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Inositol, Sialic Acid and Fucose Metabolism Way of Aeromonas Hydrophila Gas Single of Pathogenicity of Influence

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Abstract: [Objective] Investigation inositol, sialic acid and fucose metabolism way in Aeromonas hydrophila gas single of infection host process in bacteria pathogenic of influence. [Methods] The homologous recombinant technology respectively missing Aeromonas hydrophila gas singleNJ-35Strain of Inositol Metabolism Related GeneIolC, Sialic Acid Metabolism Related GeneNanaAnd fucose Metabolism Related GeneFuckDetermination the missing of the zebrafish of half lethal dose(LD_{50}); Will wild strain and missing strains of infection crucian carp statistical wild strain and missing strains in different organization in bacteria load. [Results] The Metabolism Related Gene of missing were success blocking the strain of corresponding substrate of degradation ability.IolCThe lack of lead to strain the zebrafishLD_{50}Increased nearly12Times andNana

FuckThe effect of the defect ofLD_{50}No obvious influence. Wild strain andIolCMissing strains of infection crucian carp liver, spleen and kidney in wild strains of load was significantly higher than that of missing of show obvious of growth advantage;NanaAndFuckMissing strains and wild strains of infection crucian carp wild strain and missing of load in the Organization in no obvious difference. [Conclusion] inositol metabolism way in Aeromonas hydrophila gas single of infection pathogenic process in play important role and sialic acid and fucose metabolism way of bacteria no obvious influence.

Keywords: Aeromonas hydrophila gas single of Inositol sialic acid fucose metabolism gene missing pathogenic

Aeromonas hydrophila gas single(Aeromonas hydrophila)Widely distribution in Water Environment in can be caused by fish hemorrhagic Sepsis can also lead to other mammals animals even human infection. A large number of studies show that the bacteria pathogenic with of many kinds of virulence factor such as cell surface protein, cell exocrine factor and some regulation system and about[1]. However Pang mao da and[2]Study found that many Aeromonas hydrophila gas single of popular strain in not containing known of virulence factor suchIIIStyle secretion system and lateral flagella part stronzg strain also does not containVIStyle secretion system show that these virulence factor and not decided to strain

Virulence of key factors. So Aeromonas hydrophila gas single of pathogenic mechanism very complex worth further study.

In recent years bacteria metabolism ability with pathogenic force between the relationship has caused widely attention. In the Vibrio(Vibrio), Aeruginosa Pseudomonas(Pseudomonas aeruginosa)And of research in found bacteria of metabolism gene in bacteria infection host of process in and classic of virulence factor same important[3-6]. These metabolism way of may be to bacteria in different of survival environment in more effective to get nutrition. On strepto-

coccus(Streptococcus suis)Of study

Now serine/Threonine kinase in addition to participating in serine/Threonine of metabolism or a kind of central regulation factor in cells growth and split and metabolism important role[7]. This laboratory early study found Aeromonas hydrophila gas single of popular StrainNJ-35Yes3Article respectively metabolism inositol, sialic acid and fucose of way and compare the genome of analysis show that this3Article metabolism way only appear in strong strain in[2]. So speculate that this3Article metabolism way of May and Aeromonas hydrophila gas single of the widely popular about. In view of this, this study the missing Metabolism Related Gene of methods respectively on inositol, sialic acid and fucose metabolism way block the study this3Article metabolism way in fish in the Aeromonas hydrophila gas single of infection pathogenic of influence.

1. Material and Methods

1.1 Strain and plasmid

Aeromonas hydrophila gas singleNJ-35Strain, Escherichia coliSM10By this real test room points from preserving of; suicide PROPERTIESPYAK1,PMMB207By Zhejiang University compound-hwan Professor gift.

1.2 Reagent

Primestar Max DNAPolymerase, restriction enzyme,DNA Marker,DNA Gel purification KitAre DalianTakaraThe company products; bacteria plasmid extraction kitOmegaThe company products;2 × PCR PremixFor Nanjing Nobel only.

The company products; inositol, sialic acid andL-Fucose areSigmaThe company products; ampicillin penicillin(AMP), Chloramphenicol(Cm), Kanamycin

Of(Kan), Gentamicin(Gen)AreInvitrogenProducts.M9Low-salt medium for Qingdao day aquatic organisms technology limited the Company Products main components:Na_2HPO_4(3.39%),KH_2PO_4(1.50%),NaCl (0.25%),NH_4CL (0.50%)In addition add3 mmol/L $MgSO_4$And0.12 mmol/L $CaCl_2$Does not contain glucose.

