A Preliminary Study of the Effects of gltA gene on Biofilm formation of Enterococcus

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Abstract: Aim: To investigate the effects of glutamate synthase gene (GltA) on the biofilm formation of Enterococcus (E. Fawecalis). Methods: CLSM was used to observe the biofilm formation ability of 4 strains of E. France from clinic. The number of viable cells and biofilm volume were matched with that of the standard strain v583. GltA gene expression in 4 clinical isolates were detected RT-QpcR. The gltA-High expressed strain was connected and its growth curve was detected. The formation of biofilm the viable cell number in the biofilm and biofilm volume were observed by LSCM. After 24 h culture. Results: All the 4 islands could form compact bio-Films. And the viable cells and biofilm volume of the isolated groups 250

Keywords: Biofilm formation; GltA gene; E. Faecalis; Enterococcus faecalis (E. Faecalis) is refractory root tip weeks inflammatory of main To pathogenic bacteria one its in refractory root tip weeks inflammatory cases in detection rate up 24%~77%[1]. E. Faecalis Not only can separate Formation biofilm also can with other bacteria with form multi-species students Dentin tubular in to resistance root tube antibacterial agent of killing role from E. Faecalis Formation biofilm of ability is high its pathogenicity and Drug Resistance stronger are more likely to in special environment (such as oligotrophic, High Alkali, High Salt, Hypoxia and) in survival also to be more difficult to clinical mechanical preparation and[5-6] Of Drug clear E. Faecalis Students of membrane of Formation Machine Later genea, GltA It is an important gene for glutamate metabolism in bacteria, which can be encoded by glutamate synthase (Gogat) In yakuji

In the early stage, many strains were isolated from the root canal of the teeth with refractory periapical periodontitis. E. Fawecalis And the Clinical Strains with significantly increased biofilm formation ability were sequenced. The expression of metabolic pathway was significantly different from that of the control group. GltA The gene expression level was significantly up-regulated, suggesting that GltAGene and glutamate metabolism pathway E. Fawecalis Biofilm formation may play an important regulatory role; And some research also found that GltAGenes are also important in the biofilm formation of Bacillus subtilis.

Clear its high expression on E. Fawecalis Biofilm formation, in order to find E. Fawecalis New regulation mechanism of biofilm provides experimental basis.

S13) And 1 Strain E. Faecalis Standard V583 Conventional recovery after respectively crossed inoculation in BHI Agar petri dish in and placed 37 Anaerobic conditions under the training. 24 h After respectively selected the strain of single cloning colony and will its inoculation in fresh BHI Liquid Medium in continue to anaerobic training 12 h; Use than turbidity instrument adjustment bacteria concentration

Bacteria in each 10 MuL inoculation in provided on the sterile coverslips 6 Orifice Plate in at the same time in every
hole the join6 mL Fresh BHILiquid Medium and continue to anaerobic training 24 h; Then with sterile PBS Solution Cleaning 3 Times and respectively to each hole and add 100 μL Dilution of live die bacteria dye color agent(SYTO9/PI Proportion SYTO9 pi pbs = 1.5 1.5 1 000); At room temperature under light staining 15 min After PBS Solution slow cleaning excess of stain and CLSM The observation. At the same time use CLSM Comes with software on the strain of biofilm volume and the number of viable bacteria the Quantitative Analysis.

E. Faecalis Clinical Strains GltA Gene expression level of detection

Total bacteria RNA Extraction

Press 1..2. Preparation 4. Strain E. Faecalis Clinical Strains and 1. Strain E. Faecalis The bacterial suspension of the standard strain was

Placed on sterile coverslips 6. Anaerobic incubation in orifice plates. Respectively in the cultivation 6, 12, 24 h At the point of time, all strains were collected in aseptic

EPP Pipe, and use RNA iso plus Kit Total genome of various strains RNA. All operations are strictly in accordance with the instructions, the specific steps are as follows: First to each EP Add in Pipe μL Concentration is 200 mg/mL Lysozyme, placed at room temperature

10 ~ 15 min After centrifugation, discard the supernatant and add 1 mL RNA iso plus Heavy suspension sedimentation; Ice placement 5 min After, join 200 μL Chloroform Oscillation 15 s; Ice placement 10 min After, 4. Lower centrifugal

Min And add the same amount of isopropanol in each tube; Place on ice again 10 min After, 4. Lower centrifugal 10 min And collect all strains

RNA; Finally, add to the pipes 1 mL Concentration is 750/LEthanol solution for the cleaning of various strains RNA And in 4. Lower centrifugal 5 min After the supernatant was discarded; Room temperature placement 5 ~ 10 min After that, add to each tube 20 μLDiethyl pyrocarbonate, and with a pipette repeatedly blows mix, until the tube in RNA After completely dissolved, the strains were detected by UV spectrophotometer. RNA The purity and concentration of -80 Cold Storage in refrigerator.

