimicrobial substances inhibiting adulteration due to food-borne pathogens. Thanks to the efficient utilization of organic acids, *Enterococci* contribute to the development of unique sensory characteristics in fermented dairy products. However, although they are considered to be important in foods, some strains have detrimental activities that include spoilage of foods, especially meats (Giménez-Pereira, 2005).

In most individuals, 10^5-10^7 CFU of *Enterococci* are found per gram of stool. While this may seem a large number, it is only a fraction of the total bacterial flora of the stool (10^{10}-10^{12} CFU/g, mainly composed of anaerobic Gram-negative rods) (Kayser, 2003).

Of the *Enterococci*, *E. faecalis* is often the predominant species in the human bowel, although in some individuals and in some countries, *E. faecium* outnumbers *E. faecalis* (Franz *et al*., 2001). Numbers of *E. faecalis* in human faeces range from 10^5 to 10^7 CFU/g (which could be up to 100% of the enterococcal population) compared with 10^4 to 10^5 CFU/g for *E. faecium.* Only *E. faecalis* has been isolated from the faeces of neonates (Franz *et al*., 1999). In humans, *Enterococci* are part of the normal polymicrobial intestinal flora along with approximately 450 other aerobic and anaerobic bacteria species (Kayser, 2003).

The use of *Enterococci* as probiotics remains a controversial issue. While the probiotic benefits of some strains are well known but the appearance and increased involvement of *Enterococci* with human disease and multiple antibiotic resistances have raised concern regarding their use as probiotics. The cause of fear is that antimicrobial resistance genes or genes encoding virulence factors can be transferred to other bacteria in the gastrointestinal tract contribute to this controversy (Franz *et al.*, 2003). On the other hand, their presence is unwanted in certain cheeses and in processed meat products in which they may cause spoilage problems.

Taxonaomy

Enterococci were first placed under genus streptococcus. In 1984, *Enterococci* were given a formal genus status after DNA-DNA and DNARNA hybridization studies demonstrated a more distant relationship with streptococci (Portenier *et al.*, 2003). *Enterococci* are gram positive facultative anaerobic coccoid bacteria. Enterococcal cells are ovoid and they occur singly, within pairs or in short chains. *Enterococcus* species were formerly classified in the genus *Streptococcus*, but in 1984 the *Enterococcus* was entirely separated from the genus *Streptococcus* by studies of Schleifer and Kilpper (Tendolkar *et al.*, 2003).

They are facultative anaerobes and liberal to a wide range of conditions: temperature (10 - 45°C), pH (4.5 - 10.0) and high sodium chloride concentrations (Foulquie Moreno *et al.*, 2006; Hardie and whiley *et al.*, 1997). Based on phylogenetic evidences and molecular studies (16S rRNA DNA sequencing and/ or DNA–DNA hybridization) more than 20 species were classified in the genus it is well known that *E. faecalis* strains are generally more active than other *Enterococci* strains. Citrate metabolism gives to the organoleptic properties of fermented foods by the production of diacetyl, acetaldehyde, acetoin, and 2, 3-butanediol which has very distinct

aroma properties and significantly influences the quality of fresh cheese, fermented milk, cream and butter. Besides this, carbon dioxide, another product of citrate metabolism, contributes to the texture and flavor of some fermented dairy products (Foulquie Moreno *et al.,* 2006).

Virulence factors of *E. faecalis*

As reported by Portenier *et al.* (2003), *E. faecalis* posses a number of virulence factor which includes aggreagation substance, gelatinase, a cytolysin toxin, extracellular superpxide production, capsular polysaccharides and antibiotic resistant determinant. Due to the prsesence of aggreagation substance and cell-cell contact the genetic material e.g. antibiotic resistance can be transfer between strains of *E. faecalis* (Clewell, 1981). Surface protein esp can help in biofilm formation (Toledo-Arana *et al.,* 2001). Geletinase has been produced in high amount in strain of *E. faecalis* isolated from hospitalized patients (Coque *et al*., 1995 and 1996). About 60% of *E. faecalis s*trains isolated from different strains showed hemolytic activity (Ike *et al*., 1987). The production of extracellular superoxide was higher in invasive strains than in commensal isolated (Mundy *et al.,* 2000; Huycke *et al.,* 1997 and 2002). Presence of carbohydrates in the medium would strongly increase the biofilm formation by *E. faecalis*; these have greater ability to adhere to the microtiter polystyrene plates and form a biofilm (Baldassarri *et al*., 2001). Biofilms were resistant to vancomycin in concentration of 4* MIC they were found to be also less susceptible to teicoplanin (Foley and Gilbert, 1997).

E. faecalis posses a group D carbohydrate cell wall antigen called Lancefield antigen, which is an intracellular glycerol teichoic acid associated with the cytoplasmic membrane. The cell wall contains a large amount of peptidoglycan and teichoic acid (Mathew and Bhoopathy).

Lipoteichoic acid (LTA) of *E. faecalis* was reported to be doubled in quantity during the viable but non culturable (VBNC) state suggesting a role for LTA during this period. Hyaluronidase (spreading factor); the presence of microorganisms including *E. faecalis* in periapical lesions may also be related to the activity of a degrading bacterial enzyme such as hyaluronidase. Pheromones from *E. faecalis* are chemotactic for human neutrophils and triggers superoxide production. Antibiotic resistance and other virulence traits, such as cytolysin production can be disseminated among strains of *E. faecalis* via sex pheromone system. Collagen -binding protein (Ace) helps *E. faecalis* bind to collagen in dentin.

Pheromones are small peptides (seven to eight amino acids) secreted by *E. faecalis* that promote conjugative transfer of plasmids between strains (Ike and Clewell, 1984). These peptides are chromosomally encoded and are referred to as pheromones because they elicit a specific mating response from plasmid-carrying donor cells. Typically, multiple pheromones are secreted simultaneously by a given *E. faecalis* strain. In addition to pheromones, each pheromone-responsive plasmid encodes a secreted peptide that acts as a competitive inhibitor of its corresponding pheromone (Jett *et al*., 1994).

Cytolysin is considered a virulence factor of *E. faecalis* strains in animal models (Ike *et al*., 1984; Huycke *et al*., 1991; Jett *et al*., 1992; Chow *et al*., 1993 and Singh *et al*., 1998). However, the role of this factor in enterococcal pathogenicity remains unclear. *E. faecalis* isolated from patients with endocarditis or bacteremia and from healthy volunteers were investigated for their ability to adhere to Int- 407 and Girarti heart cell lines (Archimbaud *et al.,* 2002). No link between the presence of some virulence factors such as gelatinase, aggregation substances, and cytolysin, and the ability of the strains to adhere to these cells could be found.

It should be noted that the extensive use of vancomycin has steadily raised the number of VRE over the past two decades and therefore the percentage of invasive nosocomial *Enterococci* displaying high-level vancomycin resistance (Endtz *et al*., 1999). However, antibiotic resistance as such cannot explain the virulence of *Enterococci*. Regrettably, VRE are also highly opposing to all standard anti-enterococcal drugs (Landman and Quale, 1997), and, therefore, VRE constitute a serious risk group.

Dutka-Malen *et al.* (1995) developed a PCR assay to detect glycopeptide resistance genotypes. ARE are widespread in food. They have

been found in meat products, dairy products, and ready-to-eat foods, and even within enterococcal strains used as probiotics (Franz *et al*., 2001; Giraffa, 2002).

Studies on endocarditis showed synergism between cytolysin and Agg. Until now, Agg is solely found in *E. faecalis* strains; however, its incidence among food isolates seems to be high (Eaton and Gasson, 2001; Franz *et al*., 2001). The presence of Gel production among food *E. faecalis* strains is high (Eaton and Gasson, 2001; Franz *et al*., 2001). However, Eaton and Gasson (2001) demonstrated that even when the gel gene is present, a negative phenotype can be found Studies on the distribution of this surface protein revealed a significant enrichment in infection derived *E. faecalis* isolates (Shankar *et al*., 1999; Waar *et al*., 2002). Esp is thought to play a role in adhesion and evasion of the immune response of the host. Hufnagel *et al*. (2003) compared one probiotic *E. faecalis* strain with a collection of clinical isolates and found that 89% of the clinical strains were less susceptible to killing mediated by normal rabbit sera. The results showed a strain-dependent susceptibility to opsonic killing. Moreover, opsonophagocytic killing is considered to be an important test to assess the safety of enterococcal strains.

A study carried out by Barbosa *et al.* on Hemolytic activity using sheep and human blood from two types (A and O) was evaluated. Where one isolate was b-hemolytic in human blood. Results obtained in sheep blood were totally different from those obtained in human blood. Then Biofilm production in batch and in fed-batch mode was also evaluated. In batch mode, only 28.0% and 3.9% of isolates were classified as moderate and strong biofilm producers, respectively, and in fed-batch mode, 35.7% and 63.2% of isolates were classified as moderate and strong biofilm makers, correspondingly. The existence of 13 virulence genes (efaAfs, efaAfm, esp, agg, cylM, cylB, cylA, cylLL cylLs and gelE) were inspected by PCR. Wher the most of enterococcal isolates showed the presence of one or more virulence factors, the most frequent genotype being efaAfs+ gelE+ agg+ (41.5%). *E. faecalis* isolates harbored multiple virulence traits, while *E. faecium* isolates were generally free of virulence determinants.

