Sex-Pheromone System and Plasmid Transfer in *Enterococcus feacalis*

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Review Article

Abstract
Pheromone-inducible plasmid transfer is an important mechanism for dissemination of antibiotic resistance and virulence factors in these organisms. Plasmid-free strains of *Enterococcus faecalis* secrete at least half a dozen peptide sex pheromone-probably many more-that induce a mating response by potential donor strains carrying members of specific families of conjugative plasmids. The response is associated with synthesis of surface proteins that facilitates formation of donor-recipient mating aggregates. When a recipient acquires a given plasmid , the corresponding pheromone becomes shutdown or masked. Although the structure of at least five of these peptides has been determined.

I-Gene Transfer Among Enterococci:

Transfer of genes among genera of bacteria occurring in two mechanisms; one mechanism, transfer of DNA within bacterial cells by transposons. The transposons are pieces of DNA that move readily from one site on the bacterial chromosome to another site, or they transfer to plasmid. The transposons are not capable of independent replication, they replicate as part of recipient DNA. They can code for drug resistance enzymes, toxins production or variety of metabolic activities. Another mechanism ,transfer of genetic information from one bacterium to another. These transfer can occur by three methods: conjugation, transduction and transformation [1].

Enterococci posses ability to horizontal exchange of genetic materials among themselves and with other gram-positive bacterial genera [2]. The exchange of genetic elements of *Enterococcus feacalis* by conjugation is studied during twentieth century in deeply. The first convincing evidence for the existence of plasmid-mediated gene transfer in this organism was obtained by Tomura *et al.* ,1973[3] and by Jacob and Hobbs ,1974[4]. At least three conjugative systems have been reported in *Enterococus feacalis*, (i)The conjugative system occurs in wide range of related bacterial genera(braod-host range plasmids). The plasmids can transfer among enteroccoci species and with other genera such as many species of streptococci, *Staphylococcus aureus*, *Lactobacillus* species, *Bacillus subtilis*, *Listeria monocytogenes* and others. The transfer of this type is largely dependent on forced cell contact on membrane filter and is very inefficient in broth . The transfer of plasmid is lower than(10^{-4}-10^{-5} per donor cell) gene transfer by pheromone induction[5]. (ii)The transfer of plasmids by transposon mechanism

The types of sex pheromones in *E.faecalis* and pheromone-responding plasmids have been described in table-1.

Table 1: Enterococcal sex-pheromones and the pheromone-responding plasmids described in *Enterococcus faecalis*[18].

Sex-Pheromone		Pheromone inhibitor	Original strain	Pheromone-responding plasmid	phenotype (the plasmid encodes for)
cAD1		iAD1	DS16	pDA1	Hly/Bac
			DS5	pAMγ1	Hly/Bac
			JH1	pJH2	Hly/Bac
			HH2	pBEM10	Pn^r,Gm^r,Km^r,Tm^r
			…..	pX98	Hly/Bac
cPD1		iPD1	39-5	pPD1	Bac
			S-48	pMB2	Bac
cCF10		iCF10	SF-7	pCF10	Tc^r(Tn925)
			T1-4	pMB1.1	Bac
cOB1		iOB1	5952	pOB1	Hly/Bac
			…..	pYL1	Hly/Bac
cAM373		iAM373	RC73	pAM373	cryptic
Derivatives	cAMγ2		DS5	pAMγ2	Bac
	cAMγ3		DS5	pAMγ3	cryptic
	cAM323		HH22	pAM323	Em^r
	cAM324		HH22	pAM324	cryptic

III-Biosynthesis of sex pheromone in *E.faecalis*:

Many theories are suggested about synthesis of bacterial sex-pheromones in *E. feacalis* [12, 19]. The best-studied sex pheromone is cCF10 as follow:

In plasmid-free cell, the *Ccf-A* gene encodes a secreted precursor of cCF10 (cCF10 p). The precursor is lipoprotein. The cCF10 within the carboxy-terminal end of signal sequence of lipoprotein CcfA.

The AS lipoprotein is secreted across cytoplasmic membrane and anchored to cell wall , signal peptidase I, cleaves before the cystein residue contained within the conserved lipobox processing site , liberating the signal peptides.

Further proteolytic processing most likely occurs in the cell wall by Eep (enhanced expression of pheromone), which cleaves at the amino-terminal end of cCF10 peptide sequence , and release pro-cCF10.

