Universe Scientific Publishing

ORIGINAL ARTICLE

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 enterocuccus(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 stand-ard strain v583.GltA gene expression in 4 clinical isolates were detectedRT-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 up24%~77%[1].E.FaecalisNot 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.FaecalisFormation 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. FaecalisStudents 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.FawecalisAnd 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.GltAThe gene expression level was significantly up-regulated, suggesting thatGltAGene and glutamate metabolism pathwayE.FawecalisBiofilm 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 onE.FawecalisBiofilm formation, in order to findE.FawecalisNew regulation mechanism of biofilm provides experimental basis.

S13)And1StrainE.FaecalisStandardV583Conventional recovery after respectively crossed inoculation inBHIAgar petri dish in and placed37Anaerobic conditions under the training;24 hAfter respectively selected the strain of single cloning colony and will its inoculation in freshBHILiquid Medium in continue to anaerobic training12 h;Use than turbidity instrument adjustment bacteria concentration

Bacteria in each10MuLInoculation in provided on the sterile coverslips6Orifice Plate in at the same time in every

hole the join6 mLFreshBHILiquid Medium and continue to anaerobic training24 h;Then with sterilePBSSolution Cleaning3Times and respectively to each hole and add100MuLDilution of live die bacteria dye color agent(SYTO9/PIProportionSYTO9 pi pbs = 1.5 1.5 1 000);At room temperature under light staining15 minAfterPBSSolution slow cleaning excess of stain andCLSMThe observation.At the same time useCLSMComes with software on the strain of biofilm volume and the number of viable bacteria the Quantitative Analysis.

E.FaecalisClinical StrainsGltAGene expression level of detection

Total bacteriaRNaExtraction

Press1..2.Preparation4.StrainE.FawecalisClinical Strains and1.StrainE.FawecalisThe bacterial suspension of the standard strain was

Placed on sterile coverslips6.Anaerobic incubation in orifice plates.Respectively in the cultivation6.,12,24 hAt the point of time, all strains were collected in aseptic

EPPipe, and useRNaiso plus KitTotal genome of various strainsRNa.All operations are strictly in accordance with the instructions, the specific steps are as follows:First to eachEPAdd in Pipe

μLConcentration is200 mg/mLLysozyme, placed at room temperature

10~15 minAfter centrifugation, discard the supernatant and add1 mLRNaiso plusHeavy suspension sedimentation;Ice placement5 minAfter, join200μLChloroform Oscillation15 s;Ice placement10 minAfter,4.Lower centrifugal

MinAnd add the same amount of isopropanol in each tube;Place on ice again10 minAfter,4.Lower centrifugal10 minAnd collect all strains

RNa;Finally, add to the pipes1 mLConcentration is750/LEthanol solution for the cleaning of various strainsRNaAnd in4.Lower centrifugal5 minAfter the supernatant was discarded;Room temperature placement5.~10 minAfter that, add to each tube20μLDiethyl pyrocarbonate, and with a pipette repeatedly blows mix, until the tube inRNaAfter completely dissolved, the strains were detected by UV spectrophotometer.RNaThe purity and concentration of-80Cold Storage in refrigerator.

RNaReverse TranscriptionCDNA

Reverse transcription process as per kitPerfectREal timeThe instructions are carried out in the reverse transcriptaseBio-RAd PCRMedium operation.Reaction System:RNaTemplate2.μL,5 x primescriptBuffer2.μL,Enayme mix IBuffer0.5.μL,Oligo dTPrimer 0.5.μL,RNaseSterile ultrapure water20μL;Reaction parameters: Reaction15 minAnd then85Reaction5 S.After reverse transcription, strains were collectedCDNAPlace-80Cold Storage in refrigerator.

Primer Design and Synthesis

Yin'sDNASequence whose Expected amplified fragment length 419 BP;And then To the strainsCDNAFor template23SrRNAFor in reference and in accordanceSYBRRPremix Ex Taq^{TM}(PerfectREal Time)Kit instructionsMyiq real-Time PCR

Detection SystemPCRReaction.Reaction System:CDNATemplate1MuLUpstream in(10MuMol/L)And under tour in 10MuMol/L The1MuLSYBRPremix Ex Taq^{TM}Buffer10MuLSterile ultra-pure water bu zhi25MuL.Reaction parameters:

Pre-Degeneration3 min;98Degeneration15 s55Annealing15 s

Extension30 sIn40A cycle.Reaction End after first willPCRResults production standard of melting curve to check the amplification product of quality to ensure that its specific; then again respectively determination the samplesCTValue and the compare the Threshold Method to DetermineGltA-Based

For expression level of the relative amount.