Role of *E. faecalis* as a pathogen

Non oral infection by *E. faecalis* due to the presence of addition virulence factor; very less resistant to antibiotics among all enterococcal species. *E. faecalis* is responsible for infective endocarditis and affinity to heart valve (Mandell *et al.,* 1970, Wilson and Geraci, 1983; Mouly *et al.*, 2002; Olaison and Schadewitz, 2002; McCormick *et al*. 2000; Whitener, 1993 and Vercellotti, 1984). In urinary tract infections with the presence of *E. coli.* the *Enterococci* was also found in progressive amount. In fact in case of hospital acquired bacteremia the involving percentage is low of *E. feacalis* than *E. feacium* (11% vs. 50%). They were also involved in intra abdominal as well as pelvic and soft tissue infections. In endocarditis infections 8 to 15% cases are caused by *Enterococci* than staphylococci and in case of enterococcal infections *E. faecalis* is more responsible than any aother specises (Fernandez-Guerrero *et al*., 2002).

In Oral infections caused by *E. faecalis* Williams *et al*. (1950) found that *Enterococci* were present in the saliva of 21.8% of 206 investigated persons. *E. faecalis* is clearly a part of the human oral flora. The Enterococcus most commonly isolated from subjects was *E. faecalis*, followed by *E. liquefaciens*. Sedgley *et al*. (2004) investigated the dominance, phenotype and genotype of *Enterococci* of oral cavity. *Enterococci* were detected in oral rinse samples from 11% of 100 patients receiving endodontic treatment and 1% of 100 dental students with no history of endodontic treatment. All enterococcal isolates were identified as *E. faecalis*. *Enterococci* have been isolated in small numbers from the oral cavity of a number of people (Gold *et al*., 1975). *E. faecalis* is the most commonly isolated species of *Enterococci*.

Rams *et al*., (1992) have studied the prevalence of *Enterococci* in human periodontitis. Subgingival *Enterococci* occurred in 1% of early-onset periodontitis patients and in approximately 5% of adult periodontitis patients. In this study, *E. faecalis* was the only enterococcal species recovered, and all but one isolate fit in to the similar biotype

Sood *et al*., 2008 Studies on HLAR have been done almost exclusively on *E. faecalis*. The

incidence of HLAR is increasing (approximately 50% of isolates show this resistance). In two studied conducted in Delhi, 81% of *E. faecium* and 72% of *E. faecalis* isolates exhibited HLAR in one study 15, while only 66% of HLAR isolates were detected in another 16.The three types of resistance of most significance in the *Enterococci* are high-level resistance to the aminoglycosides, ampicillin resistance caused by beta lactamase production, and glycopeptides resistance including vancomycin resistance.

AL-Khafaji *et al*., 2010 studied 276 samples collected from different sources. The samples were divided into three groups; first included 40 stool samples collected from healthy individuals; second group included 125 clinical samples from patients who admitted to teaching general Hilla hospital. The third group included 111 samples collected from environment of same hospital. The morphological characterization and biochemical reactions showed 33 isolates diagnosed as *E. faecalis*, of which, 15 isolates from normal flora of intestine, 11 isolates from clinical cases and 7 isolates from hospital environment. The findings of virulence factors proved that *E. faecalis* was having the following factors; adhesion factors (45.4%), haemagglutination (87.8%), hemolysin (15.1%), gelatinase (9.0%), lipase (6.0%) and bacteriocin (90.9%).

Presence of *E. faecalis* in root canal of teeth

Liu *et al*., 2010 the major cause of endodontic failure is the survival of microorganisms in the apical portion of root filled teeth. *E. faecalis* can adhere to the root canal walls, accumulate, and form communities organized in biofilm, which helps it resist destruction by enabling the bacteria to become 1000 times more resistant to phagocytosis, antibodies, and antimicrobials than non-biofilm producing organisms. Engstrom (1964) reported *Enterococci* in 12.1% of culture-positive teeth at the beginning of primary treatment of necrotic root canals. Siqueira *et al.* (2002) analyzed the prevalence of *Actinomyces* spp., *Streptococci* and *E. faecalis* in primary root canal infections by using a molecular genetics method.

Siren *et al.* (1997) found that *E. faecalis* in the root canal increased significantly if the canal had been left unsealed between appointments and, in particular, when appointments were many. The obvious conclusion from this study is that compromised asepsis during endodontic treatment is an important causative factor for contamination of the root canal by *E. faecalis*.

Molander *et al.* (1998) retreated 100 root-filled teeth with apical periodontitis, and found bacteria in 68% of the teeth. *E. faecalis* was the most frequent isolate, found in 47% of the culture-positive teeth. In the same study, 20 root-filled teeth without disease were similarly cultured for microbial presence. Hancock *et al*. (2001) retreated 54 root-filled teeth with post-treatment disease and obtained microbial growth from the root canals of 33 teeth (61%). They found *E. faecalis* in 10 of the culture-positive teeth (30%); in 6 teeth, *E. faecalis* was present in pure culture. Peciuliene *et al.* (2001) retreated 40 root-filled teeth with asymptomatic apical periodontitis. Microbial growth was detected in 33 teeth (83%), and *E. faecalis* was isolated in 21 teeth (64% of the culture-positive teeth). In 11 teeth, *E. faecalis* was the only isolate, and in 10 teeth it was isolated together with other bacteria or yeast. In 8 of 10 teeth where *E. faecalis* was found in a mixed infection, it was the dominant species. Rôças *et al.*, 2004 reported that *E. faecalis* is associated with different forms of periradicular disease including primary endodontic infections and persistent infections. In primary endodontic infections, *E. faecalis* is allied with asymptomatic chronic periradicular lesions significantly more often than with acute periradicular periodontitis or acute periradicular abscesses. *E. faecalis* is found in 4 to 40% of primary endodontic infections. The occurrence of *E. faecalis* found in persistent periradicular lesions has been shown to be highly elevated. In failed root canal treatment cases are nine times more probable to hold *E. faecalis* than primary endodontic infections.

Studies investigating its occurrence in root-filled teeth with periradicular lesions have demonstrated a prevalence ranging from 24 to 77% (Molander *et al*,. 1998; Sundqvist *et al*. 1998; Hancock *et al*., 2001; Rôças *et al*., 2004, Engström, 1964; Möller, 1966; Peciuliene *et al*., 2000; 2001; Pinheiro *et al*., 2003; 2003b; Siqueira and Rôças, 2004 and Gomes *et al*., 2004). The wide range of *E. faecalis* prevalence among studies may be attributed to different identification techniques, geographic differences,

or sample size (Fouad *et al*., 2005 and Baumgartner *et al.,* 2004). In some cases, *E. faecalis* has been found as the only organism (pure culture) present in rootfilled teeth with periradicular lesions (Sundqvist *et al*., 1998 and Pinheiro *et al.,* 2003). Most studies have been carried out using culturing techniques; however, polymerase chain reaction (PCR) is currently a more predictable method for detection of *E. faecalis* (Molander *et al*., 2002; Siqueira and Rocas, 2003). This method proves to be faster, more sensitive, and more accurate than culturing methods. It has enabled researchers to detect bacteria that were difficult, and in some cases impossible, to detect (Siqueira and Rocas, 2003). When compared to detection of *E. faecalis* by culturing (24-70%), *E. faecalis* has been found at consistently higher percentages (67-77%) when a PCR detection method is used (Rôças *et al*., 2004).

Kishen *et al*., 2004 An optical spectroscopy-based method has also been studied as a way to detect *E. faecalis* activity. It is possible that this detection system could be used chairside to rapidly monitor the presence or absence of *E. faecalis* in the root canal system. Because *E. faecalis* is less dependent upon virulence factors, it depends more upon its capacity to live and persist as a pathogen in the root canals of teeth (Rôças *et al*., 2004). *E. faecalis* overcomes the challenges of survival within the root canal system in several ways. It has been shown to exhibit widespread genetic polymorphisms (Sedgley *et al*., 2004).

Decontamination of *E. faecalis* infections

The size of the lesion was correlated with the microbiological findings, revealing an average lesion diameter of 6.8mm for *E. faecalis* mixed infections, 5.7mm for *E. faecalis* pure infections and 4.3mm for mixed infections without *E. faecalis*.Susceptibility of *E. faecalis* to inter appointment dressings and irrigants. A variety of antimicrobial agents have been tested for their ability to eliminate *E. faecalis* from the root canal system. These include both inter appointment dressings, such as calcium hydroxide, camphorated paramonochlorophenol, camphorated phenol and mixed antibiotic–steroid combinations, as well as irrigants such as NaOCl, chlorhexidine digluconate, chlorhexidine acetate and iodine compounds (Bystrom *et al*., 1985; Safai *et. al*., 1985; Haapasalo and Ørstavik, 1987; Ørstavik and Haapasalo, 1990; Safavi, *et al*., 1990; Heling and Pecht, 1991; Heling *et al*., 1992; Heling *et al*., 1992 and Vahdaty *et al*., 1993). *E. faecalis* is the most resistant bacterium against calcium hydroxide, both in vivo and in vitro (Bystrom *et al.,* 1985). In vitro studies show that *E. faecalis* is killed within 6–10 min in saturated calcium hydroxide (Waltimo *et al*., 1999). However, clinical experience and in vitro experiments using dentine blocks inoculated with *E. faecalis* have clearly shown that it is difficult, if not impossible, to kill *E. faecalis* in dentine.

