Characterization of adhesion (agglutination) factor of Enterococcus faecalis EM1 cells

Shatha S- Hasan & Atheer A .Majeed
Department of Biology_ college of Science_Baghdad University

Abstract

Some characters of adhesion (agglutination) factor of E.faecalis EM1 cells with Saccharomyces cerevisiae were studied . E. faecalis EM1 cells were subjected to different environmantal factors included : pHs. temperatures, NaCl concentrations & many kinds of antibiotics.

The results revealed that the maximum value of agglutination factors was at pH5 and 37c° . Agglutination can occurs at wide range of NaCl concentrations (0.9-5.4%) ,but 0.9% NaCl was the optimum.

Antibiotics in general reduced the agglutination, ampicillin was the more effective one than the others since it reduced the agglutination factor form 75 to 34 % . Treatment of E.faecalis with different sugars revealed that galactose inhibits the adhesion of bacteria with yeast cells, significantly this indicates that E.faecalis has a galactose specific glycoprotein (lectin) enabiling it to adhere with galactose containg receptors.

توصيف عامل الالتصاق (التلزن) لخلايا Enterococcus faecalis EM1

شذى سلمان حسن و اثير احمد مجيد
قسم علوم حياة- كلية العلوم-جامعة بغداد

الخلاصة

درست بعض خصائص عامل الالتصاق (تلزن) بكتريا Enterococcus faecalis EM1 مع خميرة Saccharomyces cerevisiae ، اذ تم تعريض خلايا البكتريا لظروف مختلفة تضمنت درجات حرارة وارقام هيدروجينية وتراكيز من كلوريد الصديوم ومضادات حياة مختلفة.

بينت النتائج ان اعلى قيمة لعامل التلزن تكون عند درجة ٣٧مْ ورقم هيدروجيني ٥ ، وان الالتصاق يحدث في مدى واسع من تراكيز NaCl تتراوح بين ٠.٩-٥.٤% الا ان افضل التصاق يحدث عند تركيز ٠.٩ % .

ولوحظ ان مضادات الحياة تؤدي الى اختزال عامل الالتصاق عموما وكان الامبسلين (ampicillin) اكثرها تاثيرا اذ ادى الى خفض نسبة التلزن من ٧٥ الى ٣٤% .

عند معاملة خلايا البكتريا مع سكريات مختلفة ظهر ان الكالكتوز يؤدي الى تثبيط التصاق البكتريا مع الخميرة بشكل واضح مما يدل على ان خلايا E. faecalisتمتلك عامل تلزن متخصص للكالكتوز من نوع البروتينات السكرية glycoprotein (لكتين) الذي يمكن البكتريا من الارتباط بالمستلمات الحاوية على الكالكتوز.

Introduction:

E.faecalis strains are characterized by having the ability of adhesion with different surfaces, biotic and abiotic(1).

Enterococci play a dual role in human ecology. They serve as commensal organisms of the gastrointestinal tract and also leading causes of multiple antibiotic – resistant hospital aquired infection. Many nosocomial infections result from the ability of microorganisms to form biofilm(2).

Enterococcal surface protein, Esp , has been suggested to contribute biofilm formation by E.faecalis. A review of E.faecalis virulence factors was reported (3),one of these virulence factors is the aggregation by E.faecalis substance surface adhesion.

Aggregation substance (AS) is a pheromone responsive, plasmid-encoded bacterial adhesion that mediates efficient contact between donor and recipient bacterium, facilitating plasmid exchange. In addition to its adhesion function during the bacterial conjucation process, (AS) mediates adhesion of E.faecalis to a variety of eukaryotic cells in vitro(4).

E.faecalis is capable of producing biofilms which consist of a population of cells attached irreversibly on various biotic and abiotic surface, encased in hydrated matrix of expolymeric substances. Many environmental and genetic factors are associated or have been proposed to associated with the production of biofilm formation(5).

The binding properties of lectins have been used to study the structural and functional roles of cell surface carbohydrates and to detect sugar moieties on normal and neoplastic cell surface. They exert many interesting biological effects on cells by binding to cell surface carbohydrates(6).

'The surface proteins of E.faecalis play a key role in the adhesion to bile drain associated infections(7).

Chemical compounds and environmental conditions may affect or interfer with adhesion factor, chlorhexidin is effective in

reducing the adherence of E.faecalis to bovine dentin material(8).
The aims of this study are determination of some characters of agglutination factor of a strain of E.faecalis such as the effect of pH, temperature, NaCl and its specifity for different kinds of sugars.