Base because of phase on of$2^{-[CT}$CT=

$[^{CT}$GltA(Test samples)$^{-CT}$23SrRNA(To be measured samples]$]$-$[^{CT}$GltA(Correction samples)

—$CT_{23SrRNA(Correction\ samples)}$].Correction samples of select isCTValue0Of samples set its gene copy of relative multiple1.Repeat the experiment3Times take average. GltAGene high expressionE.FaecalisStrain of Construction

GltAGene high expression vector of Construction

In accordance1.3.3Of Methods Design and SynthesisGltAGene 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 DesignSali.First onGltAGene in VitroPCRAmplification its reaction system:Q5Reaction Buffer10MuLQ5GCBuffer10MuLDNTP(10 mmol/L)1MuLQ5Enzyme0.5MuLDNATemplate1MuLAnd sterile ultra-pure water bu zhiOf10 s60AnnealingNatural 20 s72Extension2 minIn

A cycle.4Under end reaction after10 mg/mlAgarose gel electrophoresisPCRProduct the identification;At the same time use restriction enzymeSACIAndSaliOkayPCRAmplification afterGltA-Based

Because fragment and plasmid vectorPMg36eThe double enzyme cut its reaction system:Shear Buffer2MuLRestriction EnzymeSACIAndSaliThe MuLDNATemplate1MuLAnd sterile ultra-pure water bu zhiNatural 20MuL;Reaction parameters:37Under reaction1 H.ReuseT4Ligase connection its enzyme cut afterDNAFragment reaction system:T4Ligase Buffer1MuLGltAEnzyme cut Product1MuLPMG36eEnzyme cut Product

MuLT4Ligase1MuLAnd sterile ultra-pure water bu zhi10MuLReaction parameters:25Under reaction12 h.Recombinant connection complete after Will carrier electricDH5aEscherichia coli of competent in at the same time, its uniform

Coating in containing200MuG/mLErythromycinLBFlat in and placed37Under the training;16 hAfter selected single cloning colony at the same time the plasmid recovery kit recovery colony in recombinant connection success

DNACarrier and will its namedPMg36e-GltA;FinallyECORIAndSaliRestriction onPMg36e-GltAThe double enzyme cut10 mg/mlAgarose 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.FawecalisPreparation of competence

First of all, the frozenE.FawecalisStandard BacteriaV583After conventional resuscitation, the vaccination was doneBHIAgar plate, place in37Cultivation under anaerobic conditions24 hAfter that, the monoclonal colonies were selected for inoculation.

15 mlFreshBHIIn liquid medium and continue Anaerobic Incubation12 h;Take1 mLBacteria inoculation in500FreshBHILiquid

Medium, and placed in37Shaker Oscillation (250 r/min)Foster3 HAfter, take50 mlBacteria liquid and adjust its570 nmAt the wavelengthOdValue0.3.~0.4.;And then put the bacteria on ice.15 min

After,4.Lower centrifugal15 minThe supernatant was discarded and the centrifuge tube was inverted on the filter paper to dry the excess medium.0.5 mol/LHeavy suspension cell ice bath in high sucrose solution

MinAfter, in4.Collect precipitate by centrifugation and then add4 mLThe concentration of pre-cooling treatment is0.5 mol/LHigh sucrose solution, placed in-80Cold Storage in refrigerator.

Exogenous recombinationDNAIntroduction of carrier into electricity

Will be prepared aboveE.FawecalisStandard BacteriaV583After melting on ice, add1.μLLinearized recombinationDNACarrierPmg36e-GltAAnd gently make it fully blended;Ice placement

MinThen 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,25μF;Turn time4 MS).Immediately after the transferJoin1 mL sobThe solution resuspension the bacteria and37Recovery cultivation under shaker Oscillation3 HTo fully express resistance;And then take 200μLBacteria suspension, and evenly coated in containing200μG/ml

ErythromycinBHIAgar medium, placed in37Cultivation under anaerobic conditions24 hAfter, for subsequent filtering and authentication.

