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ORIGINAL ARTICLE

Velez bacillusL-1The pear Botrytis Cinerea and Penicillium Bacteria of Suppression Role evaluation and All Genome Analysis

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Abstract: [Objective] Clear Velez bacillus(Bacillus S rDNA Sequence) L-1The pear Botrytis cinerea and Penicillium bacteria of suppression role clear Bacteria

L-1Sterile fermentation broth antagonistic activity of stability and may be of Antagonistic mechanism. [Methods] by in vitro determination, living determination and pathogenic bacteria mycelium morphology observation evaluation StrainL-1The pear Botrytis cinerea and Penicillium bacteria of antagonistic activity. To pear Botrytis cinerea bacteria for try pathogenic bacteria use Oxford Cup method determination StrainL-1Sterile fermentation broth antagonistic activity of stability. UsePacbio rsiiThree generations sequencing technology determinationL-1Of all gene sequence will all gene sequence and gene protein sequence databaseBLASTComparison Analysis prediction StrainL-1May be of secondary metabolism product and potential of role mechanism. [Results] The StrainL-1The pear Botrytis cinerea and Penicillium bacteria of living inhibition rate respectively92.88%And77.47%Can caused by pathogenic bacteria mycelium enlargement, deformity. StrainL-1In10% NaClOf culture medium in can still normal growth its sterile fermentation broth high temperature resistant, acid, alkali, UV irradiation and protease degradation on pathogenic bacteria has stability of antagonistic activity. All gene sequence analysis results showed that strainL-1Yes112A Gene Involved in the many kinds of carbon source of metabolism can use many kinds of carbon source the growth; containing involved in spermidine, trehalose and strain stress resistance related compounds synthesis of gene; secondary metabolism prediction results display:L-1Containing SynthesisSurfactin,Fengycin,Bacillibactin,Bacillaene,Macolactin,Difficidin,BacilysinAnd many kinds of peptide chitosan and polyketide sugar resistance compounds of gene cluster and can degradation pathogenic bacteria cell wallβ-1,3-Glucanase and chitinase related of gene; in addition StrainL-1Containing generation acetoin and can induced Plant Resistance of gene. [Conclusion] StrainL-1Can effective antagonistic many kinds of pear of after disease resistance strong antagonistic activity stability prediction StrainL-1Can by producing many kinds of antagonistic activity compounds and cell wall hydrolysis enzymes and induced Plant Resistance implementation disease prevention effect has very big of application potential.

Keywords:Pear Botrytis cinerea pear penicilliosis biological control Velez bacillus of all Genome Sequence

And Penicillium Bacteria(Penicillium expansum)Can from pear wound infection fruit respectively pear Botrytis cinerea and penicilliosis caused serious loss[2]. At present the pear disease of control main the chemical agent such as in young fruit period, fruit growth medium-term and harvest before application of carbendazim, Tuzet, phosethyl, thiophanate-methyl and organophosphorus fungicides or Bordeaux Liquid, and on the Biological Control of research is less[3].

Author in Early Research in sieve elected on pear round with disease bacteria(Botryosphaeria berengeriana)Has significant antagonistic activity

The solution by powder bud spore rod bacteria(Bacillus amyloliquefaciens) L-1. StrainL-1Can suppression pear ring rot of extended induced by fruit Resistance Related Enzyme(Pod,CAT)Of expression and has wide of antibacterial spectrum can in fruit wound that was filled with su ke and reproductive has very big of application potential[4]. Bacillus of has very similar of physiological and molecular characteristics recent some bacillus of the genus bacteria of was re-classification[5]WhichBacillus

(B. S rDNA SequenceL-1). Velez bacillusL-1The pear Botrytis cinerea and Penicillium bacteria of prevention effect, Prevention and Control Effect of stability and Its Antagonistic mechanism still need to be further study. WithDNASequencing technology of development and high flux, fast speed, long read long and low-cost and many kinds of advantages in a of the third generation sequencing technology the emergence, for from genome of angle study microbial provide the fast is simple of way[7].

This study determination the velez bacillusL-1The pear Botrytis cinerea and Penicillium bacteria of prevention effect clear. The strain antagonistic activity of stability and use the third generation sequencing technology determination. Its all Genome Sequence, from gene angle analysis the May of role mechanism and application potential for strainL-1Of application study provide more of reference.

1. Material and Methods

1.1 Test Material

1.1.1 Test fruit: Test'Huangguan Pear'In2016Years8Month Mid-collected from Hebei Province Jinzhou City select size consistent of Health Fruit Packing, Transport(Harvest after2 DIn)To this unit Cold Storage(0 °C)Save spare.