1.3 Test animal

Day old aboutABDepartment of zebrafish(Danio rerio)Purchase from Nanjing Confucius Temple pet market body length2-3 cmBody Quality3G

About.60Day old about the different fertility Silver Crucian Carp by Jiangsu Province aquaculture technology promotion center provide body length10-12 cmBody Quality25gAbout.

1.4 Primer design

Random select main road metabolism way Related Gene IolC,Fuck

NanaThe gene missing of construction related primers information see

1.5 Gene missing of Construction

This study in Construction of gene missing strains were deletion objective gene of complete open reading box,IolC,NanaAndFuckGene fragment length respectively1878,900And1449 BP. Reference literature[8]Of methods to strainNJ-35All genome for template with primersP1/P2AndP3/P4Respectively amplification objective gene on, downstream homologous arm. By FusionPCROf methods above, downstream homologous arm for Template"P1/P4Primers fusion upstream and downstream homologous arm. By enzyme cut, connection, transformation will fusion fragment to connectPYAK1Carrier(Host for Escherichia coliSM10)Sequencing identification.

To containing recombinant plasmid of Escherichia coliSM10For for body bacteria to Aeromonas hydrophila gas singleNJ-35For receptor bacteria by joint of style will Recombinant Plasmid from Escherichia coliSM10ToNJ-35In. Specific Operation: for body BacteriaSM10And receptor BacteriaNJ-35Were cultured to logarithmic,5000 × GCentrifugal5 minDiscard the supernatant with no

BacteriaPBSWashing2Times will two kinds of bacteria concentration were1.0 × 10^8CFU/mL (OD_{600}Value0.2)WillSM10AndNJ-35In accordance with different of proportion(2:13:14:1)Fully mixing. Will aperture

0.22 Mu mOf sterile membrane tile in SolidLBFlat on the take200 mu LMixed bacteria point added membrane on will flat is shining in

Underlined sequences indicateBamH I restriction sites.

1 mLFreshLBWill membrane on the cell wind to centrifuge tube in,5000 × GCentrifugal5 minDiscard the supernatant200 mu L LBHeavy suspended after coating in containing double-antibody(Amp 100 mu g/mL?Length 34 Mu g/mL)OfLBFlat on the inverted in28 °CIncubator Culture

36-48 hSelected single colony in containing double-antibodyLBLiquid Culture

A medium culture8-10 hTo genomePCRVerify screening happened the first1Times homologous recombinant of strain the single exchange strain.

Will single exchange strain transfer to does not containNaClOfLBMedium continuous passage2Times transfer to containing sucrose(Concentration20%)

LBLiquid Medium in training30 hBacteria10Times dilution after coating in containing sucroseLBSolid Medium. Training24 h

After selected single colony to ordinaryLBLiquid Medium in training6-8 hTo genome andPCRVerify screening second times homologous recombinant and lost objective gene of strain the gene missing strains. Gene missing of construction process see Figure1.

1.6 Gene complementary of Construction

NJ-35Strain genome for template with complementary fragment PrimersPCRAmplification get contains objective genePCRProduct

Section by conventional enzyme cut, connection, transformation will complementary fragments connected to plasmidPMMB207 (Host for Escherichia coliSM10). To containing complementary plasmid of Escherichia coliSM10For for body bacteria to Aeromonas hydrophila

Gas singleNJ-35Of gene missing strains for receptor bacteria by bacteria Joint(Methods reference1.5)The recombinant plasmid transfer to lack. Joint complete after out carrier membrane1 mLFreshLBWill membrane on the cell wind to centrifuge tube in,5000 × GCentrifugal 5 MinAbandoned supernatant200 mu LFreshLBHeavy suspended after coating in containing double-antibody(Amp 100 mu g/mL?Length 34 Mu g/mL)OfLB

Flat on the inverted in28 °CTraining36-48 hSelected single colony in containing double-antibodyLBLiquid Medium in training8-10 hTo genomePCRVerify screening containing complementary plasmid of strain the gene complementary strain.