E.Fawecalis gltAScreening and Identification of strains with High Gene Expression

From the above culture24 hOfBHIAgar medium in selection single cloning colony the passage after again

extraction exogenous recombinantDNACarrier andGltABase becausePCROf of at the same time

10 Mg/mLAgarose gel electrophoresis identification its results;Then select identification results right of strain continue to the passage training continuous transfer

S after again extraction exogenous recombinantDNACarrier and

PCRAmplification and identification to verifyGltAGene high expression whether the construction success?.Verification results to electrophoresis molecular weight for standard.

GltAGene high expressionE.FaecalisStrain Growth Curve Determination

First1.2The methods respectively preparationGltAGene high expression strain and standard strainV583Of bacteria suspension then again respectively from each Bacteria

Liquid in each10MuLInoculation in provided on the sterile coverslips6Orifice Plate in at the same time in every hole the join6 mLFreshBHILiquid Medium and placed37Anaerobic conditions under the Training.Respectively in training after6,12,24 hThe time point with sterilePBSSolution wash bacteria

3. Times and in every hole the join100MuLDilution of live dead Dye

Color agent(SYTO9/PIProportionSYTO9 pi pbs =

1.5 1.5 1 000);At room temperature under light staining15 minAfter,PBSSlowly wash away excess stains and useCLSMObserve.Simultaneous adoptionCLSMQuantitative Analysis of biofilm volume and viable bacteria number of each strain by software.

Statistical Analysis

AdoptedSPSS 20.0Software-to-data (XWang Yi± S)Statistical Analysis and group comparisonTStandard of inspectionAlpha= 0.05.

gltAGene high expressionE.FaecalisStrain of Growth CurveGltAGene high expression strain and standard bacteriaV583The growth curve as shown in Figure8Shown in:Two strains were in training4 hAfter enter the logarithmic of and in14 hAt to peak;Since then its growth rate become slow and in24 hAfter were into stable.GltAGene high expression of the growth rate in enter the logarithmic the growth period was higher than that of standard

BacteriaV583But no statistical difference (P<0.05).

GltAGene high expressionE.FaecalisStrain of biofilm formation

Situation

CLSMObserve the results display,GltAHigh-expression Strains

Membrane formation rate is faster24 hWhen its biological membrane structure Density, the thickness increased significantly, while the standard strainV583Biofilm formation

Slow, cultivate24 hWhen the structure is loose, and the thickness is (Figure9.).Quantitative analysis showed that,GltAHigh-expression

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

Standard strainV583(P<0.05)(Figure10).

Figure10GltAHigh Gene ExpressionE.FawecalisStrains and standard strainsV583Comparison of the number and volume of viable biofilm (*With standard bacteriaV583CompareP<0.05)

Discussion

GltAGene YesGltabThe 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.GltAGene expression is not only activated by signal sources of carbon metabolism and nitrogen metabolism, but also by transcription activator.GltcAnd Nitrogen [12]General regulator of metabolismTnraRegulation.When there is glucose,GltcActivateGltabTranscript of Operon to meet bacterial[13.]

The need for increased glutamate to achieve rapid bacterial growth.Transcription activatorGltcThrough the Union locatedGltcAndGltAGeneBetween3.Region of a symmetric element, that isBox,BoxAndBox[14.],

Thereby adjustingGltabTranscription.Free in bacteriaGltcUsually complete sumBoxiCombined, weakly activatedGltabOf transcription; when in there Carbon Metabolism and Nitrogen Metabolism signal when and inα-Of Glutaric Acid role under,GltcThe combined withBoxiAndBoxiiRegional the regional andGltAGene Promoter-35The location completely overlap to stability.RNAPolymerase andGltAGene Promoter of each other combined with to strong ActivationGltabGene of transcription.Not only so glutamic acid same can negative feedback regulationGltAGene of expression when in glutamic acid content. For a long time its will combined withGltcThe suppressionGltAGene of promoter to end its expression.So glutamic acid concentration of change[14]

RegulationGltcThe activity andGltabGene expression is very important.At the same time when growth environment in lack of glutamine when,TnraAlso will combined withGltabOperon of downstream stopGltcOkayGltab Enterococcus faecalis biofilm formation. different substrates[J].J Endod.201339

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