1.1.2 Medium:Test in with medium raw materials were purchased from Shanghai, rope bridge biological technology limited the company including potato glucose powder, beef extract peptone powder, agar powder. Test with potato glucose medium(PDA), Beef extract peptone agar medium(NBA)And beef extract peptone(NB)Culture medium were in accordance with (the configuration.

1.1.3 For try Strain and Its Resistance of preparation: This experiment involved strain including pear Botrytis cinerea Pathogenic Bacteria(B. cinereaLHM), Pear penicilliosis

Pathogenic Bacteria(P. expansumLQM)And Velez bacillus(B.VelezensisL-1.LhmAndLqmThe pear fruit with typical gray mold and green mold symptoms during storage was isolated from the laboratory and verified by optical microscopy and pathogenicity. Pathogen Inoculation

PDAMedium, in25 °CFoster5 d, Take the bacteria cake which is the frontier of Colony Growth(Diameter6mm)For in vitro and in vivo Determination of activity.

VelezensisL-1By the author Yu2015Year9.Moon was isolated from pear rhizosphere soil,-20 °CSave in20%Glycerin. Will StrainL-1VaccinationNBAMedium,32 °CFoster2 D, Take the diameter6mmThe bacteria cake was used for the determination of in vitro activity. StrainL-1

Has been2017Year06Moon30Submitted to the General Microbiology center of the China microbial Collection and Administration Commission onCgmcc14373.

1.1.4 Strain L-1Fermentation Broth, spore suspension and sterile fermentation broth

Prepare: Using shake flask fermentation method200μLStrainL-1Spore

(20%Glycerin-20 °CSave)Vaccination100OfNBMedium,

180 r/min,32 °CFoster48 h, Get seed liquid. Will Seed

R/min,32 °CFoster48 hThe fermentation broth was obtained. Fermentation Broth passing through8000 r/minCentrifugal20 min, Collect precipitate and upper

Qing. The precipitate was diluted with distilled water, and the spore concentration was adjusted10^{8}·Cfu/mlSpore suspension was obtained.(Aperture0.22μM)The spores were removed by filtration and the sterile fermentation broth

was obtained.

1.2 StrainL-1Effects of antifungal activity and mycelium growth on Botrytis cinerea and Penicillium

Tablet confrontation Act[8]DeterminationL-1Antagonistic activity against Botrytis cinerea. Botrytis cinerea(Lhm)Mushroom cakePDAPlate Central, using sterile punch on both sides of the mushroom cake3 cmPlace symmetrical punch, in the hole inverted InoculationL-1Mushroom cakes. Inoculated plate placed25 °CCultivation.

No vaccinationL-1As a control. Repeat per Process3.Times.

The diameter of inhibition zone was measured when the control colonies were full of plates.

ReferenceWiggins & KinkelMethod[9]DeterminationL-1The Penicillium of antagonistic activity. Penicillium(Lqm)InoculationPDA25 °CCulture

5 dWith sterile knife will having colony of medium cut into small pieces and added50 mLDistilled Water with sterile blender break get Penicillium spore suspension. Will10 mlSpore suspension join500 ml 50 °C

PDAMedium in mixed uniform after pour flat. In containing Penicillium spore of flat from the plate edgeNatural 20mmThe location symmetric place2A Oxford Cup each cup join100MuLStrainL-1The fermentation broth,25 °CConstant Temperature training measurement and record antibacterial circle diameter.

Selected the above the processing and control of pear gray mold, Penicillium mycelium in optical microscope under observation mycelium and spore morphology and the compare.

1.3 StrainL-1The pear Botrytis cinerea and Penicillium bacteria of living anti-Effect

ReferenceSadeghianSuch.[10]Of methods Determination StrainL-1The pear Botrytis cinerea and Penicillium bacteria of living anti-effect. Will health fresh'Crown Pear'Fruit place in0.2% (V/V)Sodium hypochlorite in Soaking3 minOf tap water to wash after dry. With sterile punch in Pear Fruit Equatorial Both sides of the drilling1A aperture5mm, Deep3mm. Every hole In joinNatural 20MuLStrainL-1Spore suspension(1×10^8CFU/mL)To JoinNatural 20MuLDistilled Water as an negative control. Processing after the fruit With plastic wrap Sealing,Natural 20 °CMoisturizing training. Training1 dAfter in Hole posted the Botrytis cinerea(Lhm)Or Penicillium Bacteria(Lqm)Resistance of placed 20°CContinue to training timing(Every2 D/5 d)Determination of different processing of lesion diameter and by the following formula calculation anti-effect. Each to deal with the repeated3Times each repeat6A fruit. Prevention and Control Effect(%) = (1 -?Processing lesion diameter/Control lesion diameter) × 100.