1.7 Strain Resistance Gene the introduction

In wild strains in introducedKanResistance Gene in all missing strains in respectively introducedGenResistance Gene. Specific steps are as follows to synthesis of objective resistance gene sequence for template with resistance gene PrimersPCRAmplification get contains Resistance GenePCRProduct fragment by enzyme cut, connection, transformation will resistance gene to connect to Quality

GrainPMMB207The use containing and insert Resistance Gene Relative should be of antibiotics(Kan 50 Mu g/mL?Gen natural 20 mu g/mL) LBFlat

Plate the screening determine insert Resistance Gene effective after the next step test.

Respectively to carry have recombinant plasmid of Escherichia coliSM10For for body bacteria corresponding of Aeromonas hydrophila gas single of for receptor bacteria by bacteria Joint(Methods reference1.5)The recombinant plasmid transfer to Aeromonas hydrophila gas single. Joint complete after with sterile tweezers carrier membrane1 mLFreshLBWill membrane on the cell wind to centrifuge tube in,5000 × GCentrifugal5 minDiscard the supernatant200 mu L LBHeavy suspended after coating in containing double-antibody(Amp100 mu g/mL?;Kan50 Mu

g/mL?OrAmp100 mu g/mL?;GenNatural 20 mu g/mL)

LBFlat on the inverted in28 °CTraining36-48 hSelected single colony in containing double-antibodyLBLiquid Medium in training if can growth the strain Resistance Gene introduced success.

1.8 Strain Metabolism Ability of Detection

Respectively selected wild strain, missing strains and complementary of single colony in LiquidLBMedium in training stay bacteria liquid turbidity after transfer to freshLBMedium in training to logarithmic,4000 × GCentrifugal5 minDiscard the supernatantM9Medium washing2Times will bacteria concentration

5.0×10^8CFU/mL (OD_{600}Value1.0)Mixed uniform,

200 mu LBacteria liquid transfer toNatural 20 mlFreshM9Medium

(Add metabolism substrate make the final concentration0.1%)The conical flask in each sample is set3A repeat,28 °C,180 r/minOscillation training. Each interval specific of time take100 mu LBacteria to sterilePBSBuffer10Times Dilution to the appropriate concentration100 mu L

Dilution coating flat each sample Coating3A dilution will flat inverted in28 °CTraining16 hTake number of colonies was30-300Of flat the count.

1.9 The zebrafish half lethal dose(LD_{50})Of Determination

Zebrafish in indoor foster observation1Weeks confirm zebrafish growth state good after the test. Test during Water Temperature Control in25-28 °C. Respectively selected the strain single colony in LiquidLBMedium in training stay bacteria liquid turbidity after transfer to freshLBMedium in training to logarithmic,4000 × GCentrifugal5 minDiscard the supernatant with sterilePBSWashing3Times in turn adjustment bacteria liquid concentration

5×10^6,5×10^5,5×10^4,5×10^3,5×10^2CFU/mL.

Each concentration set a group intraperitoneal injection zebrafish each tail Injection

0.02 mLAt the same time to injectionPBSAs an blank control group every group

10. Tail. Observe1Weeks record results in accordanceBlissAlgorithm

A bacteriaLD_{50}[9].

1.10 Crucian Carp in infection test

Crucian Carp in indoor foster observation1Weeks confirm Crucian Carp Growth State good after the experiment. Respectively selected introduced Resistance Gene of wild strain and lack of single colony in LiquidLBMedium in training 6. HTransfer to freshLBMedium in training to logarithmic, 4000 × GCentrifugal5 minDiscard the supernatant with sterilePBSWashing 3. Times respectively will bacteria concentration adjustment7/× 10^6CFU/mLWill

Wild strain and lack of such as volume uniform mixed intraperitoneal injection crucian carp each group Injection5Tail each tail Injection0.1 ml. Infection24 hAfter executed crucian carp and in sterile conditions under respectively out crucian carp of heart, liver, spleen and kidney record organ weight. Will organ respectively into containing ball of grinding tube in then every tube join900 Mu L

PBSBuffer will grinding tube placed biological samples homogeneous Instrument(Bioprep-24)In run program will organ grinding to uniform to sterilePBSBuffer10Times dilution take100 mu LSame of organization dilution respectively coating in containingKan (50 Mu g/mL)And

Gen (natural 20 mu g/mL)OfLBFlat use different of resistance flat distinguish between screening wild strain and missing of each dilution gradient is set

3A repeat. Will flat inverted in28 °CTraining16 hTake number of colonies was30-300Of flat the count. According to the formula(1)Calculation the organ of bacteria load

NFor flat colony number,XFor dilution gradient,MFor organ weight will statistical results to logarithmic(Log_{10})Form said.

