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The effect of composite Actinomycete fertilizer for disease prevention and control of panax notoginseng

Yi Jiang [1*], Yong Wang [2], Yong Li [3], Jianzhong Yang [2], Chenglin Jiang[1], Aoluo Zhang [3]

[1]Yunnan Institute of Microbiology, Yunnan University, 650091 Kunming, Yunnan, P. R. China.
[2]Sanqi Institute of Wenshan, Wenshan College, 663000 Wenshan, Yunnan, P. R. China.
[3]Yunnan Branch, Chinese Academy of Science and Technology Development，650228 Kunming, Yunnan, P. R. China.
*Corresponding author: *jiangyi@ynu.edu.cn* Tel:+86-871-65034073

Abstract

In order to develop an effective and non-poisonous bacterial fertilizer for disease prevention and control of Panax notoginseng (Sanqi), 10300 strains of actinomycetes from soils with Sanqi planting yield with serious diseases and from animal feces were screened by using various pathogens Sanqi. Five strains of them with high and broad-spectrum anti-microbial activities were selected and prepared to the composite actinomycete fertilizer (CAF). Several tests in Sanqi continuous farming field in Wenshan were carried out by using 20 to 35kg/Chinese mu. The test results shown that the survival Sanqi plants were more average 94.69% in plot using 20kg CAF, but only 69.33% in positive control plot. The yield in field using 15, 25 and 35kg/mu CAF were 4.3, 4.7, and 5.0kg/6m^2 respectively, but the positive control was 3.9kg, and increased 11, 21 and 30 % yield respectively. Comparison with chemical pesticides even single agricultural antibiotic, composite actinomycete fertilizer had many advantages for disease (especially soil-borne disease) prevention and control of perennial crops. The CAF can be developed into an effective and non-poisonous bacterial fertilizer for disease prevention and control of Sanqi.

Keywords: Panax notoginseng disease; Actinomycetes; Fertilizer.

1. Introduction

Panax notoginseng (Chinese named “Sanqi”) belongs to Family *Araliaceae,* is one of famous and valuable Chinese herbal medicines, and one of big and specific living resources in Wenshan, Yunnan. Wenshan is a main production area of Sanqi. Planting area of Sanqi in Wenshan was 55,000 Chinese mu, Sanqi yield was 1500 ton a year, and 90 % of total yield in whole China. The gross output value of industry and agriculture of Sanqi in Wenshan was up to ten billion Yuan (RMB) a year, and had become one of genuine pillar industries in Wenshan.

Disease (especially soil-borne disease) is one of ritical problem in Sanqi cultivation [1-5]. Various diseases easily produce because of planting way of Sanqi in sunshelter and continuous cropping, therefore fast growth of pathogenic microbes. Better way is planting in new field. But the “New yield” is not easy to find Wenshan, and the cultivation cost will also increase following land exchange.

Now using pesticides for control of Sanqi diseases are about several treatments, such as mancozeb, carbendazim, triadimefon, thiophanate, pyrimethanil and pencycurn etc. Using of these pesticides mad severe environmental pollution and residue in Sanqi root and soil, and reduced the quality of Sanqi, even lead to Sanqi failure. So development of safe and non-poisonous bio-pesticides is important and urgent task for Sanqi cultivation.

Actinomycetes (Actinobacteria) are one kind of microbes producing most antibiotics [6-7]. Antibiotics as pesticide and fungicide play a major role in agriculture and animal husbandry all over the world [8-10]. But use of single antibiotic will make the pathogens to produce the resistance easily. In our view, composite fertilizer consisting of several species of actinomycetes with high and broad spectrum ant-microbial activities should be used for disease prevention and control of Sanqi. It should be effect, safe and friendly to environment. And the pathogens do not produce the resistance to the composite fertilizer. Some related results of development on the composite actinomycete fertilizer for the objective are report here.

2. Materials and Methods

2.1 Actinomycete strains

Total 10300 purified actinomycete strains were isolated. 8875 strains of them from soil samples collected from Qinghai, Sichuan and Guizhou, especially Sanqi field with serious diseases in Wenshan, Yunnan, China, and 1425 strains from 42 species of animal feces. Anti-microbial activities of these actinomycete strains were determined by using plate inhibitor zone method with 16 species of pathogens of Sanqi and other crops and 6 species of representative microbes. 970 of the 10300 strains have inhibition to one to several pathogens. Final 5 strains T004，T005，T009，T011 and T019）of *Streptomyces* were selected based on the anti-microbial activities, growth, and sporulation of the strains going through primary and repeat screen, and comparing repeatedly (Table 1).

