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Screening of Liquid Fermentation Conditions of Biocontrol Fungus Trichoderma E11 Strain

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Abstract: At present, the most convenient, easiest and easiest to achieve plant disease control effect is mainly the use of chemical pesticides. However, the large-scale use of chemical pesticides has caused serious harm to the environment and humans; biological control is less toxic to humans and animals, and it has gradually become the focus of people's research, and biological control has also become an important development trend. In order to study the optimal fermentation conditions of the Trichoderma E11 strain, this article takes the Trichoderma E11 strain as the test strain and uses the mycelium growth index as the index to determine the liquid fermentation broth, carbon source, nitrogen source, pH value, inoculation through relevant experiments The best combination of quantity, fermentation time and fermentation temperature. The research in this paper shows that the dry weight of mycelium in D-fructose culture solution is the largest at 4.0 (mg / ml); the mycelium yield of ammonium dihydrogen phosphate is 0.8 (mg / ml) and the dry weight of the mycelium is the largest; pH value is 6 The dry weight of the mycelium is the largest at 5.2 (mg / ml); the inoculation volume of 1.2mL (1.2%) or 1.7mL (1.7%) is the most suitable for mycelial growth; when the temperature reaches 25 °C, the mycelium yield reaches the maximum The value is 556mg / 50nd. Therefore, with D-fructose as the carbon source, ammonium dihydrogen phosphate as the nitrogen source, the fermentation temperature is 25 ℃, the initial pH of the medium is 6, and the inoculation volume is 1.2mL or 1.7ml.

1. Introduction

Trichodermaspp belongs to the semi-known fungus, and is a worldwide distribution of fungi. It is often found in soil and decaying wood. It has the characteristics of wide adaptability, diverse

metabolic substances, extensive parasitism, and environmental friendliness. In 1932, Weindling It was found that Trichoderma can parasitize pathogenic microorganisms and can cause their death, so it opened the prelude to the research of Trichoderma used for biological control, and it has been paid more and more attention in the prevention and control of plant diseases; because chemically synthesized pesticides are highly efficient, economical, Convenience and other advantages make chemical control an important means of preventing and controlling plant diseases, but excessive use of pesticides can cause a series of problems, such as poisoning of humans and animals, killing probiotics, making pathogens resistant, and high residues causing environmental pollution. Therefore, biological control methods that directly use biologically produced active substances or living organisms as pesticides to control plant diseases have attracted widespread attention.

Biological control uses beneficial microorganisms to inhibit the growth and spread of pathogenic bacteria, thereby preventing and controlling plant diseases efficiently and environmentally. It is a popular direction for disease control research and has broad prospects. At present, the most convenient, simple and easiest way to achieve plant diseases is to use chemical pesticides. Prevention and control, but the extensive use of chemical pesticides has caused significant harm to the environment and humans. Biological control has the advantages of less toxicity to humans and animals, no pollution to the environment, and resistance to diseases and insects. The hotspot of people's research, biological control has become an important development trend.

Imen Sellem et al. Isolated an actinomycete named TN258 from the Sahara soil in Tunisia and screened it for antagonistic activity, especially for Pythium ultimum (P.ultimum), which causes potato tuber leakage Based on the culture characteristics of TN258 strain, 16srrna gene nucleotide sequence and phylogenetic analysis results, a new isolate was proposed as Streptomyces TN258 strain. After optimizing the culture conditions, the inhibition of TN258 free cell supernatant on Streptococcus suis was evaluated effect. The research results of Imen Sellem et al. Showed that 50% of the concentration of 25 mg ml-1 can completely inhibit the growth of mycelium and destroy the hyphae; at the same concentration, the oospores deformed and germination completely stopped; in potato tubers, Streptomyces TN258 Filtering the supernatant 24 hours before inoculation, compared with the untreated group, can significantly reduce the penetration rate of pathogens by 62% and reduce body weight by 59.43% [1]. W. Dendouga et al. Isolated 14 strains of Trichoderma from Algerian desert soil, and studied their resistance to wheat Fusarium root rot and root rot disease, using in vitro and in vivo bioassay methods to study Trichoderma 3 Pathogens: biocontrol effects of stalk mold, Fusarium graminearum and Verticillium dahliae; research results of W. Dendouga et al. Showed that compared with the control, the colony diameter of Fusarium strain was significantly reduced, and the harzianum-Thr.4 colony The diameter reduction rate is the highest, and the growth of Fusarium graminearum, Fusarium graminearum and Fusarium verticillium are reduced by 70.68%, 67.05% and 70.57%, respectively; all Trichoderma strains can overgrow and sporulate above the stem mold colony, However, overgrowth was not observed in Fusarium graminearum and Fusarium verticillia; compared with untreated controls, Trichoderma isolates seeded the soil infected with pathogens before sowing, which could significantly reduce the severity of the disease; Two harzianum strains (Thr.4 and Thr.10) and T.viride Tv.6 had the largest decrease (> 70%) in the pathogenicity index of these three pathogens. Trichoderma isolates were used as the only carbon source. Fungal cell wall fluid The lyase produced by the Trichoderma isolate was tested in the nutrient medium. The activity of protease and chitinase induced by the cell wall of Fusarium graminearum was higher than that of Fusarium verticillium and Fusarium oxysporum; The enzyme activity of the mycelial cell wall of Fusarium graminearum and Fusarium stalk is the highest, but in the medium modified by the cell wall of Verticillium dahliae, the lytic activity of Verticillium dahliae Tv is the highest [2]. Gargee Dhar Purkayastha et al. Evaluated the growth promotion and biocontrol effects of Serratia marcescens ETR17 strain isolated from the rhizosphere of tea plants