E. faecalis is the most resistant bacteria species to chemomechanical preparation, including instrumentation and irrigation with EDTA and NaOCl (Molander *et al*., 1998; Sundqvist *et al*., 1998; Peciuliene, *et al.,* 2001 and Gomes *et al*., 1996), and its relative proportion in the post-debridement flora is higher than initially. Increased numbers of some other microbial species usually not present in primary apical periodontitis, such as yeast and Gram-negative enteric rods, have also been reported in teeth with post-treatment apical periodontitis (Siren *et al*., 1997, Molander *et al*., 1998; Peciuliene *et al*., 2001; Nair *et al*., 1990; Waltimo *et al*., 1997). Peciuliene *et al.* (2001) showed that the routine chemomechanical preparation with 5.25% NaOCl did not predictably eliminate *E. faecalis* from the root canal. However, 5-min irrigation with 2%/4% IKI after the chemomechanical preparation eradicated E. faecalis in four of five teeth. Molander *et al.* (1999) demonstrated that *E. faecalis* could survive in the prepared root canal even after extended periods of dressing with iodine potassium iodide and calcium hydroxide.

Estrela *et al*., 2008 reported that the efficacy of the sodium hypochlorite (NaOCl) and chlorhexidine (CHX) on *E. faecalis* was evaluated by systematic review and meta-analysis. From 41 *in vivo* studies, 5 studies met the inclusion criteria. In a sample containing 159 teeth, *E. faecalis* was found firstly in 16 (10%) teeth by polymerase chain reaction (PCR) and 42 (26.4%) teeth by microbial culture methods. After root canal disinfection, this species was observed in 11 (6.9%) teeth by PCR and 12 (7.5%) teeth by culture. Risk differences of integrated studies were combined as generic inverse variance data type (Review

Manager Version 5.0 – Cochrane Collaboration, http://www.cc-ims.net, accessed 15 May 2008), taking into account the separate tracking of positive and negative cultures/PCR. Significance level was set at $p<0.05$. It was concluded that NaOCl or CHX showed low ability to remove *E. faecalis* when assessed by either PCR or any other culture techniques.

Presence of *E. faecalis* in meat products

E. faecalis and *E. faecium* can be found in raw meat products such as beef, chicken and pork cuts, and consequently in their sub-products such as pork meat sausages. The numbers of viable *Enterococci* in contaminated poultry, pork and beef are usually in the range of 10^2-10^4CFU/ g $^{-1}$ (Hugas *et al.*, 2003). Pig carcasses from slaughtering plants can contain mean log counts of 10^4 to 10^8 *Enterococci* per 100 cm^2 of carcass surface (Franz *et al.*, 1999). *Enterococci* can also derive from cross-contamination at the final stages of meat processing, such as slicing and packaging (Hugas *et al.*, 2003).

The study done by Barbosa was to characterize *Enterococcus* sp. isolated from Alheira, Chouriça de Vinhais and Salpicão de Vinhais, fermented meat products produced in the North of Portugal, relating to their possible pathogenicity. 182 isolates were studied in which 76 were identified as *E. faecalis*, 44 as *E. facieum* and 1 as *E. casseliflavous.* 26% percent of isolates were gelatinase producers. None of the isolates produced lipase or DNase activities.

Kroko *et al.* (2007) found from 75 isolates of *Enterococci* from meat (pork, beef, poultry) 56 % resistant to tetracycline, 27 % to ampicillin, 25 % to gentamicin, 15 % to vancomycin and also 15 % to erythromycin. In study held by Koluman *et al.* (2009), 88 % of beef samples and 72 % of chicken samples were contaminated with *Enterococci* and the strains of concern were resistant to at least two types of antibiotics. Four strains were identified as vancomycin resistant *Enterococci*, four of which were *E. faecalis* and originated from chicken.

Ducková *et al.*, 2014 studied The antimicrobial effect of various concentrations of thyme essential oil against tested *Enterococci* after 24 hours of cultivation at 37 + 1 ° C was monitored by measuring the absorbance at 630 nm and comparing the measured absorbance values with the positive (sample with *Enterococci* and without thyme essential oil) and of negative (sample without *Enterococci* and thyme essential oil) controls at the beginning and end of the experiment. On the basis of these results, it can be concluded that strains *E. faecium* 43 *and E. casseliflavus* 15 isolated from poultry were the most sensitive to the thyme essential oil. The highest resistance to the action of thyme essential oil showed strains of *E. faecalis* 66 and *E. faecalis* 3M which have been isolated from poultry and *E. faecium* 184, *E. faecium* 282 and *E. mundtii* 296 which have been isolated from pork.

The study carried out by Channaiah *et al.* (2010) to determine the survival of *E. faecalis* OG1RF:pCF10 in poultry and cattle feed and its acquisition and transmission by adults of the red flour beetle, *Tribolium castaneum (*Herbst), to sterile feed.

Adult *T. castaneum* beetles were introduced into poultry and cattle feed inoculated with *E. faecalis* OG1RF:pCF10 and incubated at 28ºC with 65% relative humidity for 7 days in a growth chamber. *E. faecalis* stay alive in both poultry and cattle feed during the 7day test period. There was a logarithmic decrease in *E. faecalis* counts in poultry and cattle feed and in and on the insects. *E. faecalis* were survieved on the surface and within *T. castaneum* adults for 7 days. But adults were liberated on *E. faecalis*– inoculated poultry feed and for only 5 days on *E. faecalis*-inoculated cattle feed. The counts of *E. faecalis* decreased more slowly on poultry feed than on cattle feed, and this can be explain why adult *T. castaneum* insects were more triumphant in obtaining and moving *E. faecalis* from inoculated poultry feed to sterile poultry feed during the 7 day test period. However, *T. castaneum* adults rose on inoculated cattle feed were unable to contaminate sterile cattle feed on day 7. The *T. castaneum* was successfully acquiring antibiotic-resistant *Enterococci* from animal feed and transfer them to sterile feed. Execution of *T. castaneum* through successful integrated pest management program is therefore important to prevent the spread of antibiotic-resistant and virulent *Enterococci* in animal feed and feed manufacturing environments. The results showed that poultry and cattle feed support *E. faecalis* infection but the inoculum tends to decrease at a

logarithmic rate over time. It is during these short time periods that *E. faecalis* can be potentially acquired and transmitted to fresh feed by *T. castaneum* adults.

Presence of *E. faecalis* in dairy products

The breakdown of lactose and citrate during cheese ripening gives rise to a series of volatile compounds, such as acetaldehyde, ethanol, diacetyl, acetone, and acetoin, which may further contribute to flavour. In these regard, many *E. faecalis* and *E. faecium* strains isolated from dairy products were shown to be good producer of acetaldehyde, ethanol, diacetyl, and acetoin when grown in milk, thus further contributing in the development of aroma and flavour of cheese (Andrighetto *et al.,* 2001 and Sarantinopoulos *et al.,* 2001). From 20 tested strains of *E. faecalis* 85 % were resistant to tetracycline, 35 % to erythromycin, 15 % to ampicillin and 5 % to gentamicin. There were not any strain resistances to vancomycin detected.

In the study done by Cabrera *et al.*, *(*2000) 10 fresh cheese whey samples from local plants were analyzed to detect bacteriophages and evaluate the phage effects on the titratable acidity production in 10% sterile skim milk of 11 *E. faecalis* and 7 *Lactobacillus casei* strains, which can be used as starters for the manufacture of Palmita type Venezuelan cheese. The results showed that 4 whey samples were positive for phages with lytic acivity demonstrated by the presence of plaques from 0.2 to 0.3 mm in diameter on both M17 and MRS agar plates. The fermentative activity tests showed that 91% of the cultures with *E. faecalis* strains and 57% with *L. casei* strains were resistant to the isolated phages. Variation was observed between species as well as between strains of the same species. Such changeability recommends the use of strains resistant to bacteriophages in order to guarantee the cheese quality.

Turhan and Öner 2014 studied starter properties of 83 lactic acid bacteria (LAB) strains which were isolated from 13 cheese samples that were produced from raw milk and were characterized by using phenotypic, API and FTIR spectroscopy methods were established. Proteolytic activity, acidification and decarboxylase activity were analyzed as starter culture properties for 22 *Lactococcus* sp., 36 *Enterococcus* sp. and 25 *Lactobacillus* sp. of 83 LAB. 18 isolates could decrease pH to less than 6 in 6 hours, 38 isolates indicated lower than 20 ¼g tyrosin/ml proteolytic activity and also 42 isolates indicated no decarboxylase activity. These isolates are thought to be the appropriate starter culture for cheese industry. As a result, when acid producing capabilities, proteolytic activities and decarboxylase activities of isolates were evaluated, it was determined that ‹Lc12 (*L.lactis*) ve ‹Lc13 (*L. cremoris*) isolates among lactococci, ‹Lb74 (*Lb. fermentum*) isolate among lactobacilli and ‹E33 (*Enterococcus* sp.) isolate among *Enterococci* showed the best starter characteristics. All of these isolates could reduce < 6 pH in 6 hours, have had moderate proteolytic activity (<20 mg tyrosine / ml) and were decarboxylase negative isolates. To research possibilities of these isolates to be used as a starter culture, in terms of antibiotic resistance, phage susceptibility and aroma substances formation should be assessed.