Culture of E.faecalis
E.faecalis EM1 strain was abtained from Department of Biology- College of science – Baghdad University .The purity and characteristics of the bacterium were examined . E.faecalis EM1 was activated in brain heart infusion broth (BHIB) for 18-24 hrs at 37c° and maintained on brain heart infusion agar slants in deep freez.

Saccharomyces cerevisiae culture:-
A strain of Saccharomyces cerevisiae was abtained from a commercial sample of backery yeast, cultured on yeast extract glucose agar (YEGA) or broth (YEGB) and maintained on the same media.

Agglutination test:-
A suspension of E.faecalis EM1 and Saccharomyces cerevisiae were prepared by centrifugation of the cultured media at 5000 rpm for 30 min , the supernatent was discarded and a few milliliters of 0.2M phosphate buffer pH 7.2 were added to the precipitated cells , the step was repeated several times (washing) and centrifuged.
The washed cells were resuspended in phosphate buffer. Twenty five microliteres (25µl) of bacterial suspension were mixed with 25µl of yeast suspension on a clean slide, left for 30min and stained with crystal violate and examined by light microscope to observe the agglutination of cells , a control slide contained bacterial or yeast suspension only was prepared also.

Agglutination assay :-

Agglutination was measured according to (9) method, equal volumes of bacterial and yeast suspensions were mixed in a glass tube , other tubes contained bacterial or yeast suspension only. The tubs were vortexed fore 10 min .The suspension were allowed to setele for 10 min , the upper solution was removed and put in a new tube , the remaining steps of the procedure were accomplished and obsorbancy at 600 nm was measured. The agglutination activity was calculated as % of bacterial cells that agglutinate the yeast cells.

Determination of agglutination at different conditions - temperature and pH :-

E.faecalis EM1 was grown in BHIB+1% glucose at pH 7.5 and incubated at 37c̊ for 18 hrs. The culture was centrifuged at 5000 rpm for 30 min at 20c̊, the cells (precipitate) were separated, washed and resuspended in (0.2M) phosphate buffer at three pH valus: (5,7 and 9) in triplicate tubes, the tubes were incubated at three different temperatures 15,37,45 c̊ for 1h ,cooled in ice bath and the agglutination was assayed. . E.faecalis EM1 cells was treated with high temperature 70c̊ for 3hr and agglutination was assayed for the killed cells.

NaCl:-

Sodium chloride (stock solution) was added to E.faecalis EM1 cell suspension at pH7 to abtain different replicate concentrations (0.9-5.4%) and incubated at 37 c̊ for 30 min and the agglutination was assayed

Antibiotics:-

Stock solutions of different antibiotics included Ampicillin , Amoxicillin, Ciproflaxacin, Erythromycin, Gentamycin, Lincomycin, Methoprim, Metronidazol and Trinidazole were prepared and mixed with bacterial cell suspension to obtain a

concentration of 125µl\ml , incubated at 37c̊ for 1hr and agglutination was assayed.

Determination of the specificity of agglutination factor :-
Solution of 2% from glucose , galactose, fructose, mannose and maltose were mixed separately with bacterial suspension at 1:1 V:V, incubated at 37c̊ for 1h and agglutination was assayed.

Results and Discussion:-

- Agglutination of E.faecalis EM1 with S. cerevisiae

Agglutination of E.faecalis EM1 cells with cells of S. cerevisiae was abserved ,S. cerivisiae cells appeared as clumps of aggregated cells, this indicates that E.faecalis cells have an agglutination factor \ factors which can bind with certain receptors on cell wall of other cells.
It has been reported that E.faecalis strains may have different mechanisms for adhering with biotic or abiotic surfaces, the rate of three surface proteins of E.faecalis in the adhesion to different synthetic polymers was examined ,the presence of surface proteins increased the total number of adhering bacteria (7).
The ability of E.faecalis clinical isolate to adhere to extracellular matrixes (ECM) coating walls of microtiter plates was examined by microscopy, each isolate showed diffrerent level of adherence to ECM (10).

- Effect of temperature and pH on agglutination

Maximum values of agglutination were obtained at 37c̊ ln all pHs . However pH5 revealed the highest value of agglutination (78%) (fig.1) , minimum levels of agglutination appeared at 15c̊.
The cells subjected to 45c̊ showed different values of agglutination at different pHs , the lowes percentage (%) of agglutination was in pH9 , since it decreased from 71% to 15% at this pH.