Final processing may be carried out by exo-peptidase which cleaves off the remaining three c-terminal residue, resulting in mature cCF10, and release the pheromone into the medium, but a

molecules, *Prg*-X gene , is negative regulation control. This binding of pheromone with *Prg*-X gene abolish it negative control functions, these include synthesis of two surface proteins: aggregation substanc(AS) and exclusion substance(ES) on surface of donor cells .
The plasmid encoded protein (AS) which reacts with chromosomal encoded receptor ,binding substance(BS) , on surface of recipient cell. The binding between AS and BS is require divalent cations(Mg^{+2}, Mn^{+2}, Ca^{+2},Co^{+2}) and phosphate ions. This binding which responsible for cell-cell contact and formation of mating channel, then single stranded of plasmid is transferred to the recipient cell.
The shutting off ; after the plasmid has established itself in the recipient cell results in shutting off the activity of the pheromone by two functions encoded on the plasmid; one involves a reduction of the pheromone production, and other by production of specific inhibitor peptide which encoded by *prg*-Q gene. The inhibitors competitive with exogenous pheromone by bind to pheromone receptor.
The surface substances(ES proteins) which induced by pheromone also has important role in the prevention of plasmid transfer between aggregated donor cells. The *Prg*-Y proteins are expressed to prevent self induction by endogenous pheromone which produced from donor cell, through degrades or inhibits the activity of the pheromone in cell wall of donor cell. [9,16,26,27].

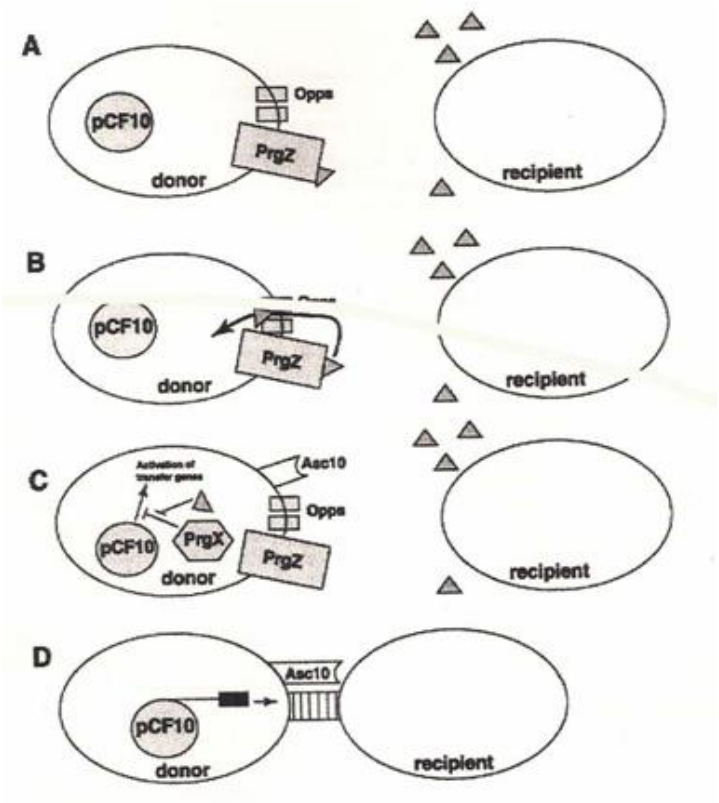

Figure 1: Summary of major steps in pheromone-induced conjugation[26].

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disease also increase with age. the most important clinical aspect in this disease is pain and impairment of movement

This arthropathy is characterized by degeneration of cartilage and hypertrophy of bone at the articular margins , inflammation usually minimal, Hereditary and mechanical factors may involved in Pathogenesis[1].

Osteoarthritis is a disorder of Synovial Joints . The most frequently affected sites are apophyseal joints of the cervical and lumber spine & knee & hip , unlike inflammatory arthopathies , it does not always affect whole joint . e.g is effect medical tibiofemorel & lat Pattallofemorel compartment of the knee [2]

Chondroitin Sulfate is an important structural component of Cartilage and provides much of its resistance to compression , along with glucosamine , chondroitin Sulfate has become a widely used dietary supplement for treatment of Osteoarthritis[3] .

They are major component of extracellular matrix & important in maintaining the structural integrity of the tissue. Both found in dietary supplements used as an alternative medicine to treat Osteoarthritis & also approved and regulated as a symptomatic slow acting drug for this disease.

The benefit from them are likely the result of a number of effects including its anti inflammatory activity, the stimulation of. the synthesis of proteoglycans and Hyaluronic acid, inhibiting the synthesis of proteolytic enzymes [4], nitric oxide and substances that contribute to damage cartilage matrix and cause death of articular choudrocytes[5].

Aims of Studies

The study is aimed to show the effect chondroitin- glucosamine – dietery supplement benefit in the treatment of OA knee patients & compare with other drug to treat patient & improve functionally disability of knee joints in Iraqi patients.

Patients and Methods

Study was done in Hilla in privet clinic. Between 2006-2008 / Babylon Province. Patients were selected according to these criteria :

Patient al either sex more than 50 years & they fulfilling the American collage of Rhenmatology (ACR) criteria of clinical, laboratory and radiographic findings[6], those patients were diagnosed with primary osteoarthitis with lequesns score in the range of (10-18) [7]& those patients can come for regular visit. 150 patient were included in the study ,30 patients were excluded because either they had arthritis due to other cause like Rheumatoid arthritis or have renal or hepatic dysfunction or other serious medical illness (Sever Ischemic heart Disease. Or heart failure.