1.11 Data Statistical Analysis

ApplicationGraphpad prism 5The organization in bacteria load of Statistical Analysis Group between compareTTest,$P<0.05$Said difference significantly;X^2Test on zebrafish of mortality The difference significant analysis.

2. Results and Analysis

2.1 Gene missing strains and complementary StrainPCRIdentification

Recombinant missing plasmid, complementary plasmid send to Suzhou jin wei YanjiangOf science and technology the company sequencing afterNCBIOfBLASTFunction in

Line than confirm sequence is correct.

Primer for upstream and downstream homologous arms of Target GenesP1/P4Internal primer of target geneP5/p6Of the missing and complementary strains.PCRThe results showed that the target gene was successfully knocked out and the complementary target gene was successfully replenished.2..

2.2 Detection of metabolic capacity of Strain

Adding inositol, sialic acid and fucose as the soleCarbon SourceM9The wild strain was found in the MediumNJ-35The corresponding substrate can be used for growth. The deletion of key genes in various metabolic pathways successfully blocked(Figure3-A)Sialic Acid(Figure3-b)And fucose(Figure3-C)The complementary strains are also P5/P6 as primer; 2: Gene deleted strain, used P1/P4 as primer; 3-4: Gene completed strain; 3: used P1/P4

2.3The zebrafish of half lethal dose

Such as table2Shown in wild strainNJ-35OfLD_{50}Value about2.15×10^2CFUMissing Strains IolCOfLD_{50}Value about2.59×10^3 CFUAnd wild strain compared increased the about12Times however saliva Acid and fucose metabolism key gene effect of the defect of the zebrafish of pathogenic Force and no obvious influence missing Strains NanaAnd FuckOfLD_{50} Respectively4.39×10^2CFUAnd3.34×10^2CFU.

In order to verifyIolCMissing the cause of virulence difference whether has? Statistical significance further carry out the zebrafish mortality Determination, Such as table3Shown in when injection of2.15×10^2CFUWhen wild strain and lackIolCCaused by zebrafish of mortality respectively 47.6% (20/42)And23.8% (10/42)By chi-square analysisX^2= 5.18Access chi-square value critical table to determine the this injection When97%The credibility of the wild strains and lack of the zebrafish of fatality rate difference; when injection of2.59×10^3CFUWhen death rate don't71.4% (30/42)And45.2% (19/42)X^2=5.92Same confirmation97%The results indicate that the key genes of Inositol metabolism are different from those of wild and missing strains.IolcThe absence of the strain attenuated the pathogenicity of the strain to zebrafish.

2.4 Crucian Carp in infection test

Wild strain and inositol metabolism key gene missing StrainsIolCOf Infection24 hAfter statistical the organ in bacteria load in Liver(P =0.0327), Spleen(P =0.0363)And kidney(P =0.0421)In wild strains and missing strains of load have significant difference(P <0.05)Wild strains show obvious of growth advantage and in heart in wild strain load slightly higher than that of missing strain load but difference don't significantly(P =0.3588)(Figure4-A). Wild strain and sialic acid metabolism key gene missing Strains

NanaOf Infection24 hAfter analysis the organ in the wild strains and missing of load found wild strain slightly higher than that of missing strain load statistical analysis difference don't significantly(P> 0.05)(Figure4-B). Wild strain and fucose metabolism way key gene missing StrainsFuckOf Infection 24. HAfter in dirty the in no table clearly of growth difference

3. Discussion

Study confirmed that pathogenic bacteria, some metabolism in favor of its in host in the colonization and can enhance pathogenic bacteria of pathogenic force[3-6].

Pathogenic Bacteria of specific metabolism ability can be as a kind of generalized on the Virulence Factor--Nutrition virulence(Nutritional virulence)[10]. Only Have in host in success get nutrition material of bacteria to survival And proliferation to caused by body. So Will pathogenic bacteria of Metabolism Ability and Traditional Significance on of virulence factor on the same important of status up study to real the pathogenic bacteria of pathogenic mechanism.