1.4 StrainL-1Salt Tolerance and Its sterile fermentation broth antibacterial role of stability

1.4.1 StrainL-1Salt Tolerance: Will differentNaClJoin

NBCulture Medium in preparation into0.1%,0.5%,1.0%,2.0%,5.0%,10.0%,12.0%,15% NaCl (W/V)OfNBCulture A liquid Inoculation1 mLStrainL-1Of seed Liquid,180 r/min,32 °CTraining48 h600 nmDeterminationODValue.

Reference Ge Pinghua[11],Olfa Kilani-FekiWait.[12]Evaluated StrainL-1Stability of antibacterial activity of sterile fermentation broth. StrainL-1The aseptic fermentation broth was treated differently.(See below description)The antagonistic activity against Botrytis cinerea was determined by Oxford Cup method. InPDAPlate central inoculation of Botrytis cinerea(Lhm)Mushroom cake, from the edge of petri dish20mmSymmetrically placed sterile Oxford cups each cup 200μLThe aseptic fermentation broth was treated, and the strains were determined afterL-1Antagonistic activity of sterile fermentation broth.

1.4.2 Acid-Base Stability:1 mol/LOfNaOHOrHCl, Will strainL-1Sterile fermentation brothPHValues are adjusted2.,3.,4.,

5,6,7,8,9,10,11And12, Static24 hFermentation Broth of the formerThe pH 6.5Spare. The effect of different pH value on the activity was determined.

1.4.3 UV Stability: StrainL-1Aseptic fermentation broth30 WUnder the UV lamp, irradiation10,20,30,40,

50,60,90,120 min, UV lamp perpendicular to the sterile fermentation broth,

Distance is30 cm. The effect of ultraviolet radiation on the activity of the sterile fermentation broth was determined.

1.4.4 Thermal Stability: Strain L-1Aseptic fermentation broth

40,50,60,70,80,90,100 °CMetal Bath20 minAfter that, cool to room temperature. The aseptic fermentation broth without high temperature treatment was taken as the control, and the changes of its activity after different high temperature treatment were determined.

1.4.5 Enzyme stability: In the strain L-1Aseptic fermentation broth

Add trypsin, pepsin and protease KAnd make the enzyme the most

Final concentration1 mg/ml,37 °CReaction1 HAnd then in80 °C

Processing30 min,4 °CImmediately cooled to determine the strains treated with different ProteaseL-1Antagonistic activity of sterile fermentation broth.

1.5 StrainL-1Genome-wide Assay

Complete Genome Sequencing and splicing of BacteriaSunWait.[13]. Using the bacterial genomeDNAQuick extraction kit(B518225Shanghai, China)Extracted StrainL-1The totalDNAAfter the concentration detection, it was submitted to the Beijing Bai Mike company, using the three generation sequencing platform.

Pacbio rsiiThe whole genome was sequenced. ViaProdigal version 2.5Software for coding gene prediction, borrowing Protein

Functional DatabaseCOG (clusters of historic groups),Go (gene ontolog)Metabolic Pathway DatabaseKegg (Kyoto)

Encyclopedia of genes and genes)The amino acid sequence predicted by the genome was compared with other functional databases, and the protein function and biological metabolic pathway were predicted.

UtilizationAntismarsh version 4.0.2Software[14](Https://antismash.secondarymetabolites.org)Analysis, bacteria, strainL-1The main secondary metabolites.

2. Results and Analysis

2.1 StrainL-1Effects on in vitro Activity and mycelial growth of Botrytis cinerea and Penicillium sp.

StrainL-1In vitro conditions can significantly inhibit, The growth of mycelium of Penicillium sp.(Figure1-A,B). Select the control and the front of the Antibacterial circle of pathogenic bacteria Mycelium **Figure 1.** The exhibition effect strain L-1 on pear gray mold and blue moldIn vivoAnd its influences on the moral characters of the pathogenes.B. cineea(A) or?P.Expansum(B) was shown by dual culture detection.B. cinerea(C e) orP. expansium(D f) absence (c d) or presence (e f) strain L-1 under microscope. Control of gray mold, Penicillium mycelium growth uniform(Figure 1-CD)And afterL-1Processing of pathogenic bacteria mycelium show Explicit of enlargement and deformity(Figure1-EF).