RNA Reverse Transcription CDNA

Reverse transcription process as per kit Perfect Real time The instructions are carried out in the reverse transcriptase Bio-RA d PCR Medium operation. Reaction System: RNA Template 2 μL, 5 x primescript Buffer 2 μL, Enzyme mix I Buffer 0.5 μL, Oligo d Primer 0.5 μL, RNA Sterile ultrapure water 20 μL; Reaction parameters: Reaction 15 min And then 85 Reaction 5 S After reverse transcription, strains were collected CDNAPlace-80 Cold Storage in refrigerator.

Primer Design and Synthesis

Yin’s DNA Sequence whose Expected amplified fragment length 419 BP; And then To the strains CDNA For template 23SrRNA For in reference and in accordance SYBRR Premix Ex Taq™ (Perfect Real Time) Kit instructions Myiq real-Time PCR

Detection System PCR Reaction. Reaction System: CDNA Template 1 μL Upstream in (10 μMol/L) And under tour in 10 μMol/L The 1 μL LSYBR Premix Ex Taq™ Buffer 10 μL Sterile ultra-pure water bu zhi 25 μL. Reaction parameters:

Pre-Degeneration 3 min; 98 Degeneration 15 s 55 Annealing 15 s

Extension 30 s 40A cycle. Reaction End after first will PCR Results production standard of melting curve to check the amplification product of quality to ensure that its specific; then again respectively determination the samples CT Value and the compare the Threshold Method to Determine GltA-Based

For expression level of the relative amount.

Base because of phase of on (\[CT_{GltA(\text{Test samples})} - CT_{23SrRNA(\text{To be measured samples})}\] + \[CT_{\text{GltA(Correction samples)}}\]) to determine the threshold number of select is CT Value 0 of samples set its gene copy of relative multiple1. Repeat the experiment 3 Times take average. GltAGene high expression E. Faecalis Strain of Construction
GltAGene high expression vector of Construction

In accordance 1.3.3 Of Methods Design and Synthesis GltAGene of upstream and downstream primers upstream primers sequence: 5'-Ttcgagctcatg-Gctagaaaaagtcgtataaaaac-3'Enzyme cut site designSACI;Downstream primers sequence: 5'-GACGTCGAC-CTAAACATTGCTTGTGACTGTTGTTTT-3'Enzyme cut

Point Design Sali. First on GltAGene in Vitro PCR Amplification its reaction system: Q5 Reaction Buffer10 MuLQ5GCBuffer10 MuLDNTP(10 mmol/L)1 MuLQ5Enzyme0.5 MuLDNA Template1 MuL And sterile ultra-pure water bu zhiO10 s60 Annealing Natural 20 s72 Extension 2 min In A cycle. 4 Under end reaction after 10 mg/ml Agarose gel electrophoresis PCR Product the identification; At the same time use restriction enzyme SACI And Sali Okay PCR Amplification after GltA-Based Because fragment and plasmid vector PMg36e The double enzyme cut its reaction system: Shear Buffer2 MuL Restriction Enzyme SACI And Sali The MuL DNA Template1 MuL And sterile ultra-pure water bu zhi Natural 20 MuL; Reaction parameters: 37 Under reaction 1 H. Reuse T4 Ligase connection its enzyme cut after DNA Fragment reaction system: T4 Ligase Buffer1 MuL GltA Enzyme cut Product1 MuL PMG36e Enzyme cut Product MuLT4 Ligase 1 MuL And sterile ultra-pure water bu zhi 10 MuL Reaction parameters: 25 Under reaction 12 h. Recombinant connection complete after Will carrier electric DH5a Escherichia coli of competent in at the same time, its uniform

Coating in containing 200 MuG/mL Erythromycin LB Flat in and placed 37 Under the training; 16 h After selected single cloning colony at the same time the plasmid recovery kit recovery colony in recombinant connection success

DNA Carrier and will its named PMg36e-GltA; Finally ECOR I And Sali Restriction on PMg36e-GltA The double enzyme cut 10 mg/ml Agarose gel electrophoresis identification its recombinant results. After the identification is correct, the recombinant vector was entrusted to Beijing hecheng gene Co., Ltd. for sequencing. E. Fawecalis Preparation of competence

First of all, the frozen E. Fawecalis Standard Bacteria V583 After conventional resuscitation, the vaccination was done BHI Agar plate, place in 37 Cultivation under anaerobic conditions 24 h After that, the monoclonal colonies were selected for inoculation.

15 ml Fresh BHI In liquid medium and continue Anaerobic Incubation 12 h; Take 1 ml Bacteria inoculation in 500 Fresh BHI Liquid

Medium, and placed in 37 Shaker Oscillation (250 r/min) Foster 3 HA after, take 50 ml Bacteria liquid and adjust its 570 nm At the wavelength Od Value0.3 ~ 0.4.; And then put the bacteria on ice 15 min

After, 4 Lower centrifugal 15 min The supernatant was discarded and the centrifuge tube was inverted on the filter paper to dry the excess medium 0.5 mol/L Heavy suspension cell ice bath in high sucrose solution

Min After, in 4 Collect precipitate by centrifugation and then add 4 mL The concentration of pre-cooling treatment is 0.5 mol/L High sucrose solution, placed in -80 Cold Storage in refrigerator.