on tea tree root rot. ETR17 cultured in vitro has obvious effects on 9 different leaf root pathogens In vitro antagonism. The phenotype and molecular characteristics of ETR17 indicate that the bacterium is Serratia marcescens, which can produce chitinase, protease, lipase, cellulase and IAA, iron carrier and other metabolites that promote plant growth; pathogenic bacteria Scanning electron microscopy studies on the interaction zone with antagonistic bacteria showed that there were serious malformations in fungal mycelia, spectroscopic analysis (LC-ESI-MS, ultraviolet-visible spectrophotometry and high performance liquid chromatography) and thin layer chromatography (TLC) It showed that the antibiotics pyrrole nitrogen and progicocin were present in the extracellular bacterial culture; the activity study of ETR17 talc formula showed that the isolate could survive for 6 months at room temperature, and the application of talc was determined by enzyme-linked immunosorbent assay (ELISA) The nutritional status of ETR17 (8-9x108 cfu g-1) in the soil after formulation, safety studies have shown that ETR17 will not produce the hemolysin observed in pathogenic Serratia strains [3].

In this paper, the Trichoderma E11 strain was used as the test strain, and the mycelial growth amount was used as the indicator. The Trichoderma E11 strain was determined by measuring different liquid fermentation broth, carbon source, nitrogen source, pH, different inoculation amount, fermentation time, fermentation temperature. The influence of silk yield, so as to study the best fermentation conditions of Trichoderma E11 strain, and lay the relevant exploration experience for the research and development of biocontrol bacteria.

2. Proposed Method

2.1. Biological Control Research

(1) Biocontrol group

1) Biocontrol fungi

Among the biocontrol fungi, Trichoderma fungus, Paecilomyces lilacinus and Arbuscular mycorrhizal fungi are the most studied. Since reported that Trichoderma lignin can parasitize a variety of pathogenic fungi and increase Trichoderma in soil the quantity can control the occurrence of certain diseases. Trichoderma fungus has become one of the biocontrol bacteria that people pay attention to; the commonly reported biocontrol Trichoderma fungi are: Trichoderma harzianum, Trichoderma lignin, Trichoderma viride, Trichoderma longiformis , Trichoderma koningii, Trichoderma polysporum, Trichoderma hookens, Trichoderma aculeatus, etc.

Trichoderma fungus is very easy to separate, it is distributed in large quantities on soil, air, withered branches and fallen leaves. Related studies have shown that Trichoderma fungi have antagonistic ability to a variety of plant pathogenic bacteria because the strains of this genus can secrete cell wall degrading enzymes, such as cellulase and protease, to partially thin and digest the cell wall of the pathogenic hyphae, which eventually leads to the cell wall of the pathogenic bacteria Rupture, mycelium rupture, and death of pathogenic bacteria; Trichoderma not only prevents and controls plant diseases, but also promotes plant growth, enhances stress resistance, and repairs polluted environments; arbuscular mycorrhizal fungi establish a reciprocal co-existence with plant roots Bio-relationship, it can symbiosis with plants, enhance the plant's resistance to Fusarium oxysporum, arbuscular mycorrhizal fungi can not only improve the nutrient microenvironment of crop roots, but more importantly, it can selectively inhibit surrounding pathogens , To reduce the probability of plants being infected by pathogenic bacteria, and reduce the morbidity and mortality of plants from a preventive level [4].

2) Biocontrol bacteria

Compared with fungi, bacteria have played an important role in biological control research due to their faster reproductive ability, stronger environmental adaptability, complex metabolic activities

and diversity of metabolites. The bacteria that are currently successfully used to control plant diseases mainly include the following genera: such as Bacillus, Agrobacterium, Amorphocystis, Pseudomonas, Azotobacter, Erwinia, Serratia, Pasteurella, Micromonospora, Enterobacter, Flavobacterium, Xanthomonas, etc. Among them, Bacillus strains are the most studied [5].

3) Biocontrol actinomycetes

Actinomycetes, as a resource-rich microorganism group, have great potential for development and application. However, at present, only a few soil actinomycetes have been isolated for biocontrol. A large number of actinomycetes have not been studied. In the current research on biocontrol actinomycetes, the most reported is streptomyces, which is widely used A class of actinomycetes for biological control of plant diseases. Among them, Streptomyces roseoflavus, Streptomyces griseostat, and Streptomyces aureus have a strong inhibitory effect on soil-borne diseases such as wilt and root rot; actinomycetes can produce A variety of secondary metabolites, such as streptomycin, chlortetracycline, actinomycin, tetracycline, etc., are widely used antibiotics, which play a huge role in the biological control of plant diseases in China.