Coppola *et al.* (1990) Natural whey cultures, a thermophilic multiple strain starter *(Lactobacillus helveticus* and *Streptococcus thermophilus)* and a more complex multiple strain starter culture together with both thermophilic and mesophilic bacteria and a yeast *(L. delbrueckii* subsp. *lactis, S. thermophilus, Lactococcus lactis* subsp. *lactis, Lactoc. lactis* subsp. *diacetylactis, Enterococcus faecalis, Leuconostoc mesenteroides* subsp. *dextranicum* and *Kluyveromyces marxianus)* and artificial acidification (addition of citric acid) were used for the manufacture of water-buffalo Mozzarella cheese. Whey acidity, fermentation end-products and microbial populations were monitored during cheese manufacture. A scorecard for sensory assessment of water-buffalo Mozzarella cheese was developed and used to compare the cheeses obtained with the different procedures. The traditional technology (raw milk and natural whey cultures) allowed shorter manufacturing times due to faster acid production during ripening.

Cheeses produced with the thermophilic multiple strain starters and citric acid addition obtained the lowest scores in sensory characteristics estimation. When the complex multiple strain starter was used scores were slightly higher and more constant than those

obtained using traditional technology.

Pirouzian *et al*. 2012 The main objective of this study was to investigate the effect of *Enterococci* isolated from traditional Lighvan cheese on the quality of Iranian UF white throughout ripening. Four samples of cheese were taken from four different cheese production units in Lighvan province. Strains of *Enterococci* in these samples were isolated by standard microbiological methods and selective medium of Kanamycin Esclin Azide Agar and then identified by biochemical methods. In the second stage of research, the effect of adding isolated *Enterococci* in traditional Lighvan cheese on the quality of Iranian UF white cheese was investigated in a 60-day period. Addition of *Enterococcus* spp. did not significantly (P> 0.01) affect the pH and percentage of pH 4.6-Soluble nitrogen/total nitrogen. But in case of cheese produced with *E. faecalis* in addition to *E. faecium* strains, lipolysis rate was higher and taste properties were getting better. Moreover, results of measuring percentage of soluble nitrogen at pH 4.6 and urea polyacrylamide gel electrophoresis indicated an increase in proteolysis rate in the cheese holding *E. faecalis* and *E. faecium* strains compared to the control cheese. Besides, the highest percentage of non-protein nitrogen was observed in the cheese containing *E. faecium*. The results showed the positive effect of the *E. faecalis* and *E. faecium* on secondary proteolysis during ripening. The proteolytic activity displayed by some enterococcal strains may contribute to cheese ripening and flavor improvement. Because of these fascinating metabolic attributes, *Enterococci* have been suggested as part of described starter culture combination for UF white cheeses. Because of their role in ripening, flavor development, and bacteriocin production in cheese, it was suggested that *Enterococci* with desirable technological and metabolic traits could be included in starter cultures of various cheeses (Foulquie Moreno *et al.,* 2006).

In this regard, recent in-depth studies of enterococcal citrate metabolism done by Sarantinopoulos *et al.* in 2001, revealed that the strain *E. faecalis* FAIR-E 229 could co-metabolise lactose and citrate in milk containing yeast extract, but could not however co-metabolise glucose (or lactose) and citrate in a more complex medium such as MRS broth, even though growth was stimulated. And obtain the metabolism into acetate and formate when citrate was present as the sole carbon source. Rea and Cogan in 2003, who revealed that glucose actually prevents citrate metabolism by several strains of *E. faecalis* and *E. faecium*, suggesting some form of repression.

Among the enterococcal species, *E. faecalis, E. faecium* and *E. durans* isolated from foods or other sources are variably capable to utilise citrate or pyruvate as the sole carbon sources, with strain-to-strain variations. Sarantinopoulos *et al.* (2001 b) found in their study that generally *E. faecalis* isolated from foods were always faster than the others in the organic acid utilisation, which confirmed a previous study result in Picante cheese reported in 1999 by Freitas *et al.* In this study, almost all isolates of *E. faecalis* utilised >84% of the pyruvate and citrate after 6 hours, and after 16 hours utilisation was complete. *E. faecium* isolates showed a variable utilisation of citrate and pyruvate after 6 hours; no correlation was observed between the ability to metabolise both substrates after 16 hours of incubation. For *E. durans* isolates, there was no relationship either between the ability of the strains to metabolise citrate and pyruvate after 6 or 16 hours.

Sarantinopoulos *et al.* (2001b) showed that *E. faecalis* isolates produced acetaldehyde and ethanol in the highest concentrations, while acetoin highest concentrations were produced by *E. faecium* isolates. The study also reported that, regarding the origin of the isolates, *E. faecalis* isolates of food origin were the main acetaldehyde producers. Ethanol concentrations were also highest among *E. faecalis* isolates of food origin, although *E. faecium* isolates showed more frequent production of this gas. Acetoin concentrations were found in the highest concentrations and more frequently among *E. faecium* strains of food origin. Generally, of all the three species, *E. faecalis,* and to a lesser degree *E. faecium*, produced the highest concentrations of these compounds and most of them were of food origin.In fact, it has been suggested that presence of strains of this species in Cebreiro cheese produced more diacetyl and acetoin than *lactococci, Leuconostocs* or *lactobacilli* (Centeno

et al., 1996).

In dairy products, both *E. faecalis* and *E. faecium* species are quite heat resistant as well. Also, most of the *Enterococci* are relatively resistant to freezing. Therefore, some investigators have associated food poisoning outbreaks with enterococcal bacteria, but definitive experiments with unequivocal positive results lack. On the other hand, many foods naturally contain from small to large numbers of *Enterococci*, especially *E. faecalis* and *E. faecium* species. Relatively low levels, 10^1 to 10^3 *Enterococci*/g, are common in a wide variety of foods and certain varieties of cheese and fermented sausages occasionally may contain more than 10^6 *Enterococci*/g (Hartman *et al.*, 2001). The higher acidifying potential of *E. faecalis* has also been confirmed in previous findings, by Villani and Coppola (1994) and Suzzi *et al.* (2000).

The frequent isolation of *Enterococci* as natural starter cultures used for the manufacture of artisan cheeses, along with the finding of strains with good acidifying and/or proteolytic properties within *E. faecium* and *E. faecalis* isolated from various cheeses such as Cebreiro cheese by Centeno *et al.* (1999) and the Italian Semicotto Caprino cheese by Suzzi *et al.* (2000) and various dairy products (raw milk, cream, butter) (Wessels *et al*., 1990), encouraged some applications of these micro-organisms as *starter cultures* (Giraffa, 2003). In Argentina, a recent study done with 122 strains of *E. faecium* indicated their high potential as non-traditional starter cultures in the manufacture of homemade Tafí cheese (Saavedra *et al*., 2003). For instances, *E. faecium, E. faecalis,* and *E. durans* strains have been proposed in combination with both mesophilic and thermophilic LAB species as part of 'defined adjunct cultures' for different European cheeses, e.g., Italian semi-cooked cheeses (Neviani *et al*., 1982) and Venaco cheese (Casalta and Zennaro *et al*., 1997); for water-buffalo Mozzarella cheese, a strain of *E. faecalis* was selected with other LAB for use in an adjunct culture preparation (Coppola *et al.*, 1988; Parente *et al.*, 1989); for Cebreiro cheese, *Enterococci* with other LAB were also suggested for use in its production (Centeno *et al.*,1996; Oumer and Gaya, 2001), as well as for Hispanico cheese (Oumer and Gaya, 2001). In all these studies, *Enterococci* showed the highest performance when being added as adjuncts.