Exogenous recombination DNA Introduction of carrier into electricity

Will be prepared above E. Fawecalis Standard Bacteria V583 After melting on ice, add 1. μL Linearized recombination DNA Carrier Pmg36e-GltA And gently make it fully blended; Ice placement

Min Then transfer it to the pre-cooling treatment of the sterile e-cup, and put it into the E-converter for e-Transformation (The transfer parameters are: 2 ~ 5 kV, 200 Omega, 25 μF; Turn time 4 MS). Immediately after the transfer Join 1 mL sob The solution resuspension the bacteria and 37 Recovery cultivation under shaker Oscillation 3 HTo fully express resistance; And then take 200 μL Bacteria suspension, and evenly coated in containing 200 μG/ml Erythromycin BHI Agar medium, placed in 37 Cultivation under anaerobic conditions 24 h After, for subsequent filtering and authentication.

E. Fawecalis gltA Screening and Identification of strains with High Gene Expression

From the above culture 24 h Of BHI Agar medium in selection single cloning colony the passage after again
3. Times and in every hole the join100MuLDilution of live dead Dye

Color agent (SYTO9/PI Proportion SYTO9 pi pbs =
1.5 1.5 1 000); At room temperature under light staining 15 min After, PBS slowly wash away excess stains and use CLSM Observe. Simultaneous adoption CLSM Quantitative Analysis of biofilm volume and viable bacteria number of each strain by software.

Statistical Analysis
Adopted SPSS 20.0 Software-to-data (X Wang Yi± S) Statistical Analysis and group comparison T Standard of inspection Alpha = 0.05.

GltA Gene high expression E. Faecalis Strain of Growth Curve GltA Gene high expression strain and standard bacteria V583 The growth curve as shown in Figure 8, Shown in: Two strains were in training 4 h After enter the logarithmic of and in 14 h At to peak; Since then its growth rate become slow and in 24 h After were into stable. GltA Gene high expression of the growth rate in enter the logarithmic the growth period was higher than that of standard bacteria V583 But no statistical difference (P<0.05).

GltA Gene high expression E. Faecalis Strain of biofilm formation

Situation

CLSM Observe the results display, GltA High-expression Strains

Membrane formation rate is faster 24 h When its biological membrane structure Density, the thickness increased significantly, while the standard strain V583 Biofilm formation

Slow, cultivate 24 h When the structure is loose, and the thickness is (Figure 9.). Quantitative analysis showed that, GltA High-expression

12, 24 h The biofilm volume and the number of viable bacteria were significantly higher

Standard strain V583 (P<0.05) (Figure 10).

Figure 10 GltA High Gene Expression E. Faecaliss Strains and standard strains V583 Comparison of the number and volume of viable biofilm (*With standard bacteria V583 Compare P<0.05)

Discussion

GltA Gene Yes GltA The important component of a manipulator, the glutamate synthase (Gogat) It is an important amide transfer enzyme for bacterial glutamate metabolism. Alpha-Two molecules of glutamic acid are generated from the ketone glutaric acid, one of which is used as a co-substrate in ammonium and is involved in the catalytic reaction of glutamine through the glutamine synthetase; the other molecule glutamate can provide ammonia for the amino transfer reaction [10]. Ki GltA Gene expression is not only activated by signal sources of carbon metabolism and nitrogen metabolism, but also by transcription activator GltC And Nitrogen [12] General regulator of metabolism Tnra Regulation. When there is glucose, Gltc Activate GltA Transcript of Operon to meet bacterial [13].
The need for increased glutamate to achieve rapid bacterial growth. Transcription activator Gltc Through the Union located Gltc And GltA Gene Between 3. Region of a symmetric element, that is Box1, Box And Box2 [14].

Thereby adjusting Gltab Transcription. Free in bacteria Gltc Usually complete sum Boxi Combined, weakly activated Gltab Of transcription; when in there Carbon Metabolism and Nitrogen Metabolism signal when and inte-Of Glutaric Acid role under, Gltc The combined with Boxi And Boxii Regional the regional and GltA Gene Promoter-35 The location completely overlap to stability. RNA Polymerase and GltA Gene Promoter of each other combined with to strong Activation Gltab Gene of transcription. Not only so glutamic acid same can negative feedback regulation GltA Gene of expression when in glutamic acid content. For a long time its will combined with Gltc The suppression GltA Gene of promoter to end its expression. So glutamic acid concentration of change [14]

Regulation Gltc The activity and GltA Gene expression is very important. At the same time when growth environment in lack of glutamine when, Tnra Also will combined with Gltab Operon of downstream stop Gltc Okay Gltab Enterococcus faecalis biofilm formation. different substrates [J]. J Endod. 2013 39

References