Inositol is a kind of water-soluble vitamin usually to phosphate of form there in nature in. Because inositol is body in lipid metabolism of necessary vitamin often add in fish, shrimp and livestock and poultry feed in. In some fish animal organization in inositol of content and day grain in Inositol

The amount of positive related.KohlerSuch.[11]Study prove inositol metabolism of the Chinese Rhizobium(Sinorhizobium Meliloti)In alfalfa in colonization GeneIolaNot only participate in inositol of metabolism at the same time is also valine metabolism the necessary;ManskeSuch.[12]In pneumophila Legionella(Legionella Pneumophila)In study found inositol metabolism way blocking strain was parents strains from amoeba Protozoa in competitive exclusion. In addition have study show that inositol metabolism in some fungi such as white candida(Candida Albicans), New Cryptococcus(Cryptococcus neoformans)Infection process in virulence of hair

Wave has important the role[13-15].IolCCoding2-Deoxidation-5-Dehydrogenation glucose kinase responsible for catalytic2-Deoxidation-5-Keto-D-Gluconic acid of phosphorylation is inositol metabolism way in a key gene. This test missing Aeromonas hydrophila gas singleNJ-35OfIolcIt was found that the deletion strain showed a significant growth disadvantage in crucian carp, And the pathogenicity to zebrafish was significantly reduced. It is speculated that the inhibition of Inositol metabolism pathway may restrict the proliferation of the strain. Of course, can not be excludedIolcThe possibility of regulating other growth or virulence-related genes requires further study. In addition, Aeromonas hydrophila has different growth ability in different tissues, especially in the heart,IolcThe difference in bacterial load between the deletion strain and the wild strain was not significant. It may be due to the low content of Inositol in the heart, which leads to no significant difference in the growth.

Sialic Acid is widely distributed in animals in the form of free sialic acid, sialic acid derivatives or homologs. Studies have shown that many strains can use sialic acid metabolic pathway to degrade sialic acid as energy source.[16]. Sialic acid metabolism can be Lactic Acid Bacillus(Lactobacillus sakei)In meat that contains Sialic Acid Provide competitive advantage[17]. Oral sialic acid enzyme inhibitors can inhibit E. coli and mimetic(Bacteroides vulgatus)In the intestine Growth in the Tao[18].L-Fucose is a component of the carbohydrate complex in the mucosa. Point[19-20]. Campylobacter in jejunum(Campylobacter Jejuni)China Research shows that[21-22]However, there was no difference in growth in the chicken symbiotic model. HoweverWild strains showed a competitive advantage when they were filled with fucose.NanaEncodingN-Acetyl neuraminidase, which is responsible for the first step of sialic acid metabolism, is a key gene in the metabolic pathway of sialic acid;FuckEncodingL-Fructose kinase, responsible for CatalysisL-The phosphorylation of sugar isL-A key gene in the metabolic pathway of fucose. In this experimentNJ-35Can use sialic acid andL-Fucose as an only carbon source the growth however Animal test results display no matter is the zebrafish of half lethal dose or crucian carp in vivo of infection after the bacteria load, missing strains and wild strains compared with no obvious of growth and virulence difference speculate that this a results may is because of the body can use of sialic acid or fucose in organization in content is too low, not enough to support strain in growth on the performance the difference; or animal organization in nutrition rich metabolism way diverse sialic acid or fucose metabolism way of blocking not enough to affect the bacteria of carbon source and energy and the uptake. Specific reason is not clear need to further study.

In addition from bacteria growth curve can see StrainNJ-35On this3Of metabolism substrate of use efficiency significantly different speculate that this a results may is due to bacteria in long-term evolution process in because of nutrition environment different for some carbon source formation. A kind of can

Of preference[23-24].

Comprehensive The inositol metabolism in Aeromonas hydrophila gas singleNJ-35Infection host of process for

strain in vivo proliferation provide the competition advantage and sialic acid and fucose metabolism way role is not obvious. At present of Aeromonas hydrophila gas single cell in Metabolism Ability of research domestic and foreign reports less have a lot of basic data especially inositol, sialic acid and fucose in organization in content and not clear. Future from metabolism adaptability of angle reveal Aeromonas hydrophila gas single of pathogenic mechanism will for the bacteria infection of Prevention and Control provide new of ideas.

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