Milk citrate catabolism by *Enterococci* may explain, among other mechanisms, the role of *Enterococci* in the development of the distinctive organoleptic properties of these cheeses. 60 years ago, Campbell and Gunsalus (1944) showed that pH had a very significant effect on product formation from citrate in *Enterococci*. Twenty-five years later, Devoyod (1969) pinpointed that *E. faecalis* subsp. *liquefaciens* had the ability to metabolize citrate in the absence of carbohydrates. Since then there was appears to have been little work on citrate metabolism by *Enterococci*, until Hagrass *et al.* (1991) studied two strains of *E. faecalis*, isolated from a fermented milk product. They showed that compounds, such as acetaldehyde and diacetyl, were produced via citrate metabolism. Raffe (1994) observed that *E. faecalis* strains, grown in skim milk, could produce lactic acid from lactose, and acetic acid from citrate. Through the same period, Urdaneta *et al.* (1995) studied citrate metabolism in three *E. faecalis* strains, grown in synthetic media, having citrate as the sole energy source. All strains not only grew in those media, but they also produced acetate as the final product. Catabolite repression by glucose and fructose occurs in *E. faecalis* strains, but this is not the case when galactose or sucrose is used as energy sources (Rea and Cogan, 2003b; Somkuti and Babel, 1969). They studied an extracellular proteinase, which was produced by an *E. faecalis* var. *liquefaciens* strain; was able to hydrolytically degrade casein in an intensive way and it was less active against h-lactoglobulin and a-lactalbumin. In another work, Hegazi (1990a) studied the proteolysis of *S. faecalis* subsp. *liquefaciens* 3/6 in skim milk with some additives. It was suggested that calcium lactate and some inorganic phosphate salts do not influence casein hydrolysis. In contrast, calcium carbonate, which is frequently used as an additive in cheese manufacturing, resulted in a markedly reduction of hydrolysis levels. Also, low NaCl concentrations (2% w/v) positively influenced casein breakdown, while higher concentrations inhibited it. The same author concluded that the activity of an extracellular proteinase produced by

the same strain (*S. faecalis* subsp. *liquefaciens* 3/6) was reduced when the strain was grown in milk that was processed at high temperature (Hegazi, 1990b). This specific proteinase was able to hydrolyze casein, h-lactoglobulin and a-lactalbumin. The activity of the enzyme was strongly reduced in the presence of EDTA and for that reason it was considered a metalloenzyme. Furthermore, Villani and Coppola (1994) examined the proteolytic activity of 24 *E. faecium* and 60 *E. faecalis* strains, after growth in skim milk, at 37 ºC for 6 h. All *E. faecalis* strains were much more proteolytic, in comparison with the *E. faecium* strains.

Andrighetto *et al.* (2001) showed that the majority of the 124 enterococcal studied, isolated from traditional Italian cheeses, displayed weak proteolytic activities in milk, but 30 of them belonging to the *E. faecalis* species were more proteolytic. Finally, the same conclusion was drawn in another systematic study performed by Sarantinopoulos *et al*. (2001), who screened 129 *E. faecium*, *E. faecalis*, and *E. durans* strains for biochemical properties relevant to their technological performance. It was found that all strains exhibited low extracellular proteolytic and peptidolytic activities, with the *E. faecalis* strains being generally more active.

The first work regarding the lipolytic and esterolytic activities of *Enterococci* was performed by Lund (1965), who determined electrophoretically the presence of esterases in cellfree extracts of *E. faecalis*, *E. faecium* and *E. durans* strains. The electrophoretic pattern of the *E. faecalis* strains was different compared to the patterns of the other two species.

Moreover, *E. faecalis* strains exhibited higher activity, as determined on the basis of the intensity of the esterolytic bands. Carrasco de Mendoza *et al*. (1992) concluded that the lipolytic activity of *Enterococci* in milk was strain-dependent. Most of the strains examined exhibited low activity and only a few strains belonging to *E. faecalis* species were typify as lipolytic.

In the same period, Tsakalidou *et al.* (1993) used synthetic substrates to detect photometrically and post-electrophoretically the esterolytic activities of *E. faecium* and *E. durans*. *E. durans* strains were active against low-molecular-mass fatty acids up to 4-nitrophenyl-caprylate (C8), while *E. faecium* was active up to 4-nitrophenyl-stearate (C18). Generally, *E. faecalis* strains were the most lipolytic and esterolytic, followed by the *E. faecium* and *E. durans* strains. Even though strains of *E. faecium* and *E. faecalis* species have been applied in human, probiotic supplements, *E. faecalis* strains have also been widely used as veterinary feed supplements.

The resistance of *Enterococci* to pasteurization temperatures, and their flexibility to many substrates and growth conditions (low and high temperature, extreme pH, and salinity) means that they can be found either in food products manufactured from raw materials (milk or meat) and in heat-treated food products. Jensen *et al.* (1975b) used two *E. faecalis* strains and two *E. durans* strains as adjunct starters for the production of Cheddar cheese. They concluded that the cheese batches manufactured with the addition of *E. faecalis* strains exhibited greater proteolytic degradation in comparison to the cheese batches manufactured without *Enterococci* or with the addition of *E. durans* strains. An increased water soluble nitrogen content and proteolytic index was also observed when *E. faecalis* strains were used as adjunct starters in cheeses such as Cebreiro (Centeno *et al*., 1999), Hispanico (Garde *et al*., 1997; Oumer *et al*., 2001) and Venaco (Casalta and Zennaro, 1997). Centeno *et al*. (1999) examined the effects of *E. faecalis* in Cebreiro cheese manufacture. It has been concluded that h-casein was broken down to a greater extent in the batches containing *E. faecalis* strains than in the control ones. Moreover, the highest level of as1-casein hydrolysis and the highest ratio of peptide as1-I/as1-casein were obtained when *E. faecalis* strains were used. The use of moderate proteolytic (and lipolytic) strains of *E. faecalis* to guarantee the quality of traditional Cebreiro cheese was recommended.

Presence of virulence factor in *E. faecalis* isolated from food stuff

Resistance of *Enterococcus faecalis* to a wide variety of antibiotics has been numerously reported. Antibiotic resistance only cannot elucidate the virulence of *E. faecalis* as an emerging pathogen of public fitness concern, causing so many type of human infections.

In the study of Olawale *et al*., 2014

incidence of putative virulence determinants among *E. faecalis* strains isolated from diverse categories of food canteens namely; primary school, fast-food and commoners' canteens (*bukataria*) in Osun States, Nigeria was examined. Six hundred and fifty-eight isolates were examined for the expressions of three putative virulence determinant factors; gelatinase, aggregation substance and cytolysin activator by phenotypic tests. In the meantime, twenty elected representative strains were examined for virulence determinant genes; gelatinase (*gelA*), aggregation substance (*asa* 1), cytolysin activator (*cylA*), enterococcal surface protein (*esp*) and collagenbinding protein (*ace*) as well as confirmation of their identity by polymerase chain reaction (PCR). Six primers were used to amplify the DNA from all the 20 chosen *E. faecalis* strains studied. Expression of putative virulence determinant factors (gelatinase, cytolysin activator and aggregation substance) in all the isolates was low (10.18, 13.83 and 29.03%, respectively). The percentage of isolates with *GelA*, *cylA* and *asa*1 genes (95, 15 and 75%) was higher compared to the isolates that show phenotypic expression (40, 15 and 30%, respectively) of the virulence determinants. No one isolates had less than two beyond the five virulence determinants investigated while, the highest was four in 1 (13%) and 4 (57%) of primary school canteen and *bukataria* isolates respectively. Moreover, there is no significant involvement ($p<0.05$) found between the virulence markers and canteen sources. It is accomplished that, potentially virulent *E. faecalis* occurred in environment of a number of canteens in Osun State, Nigeria, which may cover phenotypic expression.

Dardir *et al.,* 2011 studied phenotypic tests using API 20 S strip were used for species identification of 60 and 55 enterococcal strains isolated from dairy products and clinical samples, correspondingly. Examinations for production of gelatinase, hyaluronidase and haemolysin were performed with all enterococcal isolates, while molecular determination of virulence markers (genes of *gelE, hyl, cylA, ASA 1*and *ESP* revise in the text) using RT-PCR technique and biofilm formation were verifyed just for *E. faecium* and *E. faecalis* isolates.

Obtained results depicted that *E. faecium* (56.6 %) was the predominant species isolated from dairy products, followed by *E. faecalis* (36.6%), *E. gallinarumm* (3.3%) and (1.6%) of both *E. casseliflavous* and *E. hira.* In contrast, *E. faecalis* (76.3%) was the predominant enterococcal strain identified from human clinical isolates followed by *E. faecium* (21.8%) and (1.8%) *E. gallaniurm.* Different and diverse patterns of occurrence of virulence determinants were found for *E. faecalis* and *E. faecium* strains. In general, the incidence of virulence traits was lower among *E. faecium* strains than among *E. faecalis* from dairy products. Also the results showed that the incidence of virulence factors was highest among clinical enterococcal isolates, go aftered in decreasing order by dairy strains, recommending that the dairy strains have a lesser prospective for pathogenicity. At last, these results support and propose that the use of *Enterococcus* spp. in dairy industry as starter or probiotics culture requires careful safety evaluation.

The study carried out by Trivedi *et al.,* 2011 was to monitor the distribution of virulence factors and the antibiotic resistance of various *Enterococci* species isolated from food-stuffs. A collection of 250 *Enterococci* isolated from various food-stuffs were used to investigate seven virulence determinants and the microbial susceptibility of eight antibiotics. Species-specific PCR discovered the presence of *E. faecalis* (127 isolates), *E. faecium* (77 isolates), *E. casseliflavus* (21 isolates), *E. mundtii* (19 isolates) and *E. durans* (6 isolates). Multiplex PCR for virulence factors showed that from a total 250 isolates, 221 (88.4%) carried one or more virulence-encoding genes. ^{2}-Haemolytic activity was also marked in enterococcal species other than *E. faecalis* and *E. faecium*. Species other than *E. faecalis* and *E. faecium* isolated from food are also seen to harbor the potential for virulence. Antimicrobial susceptibility testing by the disk diffusion method showed that of the total 250 isolates, 114 (46%) were resistant to cephalothin and 94 (38%) to ofloxacin. Poorer antibiotic resistance was found with ampicillin, chloramphenicol, gentamicin and teicoplanin.