2.2StrainL-1The pear gray mold, Penicillium bacteria of living suppression Activity

In living conditions under strainL-1Can significant suppression pear Botrytis cinerea and Penicillium of extension. At room temperature conditions Inoculation Gray Mold after control of lesion diameter rapid expansion,9 dTo(39.35 ± 2. 71) mmAndL-1Strain processing of lesion diameter extended slow Inoculation9 d

When the lesion diameter(2.80 ± 3. 47) mmAnti-effect92.88% ($P \leq 0.001$)(Figure2). At room temperature conditions under Inoculation25 dAfter penicilliosis spot diameter(31.80 ± 9. 75) mmAndL-1Processing of lesion straight diameter(7.16 ± 5. 70) mmAnti-effect77.47% ($P \leq 0.001$)(Figure3).

2.3 StrainL-1Salt Tolerance and Its sterile fermentation broth antibacterial role of stability

StrainL-1In10% NaClOf culture medium in can still Growth(Figure4)Its sterile fermentation filtrate antagonistic activity of the most suitablePHFor6-7/Acid and alkali inPHFor12When the pathogenic bacteria of antagonistic activity

still40%More(Figure5-A). UV Irradiation2 hWithinL-1Sterile fermentation broth of antagonistic activity no significant influence(Figure5-B). Different high temperature treatment onL-1Sterile fermentation broth of antagonistic activity no significant shadow

2.4 StrainL-1All Genome Analysis

StrainL-1All genome inGenBankThe accession numberCP023859. The bacteria genome full-length4090582 BPGCContent46.52%. Genome contains3978A coding sequence(CDSCoding sequences)In andCOGFunction enrichment analysis,KEGGMetabolism pathway enrichment analysis,GOFunction enrichment Analysis

Such as database doBLASTComparison after have99.9%OfCDSOrder 13A Gene Involved in the non-ribosomal protein synthesis,Natural 20A gene Column get function classification. CombinedAntismarshResults Strain L-1Can produceSurfactin,Fengycin,Bacillibactin, Bacillaene,Macolactin,Difficidin,BacilysinAnd more A peptide chitosan and polyketide sugar resistance Compounds(Figure6)The Times Students metabolism product of synthesis become its Prevention Disease of main mechanism One.

StrainL-1Yes73.61%OfCDSSequence inCOGData The library gets the function taxonomy. As shown in Fig.7.As shown, which participates in the Secondary Generation Co-occurrence of genes involved in biosynthesis, transport and catabolism of Xie Products Yes.121Amino acid transport and metabolism Genes349Lipid Transport and metabolism Genes118Inorganic Ion transport and Metabolism With the synthesis of poly ketone compounds,7.Genes Involved in iron-containing non-ribose Body fat peptide synthesis. In addition, the strainL-1Can translate459A carbon Aquatic enzymes.

AccordingKeggThe results of the analysis showed that97 There are several metabolic pathways40Fructose, mannose, and half Lactose and other glucose metabolism,30Genes Involved in starch, sucrose and Metabolism of other polysaccharides;42Genes Involved in amino sugars and nucleoside Acid and carbohydrate metabolism. This kind of gene and metabolic pathway are not related to the strain? Utilization of the same carbon source and its rapid growth, cloning and Disease Control Features are closely related. GoDatabase comparison analysis showed that: StrainsL-1Have generated Yin214Carbohydrate transport and metabolism Genes 255A. Yes. Nitrile amidase(Atom09126)Feel proteins with trehalose and transport eggs

Circular mapB. velezensisL-1 genome. The Five Circles (outer to inner) present forward strand CDSs, reverse strand CDSs, nomenclature and locations of predictive secondary metabolite clusters GC content GC skew.

Bai(Atom10173,Ato10175)And other genes closely related to the stress resistance of the strain, further revealingL-1Resistance. Contains acetyl lactate Synthase(From pyruvate to acetyl lactate,Atom11909,Ato12400,Ato08987)With acetyl lactate Decarboxylase(From acetyl lactic acid to acetyl rock,Ato08986)And and Induced Resistance Related of gene from Gene angle (strainL-1Can induced by host resistance. In addition StrainL-1Containing Synthesisβ-1,3-Glucanase(ATO11163,ATO09278)And chitin combined with protein(ATO11113)And antagonistic activity related gene pointed out thatL-1Has by produce hydrolysis enzymes hydrolysis pathogenic bacteria cell wall of ability.

3. Discussion

Can use many kinds of carbon source success, rapid propagation is biocontrol Implementation biocontrol effect of Foundation[15]Early study in found StrainL-1Can in fruit wound in success, rapid colonization[4]This study from base For the perspective of further clarify the strainL-1Contains459A gene and carbohydrate compounds enzymes synthesis related,255A Gene Involved in carbohydrate transport and metabolism and have112A Gene Involved in to many kinds of Sugar Metabolism pathway in (strainL-1Has use many kinds of carbon source the rapid growth and reproduction of potential.