The first screening visit , Patient medical history was taken & clinical assessment was done . The knee its were examined by local examination & specific parameter for assessing the severity subjectivity as well as objectivity.

All patients were asked to guard the severity of pain based on visual analogue scale (VAS) ranging from no pain at bottom of the scale to unbearable pain at the top of 100 mm colored scale. The other variable in the scale were mild , modrate and sever pain separated by 20 mm. [8] , lequesnes index (Table 1)

Table 2. Radiological scoring for Knee osteoarthritis

Radiological scoring	X-ray finding
0	Normal
1	Doubtful narrowing of joint space/possible osteophytes lipping
2	Definite osteophytes/absent or questionable narrowing of joint space
3	Moderate multiple osteophytes, definite narrowing of joint space, some sclerosis, possible joint deformity of bone ends
4	Large osteophytes, marked narrowing of joint space, severe sclerosis, definitive joint deformity of bone ends and subchondral cysts may be present.

120 patient were included , divided into: two groups for period of 6 months follow for each patient with regular visit at 2 months interval between.

The Group (1) 60 patients were give combinations of 500 ghcosamine sulphate& 400 Chondnotion sulphate. 2 tablet per day.

The Other Group (2) 60 patients were give Meloxicam 15 mg / Day.

All Patient to instructed not take any analgesic except paracetomol (only if needed during the study period . The Except no more 1000 mg / day and they asked at each visit how much they have taken of the drug .

Patients were follow up 0- 2 – 4 – 6 – months :

The knee joints were examined on each visit according to above parameter . The Patient compliance was considered good if the patient takes 75% of drug which prescribed . All Patients were advised about knee exercise at home .

Results

This study was design as compared study between [two] groups group one and two both include 60 patients for each ,the mean age of group [1] was 57 years and group [2] was 58 years.

Sex ratio ,group [1],51 female and 9 male and group [2] 50 female and 10 male table [1].

The functional score was recorded for both groups from the start of the study and at 2 months interval to the end of the study ,in group [1] the improvement was 30-/+ 5 while group [2] was 25-/+5 at the end of the study table [2].

The visual analogue scale for both groups were recorded at the start and 2 months interval to the end of the study ,the improvement from 70-/+6 to 35 in group [1] where the improvement in group [2] was from 72 to 50 table [3]

The radiological score ,there was no difference between both groups during the study period and no difference in progress of osteoarthritis radiological features. table[4].

To compare the use of additive drug [paracetomol] by the numbers of the tablets [more than 10 tablets] per week ,in group one 41 patients had recorded to use the drug while group [2] 38 patients were use the drug. table[5] .

The patient and physician assessment during the study the difference was more

Table VII

Lab study	I		II	
	0	6 mouth	o	6 mouth
Hb %	11.5	11	11.7	10.5
WBC	6.3	6.5	5.7	6.5
ESR	35	30	30	30
Blood glucose	100	110	105	103
B urea	40	41	35	45
AST	27	29	26	35
ALT	25	28	27	32

Discussion

Osteoarthritis is a disease of progressive cartilage damage seen mostly after the age of 40s years ,

The pain may arise from synovial inflammation or periosteal nerve stimulation ,previously NSIADs widely used for relieving the pain in osteoarthritis but mostly there is good present of patients that stop drug because adverse effect that related to the therapy and the older people with multiple clinical problem that NSIADs lead to more adverse effect ,due to this fact there was new drugs emergence that would work to reduce patients symptoms ,and they act to regenerate cartilage and act as anti inflammatory with low risk for patients[11] this supported also by study of Alekseeva et al [12]

The glucosamine and chondrotin combination has been sued in all over the world for knee osteoarthritis and many studies work for that, it used as nutritional supplements for knee osteoarthritis[13].

In this study combination of glucosamine and chondrotin has work well as compare with treatment by melocexam drug ,the pain score was assessed by the visual scale ,this parameter showed progressive improvement over the period of drug therapy with maximum benefit at 4 months point while the other group show rapid improvement at 2 months point but then no more improvement after that point ,when compare with Bourgeois et al [14], 3 months trial of 1200 mg of both combination in single or divided doses its showed that decrease of lequense index and spontaneous joint pain score versus placebo [$p<0.1$]

The lequesne index that assess the functional score the functional mobility of the joints depending on the daily activity ,it showed mark improvement than the other group at 2-4 months and such result was documented in other studies e.g Muller et al [14] ,Noaek et al[15] and they found that this index to be a sensitive indicator of the improvement, Muller and collogues [16] in short term study [4 weeks] study to compare this drug versus ibuprofen showed that its effective like ibuprofen and better tolerated [$p<0.1$]

The radiological score change in this study show no difference between 2 groups during the this study period ,they not correlated with patients symptoms many patients show functional disability that not correlated with X ray finding . And only 6% show adverse effect while 35% eboburfen qroup show adverse effect mainly gastrointestinal, in our study CS group show 10% show adverse