Strains of *E. faecalis* isolated from dairy source; 34% among them showed ^{2}- haemolytic activity. And presence of virulence genes were

23%, 16%, 37%, 4%, 9%, 41% for hyl, asa1, esp, cylA, ace, efaA respectively. From meat origin isolated starins 2% shows gelatinase activity and 32% shows ²-haemolytic activity. And percentage presence of different virulence factor among those strain were 2%, 15%, 12%, 10%, 2%, 10% and 15% for gelE, hyl, asa1, esp, cylA, ace, efaA respectively. And strain isolated from fruit and vegetable sources were 18% shows ²-haemolytic activity. Among them presence of virulence factor were 13%, 10%, 5%, 8%, and 5% for hyl, asa1, esp, ace, efaA. There was not any vancomycin resistant gene find in *E. faecalis* strain.

In dairy origin *E. faecalis* showed resistant to CLT and OFL by 82% and 39%. In meat origin *E. faecalis* resistant to AMP, CLT, OFL, ERY, CMP, GEN, TEI were 2%, 29%, 31%, 5%, 2%, 2% and 2%. And isolates of fruit and vegetable origins were 5% resistant to AMP, 28% resistant to CLT, 44% resistant to OFL, 15% resistant to ERY and 2% resistant to TEI.

Rea *et al.* (2004) studied the effect of six strains of *Enterococci* (three strains of *Enterococcus faecalis* and one strain taken from each of *Ec. faecium*, *Ec. durans* and *Ec. casseliflavus*) on flavor development and tyramine production in Cheddar cheese during manufacture and ripening in two trials. There was no strain detected which produced gelatinase or haemolysin and all of them grew well during manufacture reaching 107 colony forming units (cfu)/g in 6 h, after which they stayed more or less stable during at least 48 weeks of ripening. There was no connection between tyramine production in a broth containing tyrosine and tyramine production in the cheese. All strains, apart from *Ec. casseliflavus*, produced tyramine in the cheese, with the maximum concentration (162 mg/kg) being produced by *Ec. durans* after 9 months ripening at 8ºC. There was no statistically significant difference ($P>0.05$) between the flavour of the control cheese and any cheese containing an enterococcus.

Nevertheless, cheese made with *Ec. faecium* E-24 received the best score in each trial at both time points. No off-flavours were found. Regarding proteolysis, only *Ec. faecalis* E-140 showed significant ($P<0.05$) increases in both phosphotungstic acid and pH 4.6 soluble N. It is concluded that *Enterococci* have little effect on the flavour of Cheddar cheese.

Tyramine can cause an increase in blood pressure and cardiac output and dilation of the eyes, lacrimation and salivation (Grind *et al.*, 1986). *Ec. faecalis* FAIR E-236 , *Ec. faecalis* FAIR E-279, *Ec. faecalis* FAIR E-315 among these strains of *Enterococci* only FAIR 279 shows presence of aggregation substance and surface protein. During 4 weeks and 36 weeks of cheese ripening at 8ºC; FAIR E236 and FAIR E315 shows average of less thymine production among three strains used.

The study performed by Riboldi *et al.*, 2009 in which 56 enterococcus spp. strains were isolated from foods in southern Brazil region. They classified by PCR as *Enterococcus faecalis* (27), *Enterococcus faecium* (23), *Enterococcus* spp. (6). The results of Antimicrobial susceptibility tests showed resistance phenotypes to a range of antibiotics widely administered in humans as gentamycin, streptomycin, ampicillin and vancomycin. In the vegetables group *E. faecium* was the most abundant species detected, mainly in beetroot and potato (100%) and parsley (80%).

E. faecalis was observed in cabbage (65%). In raw meat and colonial cheese type strain *E. faecalis* was most prevalent species. Elevated amount of High level of Aminoglycosidase resistance was observed in both *E. faecalis* and *E. faecium* strains from all the foods samples analyzed. Among them three *E. faecalis* strains isolated from cheese and meat showed ampicillin resistant pattern. In colonial cheese type one *E. faecalis* vancomycin- resistant strain was detected.

In turn, Franz *et al.*, (2001) found that of the 47 *E. faecalis* isolates of food origin they have tested, 78.7% were positive for one or more virulence determinants, compared to 10.4% of the 48 *E. faecium* isolates of food origin tested. The isolates exhibiting virulence traits were not necessarily positive for all traits; thus, the prevalence of virulence factors may be considered to be *strain or isolate specific*. In a similar manner, the Eaton and Gasson (2001) results showed that their identified virulence determinants had not previously been identified, and that this may have resulted from regional differences, suggesting a strain or isolate

specificity as well. Therefore, according to Franz *et al.* (2001 and 2003); Eaton and Gasson (2001), and Mannu *et al., (*2003), among enterococcal species, *E. faecalis* of clinical origin generally harbour more and multiple virulence determinants and with much higher frequencies than *E. faecium*, which are generally free of them. In *E. faecium* isolates of food origin, only a few have been recognised as producing either *cyl* (8.3%), *Esp* (2.1%) (Franz *et al*., 2001) or EfaAfs (Mannu *et al*., 2003). On the other hand, a study conducted by Gardini *et al.* (2001) in skim milk, reported that, although substantial amounts of tyramine (between 0.3 and 7.93 ppm) were detected in an enterococcal tested strain (*E. faecalis* EF37), the most important biogenic amine produced by the same strain was in fact the 2-phenylethylamine (up to 14.14 ppm).

Compared to other LAB, in Bover-Cid and Holzapfel study (1999), mainly *Enterococci*, carnobacteria and some strains of lactobacilli were the most intensive tyramine producers, while several other strains of *lactobacilli*, *Leuconostoc* spp., *Weisella* spp. and *pediococci* did not show any potential to produce any amines. However, even though in this study all the enterococcal strains produced tyramine, they only tested very few isolates, and this opens the suggestion of that the tyramine formation could just have been strain- or source-specific. In agreement with these findings, Sarantinopoulos *et al.* (2001 b) found in a study that the majority (96.1%) of the 129 *E. faecium, E. durans* and *E. faecalis* isolates from human, food and animal sources, tested in decarboxylase agar medium, also produced tyramine only and did not make a quantitative determination of tyramine amounts produced by the isolates of their study; therefore, they could not draw any conclusions about the possible tyramine intoxication due to the presence of *Enterococci* in cheese.

In 2000, a study done in Spain by Robredo *et al.*, chicken, pork and turkey cold meat products from 18 supermarkets, and also 50 intestinal chicken samples from one slaughterhouse were examined in order to seek enterococcal resistance. The study found that ampicillin, quinupristin/dalfopristin and high level aminoglycoside resistance were frequent among the isolated enterococcal strains, and heterogeneity was observed in susceptibility patterns among VRE strains, even in those of the same species. Thus, there was a high rate of colonisation of chicken products by VRE strains (27.2%), which was also detected in 16% of intestinal chicken samples from the slaughterhouse. No VRE were found in cooked pork or turkey products however. VRE were identified as *E. durans, E. faecalis, E. faecium* and *E. hirae.* The findings therefore suggested that chicken presence in the food chain could be a source of VRE colonisation in humans. Moreover, apparently, the VRE strains tend to remain in poultry carcasses for a long time (even years), especially if the birds received the glycopeptide 'avoparcin' as growth promoter. It is suggested that this is the result of an existing cross-resistance between vancomycin and avoparcin (Borgen *et al*., 2001).

In 2003, Peters *et al.* reported the results of a German study that attempted to determine which species of *Enterococci* could be found in food of animal origin and their significance according to their antibiotic resistance for human beings. Between 2000 and 2002, they investigated 155 samples of food of animal origin (sausages, hams, minced meat, and cheese) bought in German retail outlets. The most frequent species isolated was *E. faecalis* (299 isolates), followed by *E. faecium* (54 isolates), *E. durans* together with *E. hirae* (24 isolates), *E. casseliflavus* (22 isolates), *E. avium* (9 isolates) and *E. gallinarum* (8 isolates). Then, they focused on the resistance patterns of 118 selected *E. faecium* and *E. faecalis* isolates to 13 antimicrobial active agents. All the selected isolates were sensitive to the glycopeptide antibiotics, vancomycin and teicoplanin. Only one *E. faecalis* strain (among the 118 examined isolates) isolated from ham showed high-level resistance to gentamicin. All *E. faecalis* strains and 94% of the *E. faecium* strains were sensitive to penicillin. The study suggested that the situation of antibiotic resistance, with regards to the examined antibiotics, seemed to be favourable and that the investigated strains were sensitive to ampicillin and amoxicillin/ clavulanic acid which in combination with an aminoglycoside such as gentamicin are agents of choice for the treatment of presumptive enterococcal infections in human medicine.