Has application potential of biocontrol strain need to be able to tolerance Extreme Environment[15]. This study in strainL-1Can in containing10% NaClCulture Medium in Growth Reproduction metabolism product high temperature resistant, UV Irradiation, acid, alkali and protease degradation. In addition StrainL-1Has the spermidine of Nitrile

amidine synthase and trehalose transport and feel protein of gene spermidine not only is plant growth regulator and has protection body tolerance high salt, low temperature, low humidity and peroxide Function Classification, percentage (A-T) strain L-1 genome genes according. COG database.

Chromatin structure, dynamics; B: energy production, conversion; C: Cell Cycle Control Cell Division chromosome partitioning; D: amino acid transport,. metabolism; E: nucleotide transport,. metabolism; f: carbohydrate transport,. metabolism;

Coenzyme transport,. metabolism; H: lipid transport,. metabolism; I: Translation ribosomal protein structure, biogenesis; J: transcription; K: Replication Recombination, repair; l: cell Wall/membrane/envelope biogenesis; M: Cell Motility; N: post translational modification Protein Turnover chaperones;

Inorganic Ion transport,. metabolism; P: Secondary metabolites biosynthesis transport, catabolism; Q: General function prediction only; R: function unknown; s: Signal Transduction mechanisms;

Tracellular traffic, creation, and vesicular transport; U: defense mechanisms.

Environment[16], While trehalose(Trehalose)As a osmotic protective agent, it has the function of protecting organisms from high salt, low temperature and low humidity environment.[17]. All the above results reveal that the strainL-1And their metabolites are resistant to extreme environments.

Strains found in previous studiesL-1Antagonistic to pear ring rot fungus[4]In this studyL-1Pear gray mold, Penicillium also table

It showed significant antagonistic activity and wide antibacterial spectrum. Genome-wide results show that the strainL-1It has a gene cluster related to the production of a variety of resistant compounds such as polyketide, peptidoglycan and peptides, andBeta-1,3-Genes Related to glucanase and chitinase biosynthesis. Surface active peptide(Surfactins)And other non-ribosomal peptide compounds can be used as signaling molecules to induce Plant Resistance by acting on biofilms to achieve disease prevention.[18],BacillibactinIt can be used as an iron carrier to achieve disease prevention through competition for iron ions. A variety of lipid peptides and peptide polysaccharides have antagonistic effects on pathogenic bacteria.[19].Beta-1,3-Glucanase and chitinase are enzymes that act on the cell wall of pathogenic bacteria, which can catalyze the hydrolysis of the cell wall of pathogenic bacteria, causing the necrosis and cell wall degradation of pathogenic bacteria, thereby reducing the occurrence of diseases and fruit decay.[20-21]. All the above results indicated that the strainL-1It has the ability to produce antibiotics and hydrolytic enzymes against pathogenic bacteria to achieve disease control.

In addition, the whole gene sequence showed that the strainL-1It contains genes related to the activity of acetyl Lactic Acid Decarboxylase and can be degraded to form acetoin.(Acetoin)The ability of acetoin to induce Plant Resistance[22], Indicating StrainsL-1Induction of plant resistance to achieve the purpose of disease prevention. The results are consistent with the previous studies.L-1Expression of resistance-related enzyme activities in Crown Pear[4]The results are consistent.

Pang xuequn, *et al*.[23]To summarize the mechanisms of antagonistic bacteria controlling Postharvest Diseases of fruits and vegetables, including: producing extracellular antibiotics, space and nutrient competition among microorganisms, acting directly on pathogenic bacteria, extracellular enzymes, such as chitinase and glucanase, that can degrade fungal cell walls, inhibit the leakage and rupture of pathogenic bacteria cells. Preliminary results show that the strainL-1It can significantly resist the pear ring rot bacteria, quickly colonize the fruit wound, and induce the expression of Plant Resistance Related Enzyme Activities.[4]The results of this study further show that the strainL-1There were also significant antagonistic activities against Botrytis cinerea and Penicillium. The strain was identified from the stability of antagonistic activity of sterile fermentation broth and the whole gene sequence analysis.L-1Can use a variety of carbon sources for growth, strain

Through the production of various secondary antagonistic compounds and enzymes for the hydrolysis of pathogenic bacteria, and the induction of various mechanisms of host resistance, the purpose of disease prevention is achieved. Peles spore pole

L-1It is a biocontrol strain with great potential for application. The optimal fermentation conditions, application forms and separation of main secondary metabolites need to be further studied and explored.

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