It can be concluded that agglutination or adherence of E.faecalis can accures at wide rang of temperatures and pHs ,but it favoured 37c° and pH 5,9.Agglutination – ability of E.faecalis EM1 killed cells was determined afte subjection cells in 70c° , the results showed that cells , (dead cells) have the ability to agglutination yeast cells but at low level is (43%). (fig.2)

Effect of NaCl:-

Treatment of E.faecalis EM1 cells with different concentrations of NaCl had little effect on agglutination the agglutinatoin percentage (%) decreased slightly as NaCl concentration increased (fig.3). The lowest value was 60.5 % at 5.4% NaCl , while it was 75% at 0.9% NaCl.

The results indicate that adherence (agglutination) of E.faecalis with Saccharomyces cerevisiae is not inhibited by NaCl concentration above the physiological concentration (approxamility 0.9%) .However increasing of NaCl about 5% may interfere with the adherence factors to some extent

Effect of antibiotics:-

The antibiotic used in this study deacreased the agglutination in general, the most effective one was ampicillin , it reduced the agglutination from 75% to 32% , follow by methoprim 39%. The remaining antibiotics reduced it to 48-65.5% (fig.4).

Specificity of adherence factor:-

One of mechanisms of cell adhesion is the presence of specific types of proteins which have affinity to bind with specific kinds of sugars (or carbohydrates), these proteins are called lectins.

In this study, the expected adhesion factor(s) is a kind of these proteins. To identify this factor, cells of E.faecalis were treated with different kinds of sugars befor mixing with yeast cells . It was found that galactose has an inhibitory effect on cells agglutination.

Galactose reduced the agglutination from 75% to 22.5% . Mannose had no effect , the remaining sugar slightly decreased the agglutination (fig.5).
This indicates that E.faecalis EM1 has a kind of galactose specific lectin (glycoprotein) responsible for agglutination of the bacterium with other cells containing galactose receptor.

Discussion :-

Enterococci are able to survive a range of stresses and hostile environments including those of extreme temperature (5-65 c°) , pH 4.5 -10 and high NaCl concentration, enabiling them to colonize a wide range of niches (11).
For many years Enterococcus species were believed to be harmless to humen, they have been used widely in the food industry (12). Recently , enterococci have become one of the most common nosocomcial pathogens(3).
E.faecalis has the ability to produce adhesion factors and aggregate with prokaryotic and eukaryotic cells. The aggregation substance (Agg) on the surface of E.faecalis has been shown in vivo to from large aggregates and hence may contribute pathogenesis . The presence of (Agg) increases the hydrophobicity of the enterococcal cell surface (12).
Extracellular surface protein (Esp) is a cell wall – associated protein desecribed in enterococcus spp.(13). Esp also contribute to enterococcal biofilm formation which could lead to resistance to environmental stresses, and adhesion to enkaryotic cells(14).
The binding (adherence) ability of Enterococcui with different matrix may affected by different substances , tunicamycin had a blocking effect on some strains of enterococci binding. Treatment of six strains .with proteolysic enzymes and sodium metaperiodate significantly decreased their binding . This suggests that both proteins and carbohydrate moieties are involved in binding.(15).

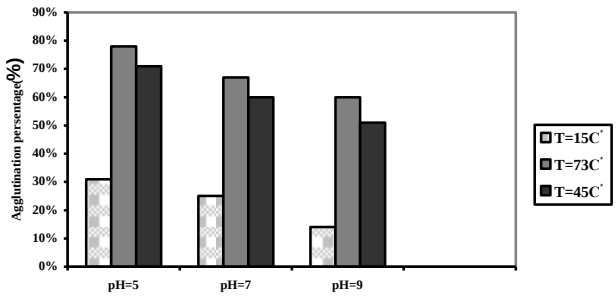

Fig. (1) :- Agglutination of bacteria E.faecalis EM1 with S. cerevisiae
at different temperature and pHs :-