(2) Biological control mechanism

1) Competition

Competitive action means that the biocontrol microorganisms control the growth of plant pathogenic microorganisms by grabbing the nutrients secreted from the plant roots and seeds, etc .; the beneficial microorganisms can colonize the rhizosphere in large numbers, resulting in plants Nutrients such as limited carbon and nitrogen sources in the rhizosphere are more scarce, which reduces the number of pathogens that survive and colonize the plant rhizosphere; the cell junctions at the root tip, the germination points of the lateral roots and other parts are due to the root secretions. The ideal place for the colonization of various microorganisms including pathogenic bacteria, the introduction of biocontrol bacteria in the plant root system can prevent or reduce the colonization of harmful rhizosphere microorganisms at these sites, thereby playing the role of site exclusion [6].

2) Antagonism

Antagonism refers to the production of one or more antibacterial substances by microorganisms, acting on the cell wall, cell membrane, protein synthesis system, energy metabolism system, cell division, etc. of the pathogenic bacteria, which can directly inhibit or kill the pathogenic bacteria; most of them in nature have bacteriostatic effects The antagonistic substances produced by the fungi and bacteria mainly include antibiotics, degrading enzymes and other antibacterial substances, such as xylemmycin and antibacterial peptides.

3) Heavy parasitism

Heavy parasitism is the main mechanism by which parasitic fungi antagonize pathogenic fungi. It is not an accidental phenomenon, but a complex process that includes a series of successive steps of invasion, identification, contact, winding, penetration and parasitization of pathogenic bacteria [7].

2.2. Application of Trichoderma

Trichoderma has the characteristics of strong adaptability, strong proliferation, high nutrient utilization rate, strong ability to improve rhizosphere, strong inhibitory activity against plant pathogenic fungi, and good plant growth promotion effect. It is used in soil remediation, disease control, and plant growth promotion. All aspects are widely used [8].

(1) Soil treatment

In terms of environmental soil remediation, Trichoderma can significantly improve the effectiveness of soil copper and promote the absorption of copper by plants, and can be used as a

remediation agent for heavy metal contaminated soil. At the same time, Trichoderma secretes organic acids such as fumaric acid, citric acid or gluconic acid through the carbon source to dissolve mineral cations, including manganese, magnesium, iron, other micronutrients and phosphates, adding Trichoderma to the lack of these metals Cationic soil can effectively dissolve these metals, thereby improving crop yields. For example, solid preparations of Trichoderma reesei spilled into contaminated soil can significantly increase the concentration of plants on Cd; Trichoderma koningii (NBRI-PR5) Plant pathogens have antagonism and phosphorus solubilization. The organic acids produced can be used to dissolve insoluble tricalcium phosphate (TCP) at high pH. Phosphorus solubilizing fungi have a great effect on the activation of microbial phosphorus. Next, NBRI-PR5 produces alkaline phosphatase to solubilize phosphorus. Therefore, this strain has broad application prospects in the reclamation of degraded soils with high acidity and drought conditions. Trichoderma as a phosphorus solubilizer for grasslands lacking soil nutrients, can be used for soil treatment and improving the biomass of grassland plants [9].

(2) Disease control

Trichoderma has a significant control effect on some common diseases in crops, such as Trichoderma harzianum has a good biocontrol effect on tomato and pepper major diseases, tomato blight and pepper blight; Trichoderma virens and Trichoderma harzianum are withered to poplar leaves The growth of pathogenic strains has an antagonistic effect, Trichoderma has a certain space and nutrition competition, and it has a certain inhibitory effect on the growth of pathogens; Trichoderma GDFS1009 strain can effectively prevent straw rot, the inhibition rate is 60%, 3g / pot Trichoderma granules have the best control effect on corn stalk rot (40.95%). Trichoderma granules have been used continuously for 3 years, which has obvious control effect on straw rot; Trichoderma harzianum mainly achieves bacteriostasis by infecting anthracnose mycelium Activity, the inoculation time significantly affects the activity of the strain. Inoculation with Trichoderma viride before 24h will reduce the size of lesions the most, and it is not easy to cause discoloration of papaya fruit.

(3) Promote plant growth

In terms of promoting plant growth, Trichoderma amino acid organic fertilizer has a good growth promoting effect on tomatoes and can be used as a microbial fertilizer; Trichoderma can also be used on various flowers and horticultural crops to increase the germination rate and the flowering period is getting earlier , Fresh weight and height increase; Trichoderma harzianum and Trichoderma viride are used in calendula, petunia and verbena to increase the number of flowers, the weight of stems and flowers; Trichoderma can increase the leaves and branches of Jianlan The quantity and prolonged flowering time have certain promotion effect on Jianlan's vegetative growth and reproductive growth. Trichoderma can be used in the production of vegetables, such as radish, soybean, tomato, cucumber, pepper seedlings, to increase the emergence rate, leaf area, plant height and dry weight; Trichoderma fortified biological fertilizer (bio / compost) makes ripe tomatoes resistant Increased oxides and minerals have an improved effect on soil nutrient utilization and microbial population [10].