Experimentally, an Italian study

conducted by Cocconcelli *et al.* in 2003 assessed the frequency of gene transfer of virulence determinants and antibiotic resistance factors among *E. faecalis* of clinical and food origin, during cheese and sausage fermentations. They found that even in the absence of selective pressure with antibiotics, plasmids carrying antibiotic resistance could be transferred to food strains and that the plasmid subsequently persisted in the new receptor. Very high frequencies of transfer were observed in sausages if compared to cheese, and the highest frequencies were observed during the ripening of fermented sausages. In this study, antibiotic resistances transferred were to tetracycline and vancomycin. So, the study showed that even in the absence of selective pressure with antibiotics, mobile genetic elements carrying antibiotic resistance and virulence determinants could be transferred at high frequency to food related *Enterococci*, during the fermentation of cheese and sausage.

According to a report of multiple vancomycin-resistant genes found in *Enterococci* isolated from poultry and pork in Germany by Lemcke and Bülte in 2000, when comparing food isolates with human isolates by means of PFGE they did not show homologous fingerprints according to their source of origin, and therefore it is unlikely that there is a close genetic relationship between enterococcal isolates from animal foodstuff and humans. Nevertheless, *Enterococci* in processed food still may indicate a possible route for the acquisition of antibiotic-resistant strains by vulnerable hospital patients, for example those with haematological malignancy, and precautions with them should be taken seriously (Curtis *et al.* 2001).

A study done by Teuber *et al.* in 2003 showed that plasmid pRE25 of *E. faecalis* (isolated from a raw-fermented sausage) transfers resistance against several antimicrobials, and those identical resistance genes were found in other pathogens, namely *S. pyogenes, S. agalactie, S. aureus, Campylobacter coli, Clostridium perfringens,* and *Clostridium difficile*. Given that in the gastrointestinal tract of animals and humans, a unique ecologic niche exists, where they come into close contact with other Gram-positive or Gram-negative bacteria, it is feared that antibiotic resistance genes could be interchanged.

Kayagil in 2006 studied out starter culture combinations in White cheese production, white cheeses produced without using starter cultures were examined. First time Özer (1964) suggested that fecal streptococci (*Enterococcus faecalis, E. faecium, E. durans*) could be combined with lactobacillus. Also Yorganc1olu (1986) suggested *E. faecalis* and *E. faecium* which have high acid production rate, are resistant to salt but have low proteolytic activity. *L. lactis subs. lactis, E. faecalis, E. durans* and *L. plantarum* combination was found to be successful when compared with commercial *Lactococcus, Enterococcus* and *Lactobacillus* combinations in lactic acid production and inhibition of other microorganism's aspects (Gürsel *et al.* 1994). *E. faecalis* is used as starter culture in Cheddar cheese, Mozzarella, Provolone production (Tekin_en and Atasever, 1994; Tamime 1983). It is also important that these bacteria are resistant to high salt concentrations which are an asset for Salted White cheese can adapt bad conditions easily and produce antimicrobial substance.

However, the most important disadvantage of them is that, some strains of these bacteria produce enterotoxin rarely and most strains produce biogen amine related to their amino acid decarboxylase activity (Tunail, 1999). *Enterococci* can cause food intoxication through production of biogenic amines and can be a reservoir for worrisome opportunistic infections and for virulence traits (Giraffa *et al.*, 2002).

In previous studies on European cheeses, *Enterococci* mainly belongs to *E. faecalis* and *E. faecium* and resistant to penicillin, tetracycline, chloramphenicol, erythromycin, gentamicin, lincomycin, rifampicin, fusidic acid and vancomycin in different proportion were detected; a prevalence of multiple drug resistance was also observed (Teuber *et al*., 1999). Although ARE found in both pasteurized and, to a much superior degree, raw milk cheeses, their occurrence in these second products may represent a more serious risk of expanding antibiotic resistance through the food chain. There were strains with high-level resistance to kanamycin and gentamicin was isolated from French raw milk cheeses and hospitalized patients (Bertrand *et al*., 2000).

A recent epidemiological study carried out in France, which explained regular pulsed field

gel electrophoresis (PFGE) patterns in antibiotic-resistant *E. faecalis* from humans and cheeses, recommends that cheeses may serve as a reservoir of ARE with characteristics that allow them to persist and spread in the community (Bertrand *et al*., 2000). Food-associated *Enterococci* could therefore be a reservoir for antibiotic resistance. Once ingested, ARE can survive gastric passage and multiply, thus leading to maintained intestinal carriage (Strensen *et al*., 2001). The presence of safer strains within food-borne *Enterococci* was also emphasised (Girafa *et al.*, 1997; Giraffa and Sisto, 1997).

Study of Antibiotic resistance of *E. faecalis*

Cytolysin produced by *E. faecalis* is a two-peptide bacteriocin (lantibiotic-type) which possesses lytic activity against erythrocytes and prokaryotic cells (Booth *et al*., 1996). Among the diverse fully characterized enterocins, it is important to highlight the enterocin AS-48 produced by *E. faecalis* S-48. This cyclic peptide was the first enterocin purified to homogeneity which exhibits bactericidal activity against a wide variety of Gram-positive bacteria, including food spoilage and pathogenic bacteria such as *Bacillus cereus*, *Clostridium botulinum*, *C. difficile*, *C. perfringens*, *Staphylococcus aureus* and *L. monocytogenes*. It also showed activity against some Gram-negative species (Foulquie Moreno *et al*., 2006; franz *et al*., 2007; Abriouel *et al*., 2003; Ananou *et al*., 2005; Lucas *et al*., 2006 and Gong *et al*., 2010). Some features of AS-48 render this bacteriocin a promising alternative to chemical preservatives (Ananou *et al*., 2005; Lucas *et al*., 2006 and Ananou *et al*., 2005).

Antibiotic multi-resistance has been more commonly reported for *E. faecalis* due to its tarnished capacity to obtain and move antibiotic resistance genes (Citak *et al*., 2004 and McBride *et al*.). It is well known that *Enterococci* may express high-level resistance to glycopeptides mainly associated with excessive use of vancomycin in hospitals as well as the use of the animal growth promoter avoparcin (Khan *et al*., 2008). Vancomycin resistance can be intrinsic (vanC) or acquired (vanA, vanB, vanD, vanE, vanG) with vanA and vanB being the most frequent transferable vancomycin-resistant phenotypes (Ogier and Serror, 2008). Moreover, according to Foulquie-Moreno *et al*. (2006) some strains of *Enterococcus* spp. may exhibit resistance towards streptogramins (*E. faecalis*) isoxazolylpenicillins, cephalosporins, monobactams, aminoglycosides (low level), lincosamides (mostly), and polymyxins. Resistance to ampicillin, tetracyclines, macrolides, aminoglycosides (high level), chloramphenicol, trimethoprim/ sulfamethozaxole, quinolones, and streptogramins also stand for acquired resistance of *E. faecium* and related species.

Among the virulence factors described for *Enterococci*, the cytolysin of *E. faecalis* (²-hemolysin/bacteriocin activity linked to the same genetic determinant) may be easily transferred by means of conjugative plasmids (Ike *et al*., 1987) and for this reason β-hemolytic isolates are considered undesirable in foods. According to Fifadara *et al*. (2003) their use as starters in food fermentation is unsuitable. The aggregation substance (AS) is a surface-bound protein of *E. faecalis* responsible for bacterial aggregation which facilitates plasmid transfer (Franz *et al*., 2001 and Wells *et al*., 2000). Some authors demonstrated that there is an elevated prevalence of Gel production among *E. faecalis* strains isolated from food samples and even when a negative phenotype is obtained, the strain may dock silent genes for this trait (Eaton and gasson, 2001; Franz *et al*., 2001; Gomes *et al*., 2008).

It is important to point out that the incidence of virulence determinants is higher in clinical isolates of *Enterococci* followed by animal and food isolates (khan *et al*., 2008; Ben Omar *et al*., 2004), however, it is difficult to separate safe and non-safe enterococcal strain, since virulence genes can be easily exchanged between strains (Eaton and gasson, 2001; Robredo *et al*., 2000; Messi *et al*., 2006; McGowan *et al*., 2006).

The observation that some *E. faecalis* strains produced zones of hemolysis on blood agar plates led to the first comprehensive study of the hemolysin molecule (Todd, 1934). Subsequently, hemolysis was found to be caused by a exclusive toxin; cytolysin, as it lyses a wide range of target cells including both Gram-positive bacteria and eukaryotic cells (Todd, 1934; Kobayashi, 1940; Brock *et al*., 1963; Roelofsen *et al*., 1964; Basinger and Jackson, 1968). The cytolysin is now known to make a large

contribution to the pathogenicity of *E. faecalis* (Elsner *et al.* 2000; Karen Carniol, 2006). The cytolysin has also been shown to be associated with increased toxicity in human infection.

A retrospective study analyzed 190 clinical *E. faecalis* isolates and found that 45 percent of isolates were cytolysin positive. additionally, after controlling for treatment modality and drug resistance, patients who were get infected with cytolytic *E. faecalis* were at a five-fold increased risk of an acutely terminal outcome (death within three weeks of diagnosis) compared to patients infected with non-cytolytic strains (Huycke *et al.*, 1991). *E. faecalis* can cause a severe postoperative endophthalmitis, and cytolytic strains have been established to be common in these infections (Booth *et al*., 1998). Epidemiological studies from Japan found that 60 percent of *E. faecalis* isolates investigated from two hospitals were cytolysin positive (Ike *et al*., 1987). In addition to causing increased toxicity of infection, the bacteriocin movement of the cytolysin may well be a key colonization factor of *E. faecalis* in the intestine, earlier to establishment of infection at another sterilized body site. *In vitro* experiments demonstrated that cytolytic strains can outcompete bacteriocin-sensitive *Enterococci* and other Gram-positive bacteria in liquid broth culture (Brock and Davie, 1963). Cytolysin was also observed to be formed by *E. faecalis* isolated from nine out of 31 healthy infants in Norway (Solheim *et al*., 2009).