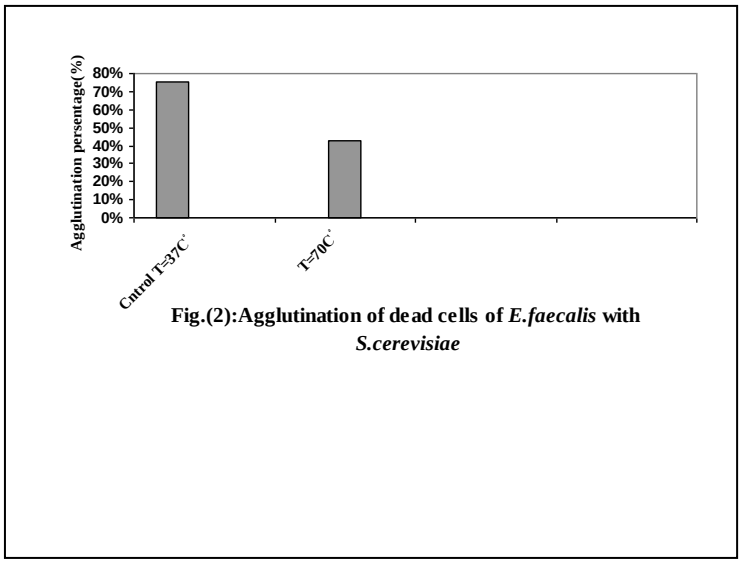

Fig.(2):Agglutination of dead cells of *E.faecalis* with *S.cerevisiae*

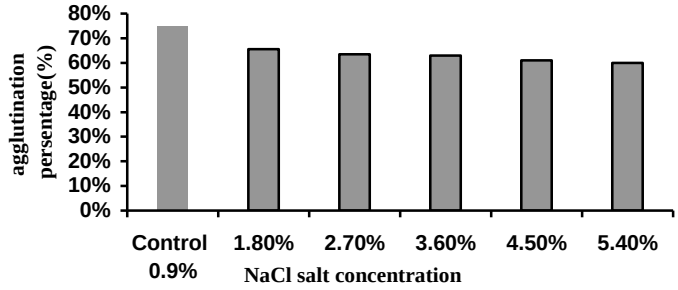

Fig.(3)The replicat salt concentrations effect on agglutination factor persentage (%) of bacteria *E.faecalis* EM1 with *S.cerevisiae*

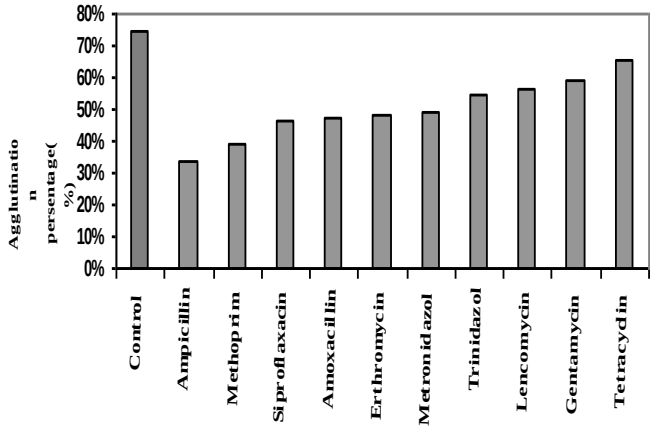

Fig.(4):Anitibiotic effects on agglutination factor of *E.faecalis* EM1 with *S. cerevisiae*

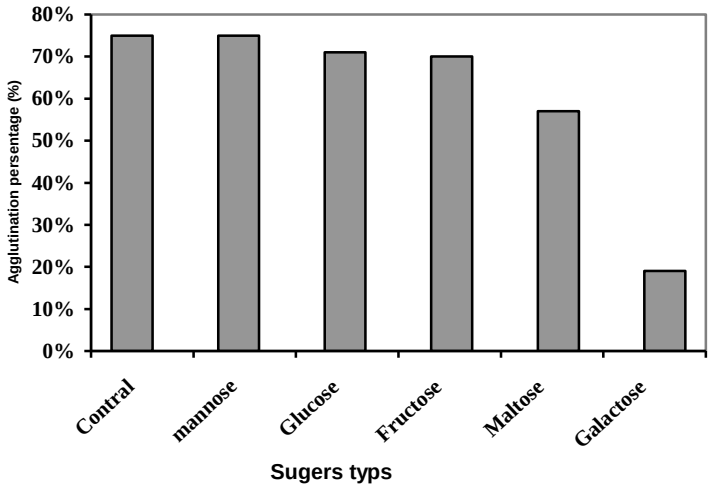

Fig.(5):Effect of sugers on agglutination factor of bacteria *E.faecalis* EM1 with *S. cerevisiae*

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