(4) Industrial production

In terms of industrial production, Trichoderma spp. Is an efficient, economical, and environmentally friendly raw material with great production potential. Laccase has a potential role in dye repair. The enzymes Xyl1 and Xyl2 in the substrate Trichoderma can effectively degrade beech xylan and prepare wood oligosaccharides. During the baking process, adding Xyl1 can increase the softness of wheat bread and whole wheat bread Degree and volume; the use of Trichoderma brevis KT693225 solid state fermentation low-cost disposable agricultural waste to produce xylanase to solve the two major problems of environmental pollution caused by agricultural waste and high production cost of industrial xylanase; keratin It is the main component

of feathers and has a great impact on environmental pollution. It uses Trichoderma harzianum HZN12 to produce the largest yield of keratinase, and uses keratinase to dehair goat skin. Compared with chemical treatment, keratinase depilated skin shows superior In addition to performance, keratinase also shows good stability and compatibility with commercial detergents tested, making it suitable for industrial applications, such as leather and detergent industries.

(5) Medical

In terms of medical treatment, Trichoderma fungus, as a fruitful resource, has been widely used in the pharmaceutical industry. A new epoxide hydrolase isolated from Trichoderma reesei is a promising anti-infectious disease molecule that can participate in endogenous and exogenous epoxide metabolism. The development of its inhibitors is in It has important applications in medicine; a new type of water-soluble polysaccharide TPS was isolated from the mycelium of Trichoderma koningii, which is non-toxic in normal cells (lo2 cells) and can inhibit mouse colon cells (ct26 cells)), It also has the effect of scavenging free radicals on hydrogen peroxide, and has potential application prospects in the field of biomedicine.

2.3. Trichoderma Degradation Mechanism and Genetic Engineering Breeding

(1) Mechanism of degradation of pomace by Trichoderma viride

During the growth process, Trichoderma viride secretes a lot of extracellular enzymes, not only cellulase but also pectinase, chitinase, glucanase, etc .; the most recognized degradation mechanism of cellulase is synergy Theory of action, cellulase components play a synergistic role in the hydrolysis of cellulose, there are currently four kinds of synergistic effects [11]:

1) Relaxation of intermolecular hydrogen bonds between adjacent cellulose chains;
2) Synergy of β-glucosidase and endo- or exo-glucan cellulase;
3) Synergy between substrate and exoglucan cellulase;
4) Synergy between endo- and exo-glucan cellulase.

The β-glucosidase produced by Trichoderma viride is regioselective and stereoselective for the hydroxyl group of sugar during the transglycosylation process. Therefore, this enzyme can be used for the regioselective and stereoselective synthesis of oligosaccharides; Endoglucanase I (endoglucanase, EGI) is only in the space around the catalytic residue and does not directly affect the transglycosylation reaction of EGI, but several amino acid residues in the active site are involved in the transglycosylation of EGI Reaction, it not only has hydrolase activity, but also has the role of transglycoside; in recent years, there have been many researches on the degradation function of Trichoderma viride, and the focus is on the degradation and reuse of residues, such as protein production, dietary fiber, enzyme production, etc. This provides a theoretical basis for the application of Trichoderma viride to actual industrial production.

(2) Mechanism of Trichoderma reesei degrading pomace

The cellulase secreted by Trichoderma reesei is an extracellular enzyme. Its enzyme system is complete, high in content, strong in stability, safe and non-toxic. It is mainly divided into three categories: endoglucanase and exoglucanase. And β-glucosidase. The mechanism by which Trichoderma reesei degrades cellulose is generally considered to be a "endo-exo" synergetic mechanism. Endoglucanase mainly hydrolyzes the β-1,4-glycosidic bond in the non-crystalline region to shorten the cellulose molecule; The exoglucanase then hydrolyzes the β-1,4-glycosidic bond at the end of the cellulose linear molecule to produce a cellobiose molecule; β-glucosidase hydrolyzes the products of the previous two steps into glucose molecules, but β -The activity of glucosidase is relatively low, which causes cellobiose to hydrolyze and accumulate, thereby affecting the degradation effect of cellulose. Methods to improve β-glucosidase enzyme activity mainly include breeding, mutagenesis, genetic recombination, etc. Cellulase secreted by

Trichoderma reesei also degrades pomace through synergistic action [12].