Other characteristics of *E. faecalis*

This problem is circumvented by exploiting the unique features of *Enterococcus faecalis* heme metabolism. The bacterium does not require heme for growth, but if supplied with heme, it can synthesize hemoproteins (Knowles, 1980).

However, experimental work done in the 1960'ies and 1970'ies indicated that *E. faecalis* cells are capable of aerobic respiration if supplied with heme in the growth medium (Bryan-Jones and Whittenbury, 1969; Pritchard and Wimpenny, 1978; Ritchey and Seeley, 1974).

The presence of unspecified cytochromes and catalase activity was reported and confirmed these observations and characterized an enterococcal cytochrome *bd* (Winstedt *et al.*, 1999) and a heme-containing catalase (Frankenberg *et al.*, 2002) in *E. faecalis*. After demonstrating the presence of these hemoproteins, *E. faecalis* was for synthesis of artificial catalases (Brugna *et al*., 2003) and constructed a catalase-deficient *E. faecalis* mutant strain (Frankenberg *et al*., 2003). *E. faecalis* does not require heme for growth, but the growth yield is slightly promoted if hemin is added to the medium (Bryan-Jones and Whittenbury, 1969). This effect is observed with hemin concentrations ranging from 2 mg/l to 20 mg/l. *E. faecalis* catalase activity was briefly described in the literature in the early 1980'ies (Pugh and Knowles, 1982 and 1983). In line with work on hemoproteins in *E. faecalis*, performed a characterization of this enzyme (Frankenberg *et al*., 2002). Cloning of the gene, *katA*, and homologous and heterologous expression, purification and biochemical characterization allowed identification and description of the KatA enzyme. *E. faecalis* can take up and use heme from several different sources, e.g. hemin, haematin, blood (Ritchey and Seeley, 1974; Sijpesteijn, 1970) and hemoglobin. Rather high concentrations of hemin in the growth medium (>5 ¼M) are necessary to achieve maximal production of catalase (Frankenberg *et al.*, 2002). This may indicate a low affinity of the uptake system for free heme. Hemin was supplied from a stock solution prepared with water, detergent and NaOH, or dissolved in dimethyl sulfoxide (DMSO). As *E. faecalis* does not depend on hemoproteins for growth under aerobic conditions, it could be expected that this bacterium would be resistant to the toxic effect of heme analogues. *E. faecalis* was found resistant against many metalloporphyrins being bacteriocidal for other gram-positive bacteria (Brugna *et al*., 2003). The assays were conducted under conditions where *E. faecalis* is known to take up heme from the growth medium.

Allameh *et al*. (2014) studied the isolation and characterization of lactic acid bacteria from the intestine of snakehead (Channa striatus) fingerling to be used as new probiotic in aquaculture. The viable counts of bacteria in the fish intestine was 2.1×10^6 cfu/g. five LABs were isolated from the intestine of twenty fish and one of these isolates, LAB-4 was identified as *E. faecalis* by conventional and molecular

techniques. Probiotic properties showed that these bacteria could grow from pH 3 to 8 but best grow at pH 7. *E. faecalis* grew at 0.15 and 0.3% bile salt concentration from 15 to 45 C and at 4% NaCl in de Man Rogosa and Sharp (MRS) broth. This bacterium showed in vitro inhibitory activity against three fish pathogens e.g. Aeromonas hydrophila, Pseudomonas aeruginosa and Shewanella putrefaciens. After performing antibiotic sensitivity tests it was cleared that *E. faecalis* was resistant to streptomycin, gentamycin and kanamycin; Intermediate response to tetracycline and sensitive to chloramphenicol, amoxicillin and ampicillin antibiotics. Existence of fish were significantly improved ($p<0.05$) when fed with *E. faecalis* fortified diet in an in vivo challenge test using *A. hydrophila*. So it was concluded that *E. faecalis* is a promosing probiotic for snakehead fish against pathogenic infestation.

Bacteriocin production by *E. faecalis*

The bacteriocins produced by *E. faecalis* strains are referred to as following type

Type 1: cytolysin (bacteriocin/ hemolysin) from *E. faecalis* DS16 (Gilmore *et al*., 1994). This two-component lantibiotic displays both hemolytic and bacteriocin activity.

Type 2: cyclic peptide antibiotic AS-48 (enterocin AS- 48) got from the *E. faecalis* S-48 (Mart1nez-Bueno *et al*., 1994). This compound is active towards both Gram-positive and Gram-negative bacteria. In contrast, the identical enterocin 4 produced by *E. faecalis* INIA 4 is only active towards Gram-positive bacteria (Joosten *et al*., 1996). Nunez *et al*. (1997) concluded that *E. faecalis* INIA 4 is able to produce its enterocin in competition with the milk native microbiota during the manufacture of Manchego cheese.

Type 3: bacteriocin 31 from *E. faecalis* YI17 (Tomita *et al*., 1996) with a narrow antibacterial spectrum.

Type 4: enterocin 1071A and enterocin 1071B from *E. faecalis* BEF 1071 (Balla *et al*., 2000). These enterocins present an activity spectrum narrower than Type 2 and broader than Type 3 enterocins produced by *E. faecalis*. Enterocins, as most bacteriocins, have the cytoplasmic membrane as their primary target.

E. faecalis B114 produce Enterocin not known but used in Camembert cheese (Sulzer and Busse, 1991). *E. faecalis* INIA 4 produces Enterocin 4 used in Hispano cheese (Garde *et al*., 1997; Oumer *et al*., 2001). *E. faecalis* TAB 28 produces Enterocin AS-48 used in raw milk cheese (Rodr1guez *et al*., 2001).

Applications of enterocins as additives in food are Enterocin 226 NWC used in Mozzarella cheese (Villani *et al*., 1993); Enterocin 4 used in a model dairy system (Rodr1guez *et al*., 1997); Enterocin CCM 4231 in Cattle slurry environment (Laukova *et al*., 1998); Enterocin CCM 4231 Soy milk (Laukova and Czikkova, 1999); Enterocin CCM 4231used in Dry fermented Hornadsalami (Laukova *et al*., 1999); Enterocin CCM 4231 Bryndza, a traditional Slovak dairy product from sheep milk (Laukova and Czikkova, 2001); Enterocin CRL 35 in Goat cheese making (Far1as *et al*., 1999); Enterocin CRL 35 in Meat system (Vignolo *et al*., 2000);Enterocin CTC 492 Meat products (Aymerich *et al*., 2000b) and Enterocin CTC 492 used in Cooked pork (Aymerich *et al*., 2002).

CONCLUSION

As we discussed the contradictory nature of *E. faecalis* in the review so it is concluded that *E. faecalis* is the most controversial microorganism. The pheromones present in the *E. faecalis* makes conjugative transfer of plasmid from one strain to another (with in *E. faecalis* or in some other microorganism like *Staphylococcus aureus)*. But the non virulence strain of *E. faecalis* can be protected and maintained in strict laboratory conditions.

Presence of virulence factors; aggreagation substance, gelatinase, a cytolysin toxin, extracellular superpxide production, capsular polysaccharides, phermones and antibiotic resistant determinant make it fecal contaminant in nosocomial infections. Tyramine production by *E. faecalis* causes lethal contamination in meat products and a matter of concern in packed food. Infections of *E. faecalis* in root canal of teeth after treatment make it highly susceptible contaminant. Decontamination by some agents like NaOCl, chlorhexidine digluconate, chlorhexidine acetate and iodine compounds is not easy task because *E. faecalis* are resistant to high salinity.

But there is also a history of constant and safe use of *E. faecalis* in dairy products e.g.

cheese, butter, cream etc. In some special kind of cheese like cheddar, machengo, venaco type cheese etc it is use as unique flavor enhancer by the activity of lipolytic , proteolytic, esterolytic it produces diacetyl, acetaldehyde, acetoin, and 2, 3-butanediol etc. which enhances the organoleptic characteristics of food products so used as an adjunct culture. But its' property of resistant to high temperatures make it difficult for sterilization of milk products by using techniques like pasteurization etc.

The bacteriocin produced by *E. faecalis* known as enterocin inhibit the growth of undesirable bacteria in food and makes food perishable e.g. Enterocin 4, Enterocin AS-48, Enterocin 1071A, Enterocin 1071B. Some enterocin like Enterocin 226 NWC, Enterocin CCM 4231, Enterocin CRL 35, Enterocin CTC 492 are also used as food additives.

There are some other characteristics which studied by the researcher like heme metabolism and probiotic supplement in fish diet. So it should necessary to reach the useful aspects of *E. faecalis*; which keep them in our good books but it should be more necessary to prevent the growth of vancomycin, ampicillin resistant *E. faecalis* by inhibiting there gene transfer because there outbreak is more hazardous.

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