(3) Status of genetic engineering breeding of Trichoderma

With the continuous development of microbial technology, the fungal genome sequencing work is gradually carried out. At present, the transcriptome sequencing technology (RNA-seq) is mainly used to complete the determination of the gene sequence of Trichoderma. The determination of the gene sequence makes Trichoderma in enzyme production and industrial application more The more and more important role is also through genetics to make it possible to control the growth and development of microorganisms and promote product production through genetic engineering to regulate gene sequences. In the process of gene breeding, we currently mainly use RNA-seq to analyze gene sequences, and then achieve our goal through gene recombination or gene knockout; at present, the mechanism of Trichoderma degrading pomace is not thorough, and the composition of pomace It is more complicated, and it is not a single enzyme that can achieve good degradation effects. Therefore, we must not only explore its mechanism of action, but also select strains more suitable for degrading pomace, and further explore the synergistic effect between Trichoderma spp.

3. Experiments

3.1. Research Materials

(1) Test strain

Trichoderma E11

(2) Liquid fermentation broth

PD (Potato Glucose Culture Solution): 20% potato dip, 2% glucose, deionized water 1000mL;

PS (potato sucrose culture solution): 20% potato dip, 2% sucrose, deionized water 1000mL;

CD (Carrot and Glucose Culture Solution): 20% carrot juice, 2% glucose, deionized water 1000mL;

BD (Banana Glucose Culture Solution): 20% banana juice, 2% glucose, deionized water 1000mL;

Culture medium A: glucose 10.0g, peptone 5.0g, K2HPO 41.00g, MgSO4.7H2O 0.50g, deionized water 1000mL; CM: sucrose 30.00g, NaNO 32.00g, K2HPO4 1.00g, KCl 0.50g, MgSO4.7H2O 0.50g , 1000mL of deionized water.

(3) Medicines and reagents

Carbon sources include maltose, sucrose, glucose, D-fructose, α-lactose, soluble starch, D-mannitol, nitrogen sources include sodium nitrate, ammonium nitrate, ammonium sulfate, urea, ammonium dihydrogen phosphate, potassium dihydrogen phosphate, glycine , Yeast extract, beef extract, peptone, acid salt, sodium hydroxide.

3.2. Research Methods

(1) Determine the effect of different liquid fermentation broths on the mycelium output of Trichoderma E11 strain

Place the Trichoderma E11 strain in a PDA plate medium at 28 ℃ and expand the culture at a constant temperature for 3 days. Rinse the spores with deionized water to prepare a spore solution (1.5 × 106 / mL) for use. 6 different liquid fermentation broths (autoclaved at 121 ° C for 25min), inoculated with 1mL of the above prepared spore suspension at the same time, cultured at 28 ° C, alternating light and dark, 180r / min shaker for 7d, then vacuum filtered mycelium The body is dried in a 60 °C oven to constant weight and weighed.

(2) Effects of carbon and nitrogen sources on the mycelium production of Trichoderma E11 strain

Using PD as the basic medium, use equal mass fractions of carbon (maltose, sucrose, glucose, D-fructose, α-lactose, soluble starch, D-mannitol) and nitrogen (sodium nitrate, ammonium nitrate, ammonium sulfate, urea , Ammonium dihydrogen phosphate, potassium dihydrogen phosphate, glycine, yeast extract, beef extract, peptone) replace sucrose and sodium nitrate, at the same time inoculate 1mL of the above prepared spore suspension, shaking at 28 ℃, alternating light and dark, 180r / min The bed was cultured for 7 days, then the mycelium was vacuum filtered, dried in a 60 ° C oven to constant weight, and weighed.

(3) The effect of pH on the mycelium production of Trichoderma E11 strain

Using PD as the basic medium, adjust the pH to 3, 4, 5, 6, 7, 8, 9, and 10 with 0.1% hydrochloric acid and 0.1% sodium hydroxide, and inoculate 1mL of the above prepared spore suspension at the same time. 28 ° C, alternating light and dark, 180r ／ min shaker culture for 7d, and then vacuum filtration mycelium, oven drying at 60 ° C to constant weight, weighing.

(4) The effect of different inoculation doses on the mycelium output of Trichoderma E11 strain

Using PD as the basic medium, inoculate 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mLP3.9 Trichoderma spore solution, and inoculate 1mL of the above prepared spore suspension at 28 ℃, alternating light and dark, 180r ／ min. After shaking culture for 7 days, the mycelium was vacuum filtered, dried in a 60 ° C oven to constant weight, and weighed.

(5) Effect of fermentation time on the mycelium production of Trichoderma E11 strain

Using culture medium A as fermentation broth, take 0.8ml of the spore suspension with the adjusted concentration, connect it to a triangular flask containing 50ml of culture medium, and shake and culture on a shaker with a temperature of 25 ℃ and a rotation speed of 180r / min. 1-7d. Then the mycelium is vacuum filtered, dried to constant weight, and weighed.

(6) Effect of fermentation temperature on the mycelium output of Trichoderma E11 strain

Using culture broth A as fermentation broth, take 0.8ml of the spore suspension with the adjusted concentration, and insert it into a triangular flask containing 50ml of culture broth at a temperature of 10, 15, 20, 25, and 30 °C, respectively. Cultivate with shaking at 180r / min for 3d, then vacuum filter the mycelium, dry to constant weight. Weigh.

3.3. Data Sources

The data used in this article comes from China Agricultural Science, Institute of Land and Natural Resources, Forestry Science and Technology, "China Land Press", "Journal of Mycology", "Chinese Journal of Oil Crops", "Chinese Journal of Biological Control", "Journal of Plant Protection" "," Chinese Agricultural Science Bulletin ".

4. Discussion

4.1. Analysis of the Effect of Different Liquid Fermentation Broths on the Mycelial Yield of Trichoderma E11 Strain

The effect of different liquid fermentation broths on the mycelium production of Trichoderma E11 strain is shown in Table 1.

Table 1. Effect of different liquid fermentation medium on mycelial yield of Trichoderma E11

Culture medium	Dry weight of mycelium (mg / ml)	Culture medium	Dry weight of mycelium (mg / ml)
PD	3.0aA	BD	0.4dD
CD	1.5bB	Culture medium A	0.2eE
PS	0.5cC	CM	0.1fF

It can be seen from Table 1 that the E11 strain can grow in different culture solutions, but its

mycelial output is significantly different; among them, the potato mycelium culture medium has the largest dry weight of mycelium, which is 3.0 (mg / ml), indicating that potato glucose culture solution The most suitable for the growth of the mycelium, the dry weight of the rest of the culture medium is CD> PS> BD> culture medium A> CM.

4.2.Analysis of the Effect of Different Carbon and Nitrogen Sources on the Mycelial Yield of Trichoderma E11 Strain

(1) Analysis of the effect of different carbon sources on the mycelium production of Trichoderma E11 strain

The effect of different carbon sources on the mycelium production of Trichoderma E11 strain is shown in Figure 1.

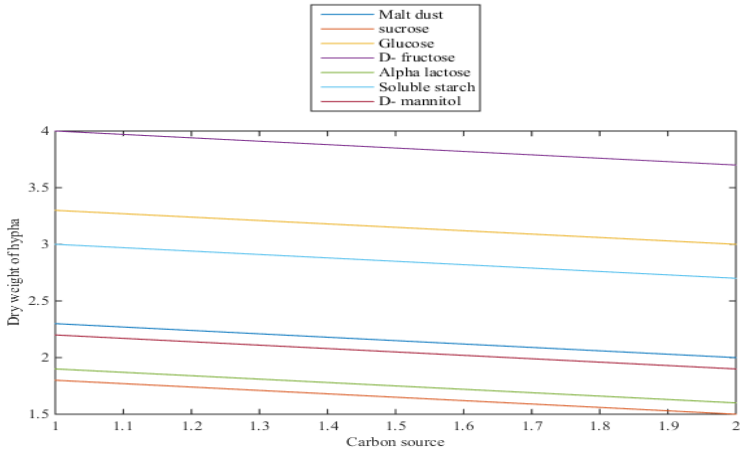

Figure 1.Effect of different carbon sources on mycelial yield of Trichoderma E11

It can be seen from Figure 1 that E11 strain can grow in different carbon source culture solutions, but its mycelial output is significantly different; among them, the dry weight of mycelium in D-fructose culture solution is the largest, which is 4.0 (mg / ml), indicating that D -Fructose is the most suitable for its mycelial growth. The dry weight of mycelium in the remaining carbon sources is glucose> soluble starch> D-mannitol> maltose> α-lactose> sucrose.

(2) Analysis of the effect of different nitrogen sources on the mycelium production of Trichoderma E11 strain

The effect of different nitrogen sources on the mycelium production of Trichoderma E11 strain is shown in Table 2.

Table 2. Effect of different nitrogen sources on mycelial yield of Trichoderma E11

Nitrogen source	Dry weight of mycelium (mg / ml)	Nitrogen source	Dry weight of mycelium (mg / ml)
Sodium nitrate	0.5dD	Potassium dihydrogen phosphate	0.5dD
Ammonium nitrate	0.5dD	Glycine	0.5dD
Ammonium sulphate	0.7bB	Yeast extract	0.7bB
Urea	0.3fF	Beef paste	0.6cC
Ammonium dihydrogen phosphate	0.8aA	Peptone	0.4eE

It can be seen from Table 2 that the E11 strain can grow in different nitrogen source culture solutions. Among them, the mycelium output in ammonium dihydrogen phosphate is 0.8 (mg / ml), which is significantly different from other nitrogen sources. The dry weight of the mycelium is the largest, indicating that Ammonium dihydrogen phosphate is the most suitable for its mycelial

growth. The difference in mycelium output between ammonium sulfate and yeast extract is not significant, both are 0.7 (mg / ml). The difference in mycelium output between sodium nitrate, ammonium nitrate, potassium dihydrogen phosphate and glycine. Not significant, all are 0.5 (mg / ml). The difference between beef extract, peptone and urea and other nitrogen sources is extremely significant. Among them, the dry weight of mycelium in urea is the smallest, indicating that urea is the most unsuitable for its mycelial growth.

4.3. Analysis of the Effect of Different pH on the Mycelium Output of Trichoderma E11 Strain

The effect of different pH on the mycelium production of Trichoderma E11 strain is shown in Table 3.

Table 3. Effect of different pH on mycelial yield of Trichoderma E11

PH	Dry weight of mycelium (mg / ml)	PH	Dry weight of mycelium (mg / ml)
3	4.3eE	7	5.0aA
4	4.6dD	8	3.9fF
5	4.7cC	9	3.7gG
6	5.2bB	10	2.1hH

It can be seen from Table 3 that the E11 strain can grow in different pHs, but its mycelial yield is significantly different. Among them, when the PH value is 6, the dry weight of the mycelium is the largest at 5.2 (mg / ml), and the dry weight of the remaining mycelium is shown as pH7> pH5> pH4> pH3> pH8> pH9> pH10. Its hyphae grow, followed by pH7 and pH5.

4.4. Analysis of the Effect of Different Inoculation Doses on the Mycelium Output of Trichoderma E11 Strain

The effect of different inoculation doses on the mycelium production of Trichoderma E11 strain is shown in Figure 2.

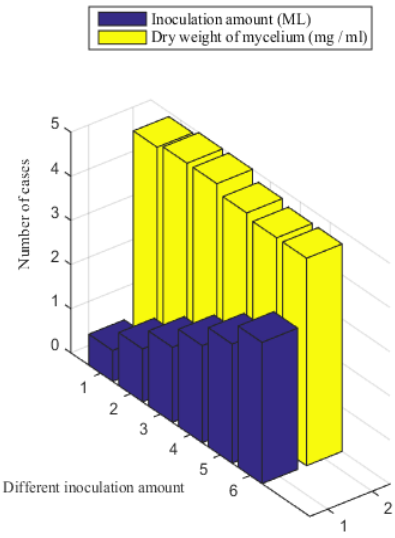

Figure 2. Effect of different inoculation amount on mycelial yield of Trichoderma E11

It can be seen from Figure 2 that when the inoculation volume of E11 strain is 1.2 and 1.7mL, 2.7 and 3.2mL, the difference in mycelial output between the two pairs is not significant, and the

inoculation volume is 0.7mL and 2.2mL and the difference in the mycelial output in the remaining inoculation volume Significantly, the dry weight of mycelium showed 1.0mL = 1.5mL> 0.5mL> 2.0mL> 2.5mL = 3.0mL. It showed that the inoculation volume of 1.2mL (1.2%) or 1.7mL (1.7%) was the most suitable for its mycelial growth, followed by the inoculation volume of 0.7mL (0.7%).

4.5. Analysis of the Effect of Different Fermentation Time on the Mycelium Production of Trichoderma E11 Strain

The effect of different fermentation time on the mycelium production of Trichoderma E11 strain is shown in Figure 3.

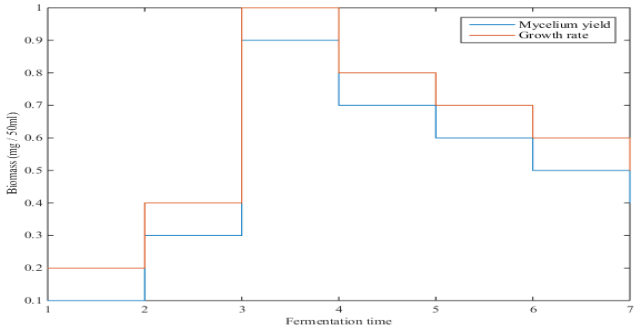

Figure 3. Effect of different fermentation time on mycelial yield of Trichoderma E11

It can be seen from Fig. 3 that as time goes on, its production gradually rises. By the third day, the mycelium production increased significantly and quickly reached the maximum value, which was 890mg / 50ml; after that, the mycelium production started with time. Decreased, but at a slow rate; in addition, from day 4 on, particulate matter was clearly visible in the vacuum suction filter. Microscopic examination revealed spores of strain E11, indicating that day 3 was the production of Trichoderma mycelium. The best time out.

4.6. Analysis of the Effect of Different Fermentation Temperatures on the Mycelium Yield of Trichoderma E11 Strain

The effect of different fermentation temperatures on the mycelium production of Trichoderma E11 strain is shown in Figure 4.

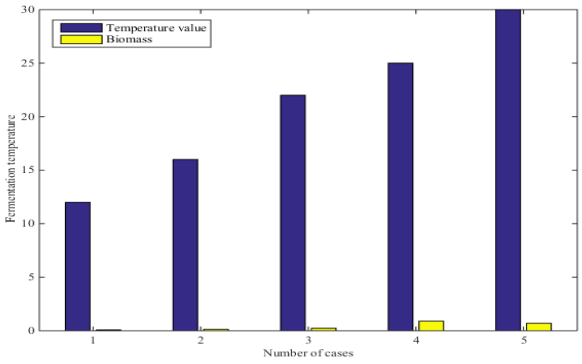

Figure 4. Effect of different fermentation temperature on mycelial yield of Trichoderma E11

It can be seen from Fig. 4 that the mycelium biomass of Trichoderma gradually increases with increasing temperature. When the temperature is lower than 20 °C, the strain grows slowly or

hardly, and the measured mycelium production is also low. Only 28mg / 50ml; when the temperature reaches 25 ℃, the strain is most suitable for growth, the mycelium production reaches the maximum value, 556mg / 50nd; the temperature continues to increase, the mycelium production decreases instead, indicating that the temperature is too high or too high Low will hinder the growth of Trichoderma, the optimal fermentation temperature of E11 strain is 25 ℃.

5. Conclusion

Trichoderma is one of the important biocontrol bacteria. It can repair heavy metal pollution and pesticide pollution in soil. It has the functions of phosphorus, potassium and nitrogen fixation. It has high application value in controlling plant diseases and insect pests. In addition, it can promote plants Grow. Although Trichoderma has obvious advantages and is widely used, it is easily affected by the external environment, resulting in limited viability and colonization ability, and unstable biocontrol effects. At present, the main problems of the Trichoderma research are as follows: the field test effect is unstable; there is no clear standard for the dosage control of the Trichoderma preparation. In view of the problems in the application of Trichoderma, we should focus on strengthening the following aspects of research: improving Trichoderma strains and screening out high-efficiency strains; exploring the combined effect of Trichoderma and other microorganisms; research on Trichoderma biocontrol genes In this paper, the best combination of liquid fermentation broth, carbon source, nitrogen source, pH value, inoculation amount, fermentation time and fermentation temperature was determined by using Trichoderma E11 strain as the test strain and the mycelium growth index as the index.

Trichoderma E11 strain grows well under liquid culture conditions, and the mycelial biological yield is closely related to the culture conditions, especially affected by the type of carbon source, nitrogen source and fermentation temperature. Therefore, choosing the appropriate fermentation conditions is sufficient Quantitative fermentation of biological output is of great significance. The research in this paper shows that the dry weight of mycelium is the largest in D-fructose culture fluid, which is 4.0 (mg / ml), indicating that D-fructose is the most suitable for mycelial growth; the mycelium production of ammonium dihydrogen phosphate is 0.8 (mg / ml), Compared with other nitrogen sources, the mycelium has the largest dry weight, indicating that ammonium dihydrogen phosphate is the most suitable for mycelial growth. Among them, urea has the smallest dry weight of mycelium, indicating that urea is the most unsuitable for mycelial growth; when the pH value is 6, , The dry weight of mycelium is the largest, 5.2 (mg / ml), indicating that pH6 is the most suitable for mycelial growth; when the inoculation volume is 0.7mL and 2.2mL, the mycelium yield is significantly different from the rest of the mycelium yield, the dry weight of the mycelium 1.0mL = 1.5mL> 0.5mL> 2.0mL> 2.5mL = 3.0mL, indicating that the inoculation volume of 1.2mL (1.2%) or 1.7mL (1.7%) is the most suitable for mycelial growth, followed by the inoculation volume of 0.7mL (0.7%)); With the extension of time, its production gradually increased. By the third day, the mycelium production increased significantly, and quickly reached the maximum value of 890mg / 50m, indicating that the third day is the best period for the production of Trichoderma mycelium; When the temperature is lower than 20 ℃, the strain grows slowly or hardly, and the measured mycelium production is also low, only 28mg / 50ml; when the temperature is When the temperature reaches 25 ℃, the strain is most suitable for growth. The mycelium output reaches the maximum value of 556mg / 50nd. When the temperature continues to increase, the mycelium output decreases, indicating that too high or too low temperature will hinder the growth of Trichoderma, E11 The optimum fermentation temperature of the strain is 25 ℃.

The research in this paper shows that with D-fructose as the carbon source, ammonium dihydrogen phosphate as the nitrogen source, fermentation temperature of 25 ℃, initial pH of the

culture medium 6, inoculation volume of 1.2mL or 1.7ml, the highest mycelium yield. Trichoderma is an important antagonistic fungus for controlling soil-borne diseases of crops. Related studies have found that endophytic Trichoderma is present in plants. Trichoderma is successfully isolated from cocoa, tea, reed, aloe, yew and other plants; from reed and The endophytic Trichoderma yew isolated from Taxus chinensis has good control effects on tobacco brown spot disease and rice leaf sheath, respectively. The study also found that Trichoderma strains isolated from other places can be colonized on cocoa stems and form with cocoa stems the symbiotic relationship eventually becomes the endophytic fungus of the host. In recent years, the use of host endophytic Trichoderma to control host plant diseases has become a hot research topic in green control, such as tea endophytic Trichoderma for tea spot disease, cucumber endophytic Trichoderma for cucumber wilt, banana endophytic Trichoderma for control Banana wilt and so on.

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Data Availability

Data sharing is not applicable to this article as no new data were created or analysed in this study.

Conflict of Interest

The author states that this article has no conflict of interest.

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