2.2 Fermentation and preparation of composite Actinomycete fertilizer (CAF)

The 5 selected strains were fermented in YIM 61 broth (Soybean meal 20g；peptone 2g；glucose 20g; starch 5g；yeast extract 2g；NaCl 4g; K_2HPO_4 0.5g; $MgSO_4 \cdot 7H_2O$ 0.5g; $CaCO_3$ 2g. pH 7.8） on a shaker (220/rpm) at 28° C for 7 day. The equal volume ferments of 5 trains were mixed with 10 times of brown coal, and became “the composite actinomycete fertilizer (CAF)”.

3. Results

3.1 Similar species of 5 Streptomyces strains and antimicrobial spectrum

5 strains were identified by using international commonly procedures [10, 11]. Their similar species with known species is showed in Table 1.

Table 1. Similar species of 5 strains Actinomycetes

YIM No.	Sources	Similar species	Similarity %
T004	Sanqi serious disease yield (soil)	*Streptomyces griseus*	100
T005	*Elephas maximus* feces	*Streptomyces violascens*	99.427
T009	*Elaphurus davidianus* feces	*Streptomyces rutgersensis*	99.727
T011	*Rhinopithecus roxellanae* feces	*Streptomyces koyangensis*	99.839
T019	Sanqi serious disease yield (soil)	*Streptomyces psammoticus*	99.707

The anti-microbial spectrum of the 5 actinomycete strains is show in Table 2. It shows that the 5 strain had higher and broad spectrum anti-microbial activities to mostly pathogens tested.

Table 2. Anti-microbial spectrum of 5 strain actinomycetes (*inhibition zone diameter mm)*

YIM No.	1*	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
T004	28	21	11	30	-	10	14	12	10	-	14	24	-	26	-	36	18	24	18	11	30	16
T005	22	20	26	42	20	18	10	-	42	26	26	-	-	-	-	36	24	25	30	20	26	24
T009	24	10	35	8	-	32	32	40	36	38	30	34	26	-	46	-	13	-	15	10	35	17
T011	12	12	24	-	-	42	22	40	46	20	56	52	-	-	-	-	28	-	30	-	24	13
T019	28	-	23	-	-	-	14	-	32	16	22	22	16	-	-	-	21	-	16	-	23	17

*1.*Rhizoctonia solani* from Sanqi；2. *Colletotrichum gloeosporiodes* from Sanqi；3. *Fusarium solani* from Sanqi；4. *Erwinia carotovora* subsp. *carotovora* strains 1; 5. *Erwinia carotovora* subsp．*carotovora* strains 2; 6. *Fusarium solani* from *Solanum melongena*; 7. *Alternaria alternata*; 8. *Fusarium moniliforme*）; 9. *Glomerella cingulata*; 10. *Gonmatopyricularia am-omi*); 11. *Polyscytalum pustulans*; 12. *Phytophthora nicotianae*; 13. *Tolypocladium inflatum*）; 14. *Mycocentrospora acerina*; 15. *Rhizoctonia solani*; 16. *Piricularia oryzae*; 17. *Bacillus subtilis* subsp. *Subtilis*; 18. *Staphylococcus aureus* subsp. *aureus*; 19. *Escherichia coli*); 20. *Enterococcus faecium*; 21. *Candida albicans*; 22. *Aspergillus niger*.

3.2 Effect of CAF for disease prevention and control of Sanqi

3.2.1 Primary field test

Test place: Field of continuous cropping Sanqi in Experimental area of Wenshan Sanqi Institute Test acreage: 5 m^2 for each treatment

Test treatment:

1=CAF 3.3kg/ 666m^2 (Chinese mu);
2=CAF 6.7kg/mu;
3=CAF 10kg/mu
4=positive control (oxadixyl mancozeb 0.5kg/mu+ 40 % Benzimidazole 120g/mu)
Test repeat: 3 times for each treatment

Fertilizing method: CAF was applied in the germchit hole, and mixed with soil when transplant Test duration: transplant at January 8; Observe result at August 26, 2014

Results of the primary field test are show in Table 3. The results indicate that 10.kg CAF/mu can increase percentage of seedling emergence for 15% (average 120 plants for positive control versus 141 for 10kg CAF), and the incidence percentage reduced 25.6% from 90.74% to 5.09%. But it is apparent that using amount of CAF was too low. Therefore 3.3kg and 6.7kg/mu were not effect for control Sanqi disease.

Table 3. Control effects of CAF on disease of Sanqi (Amount of plants/m2)

Treatment	Test plot I			Test plot II			Test plot III			Average incidence%
	Amount* of plant	Amount of disease	Incidence %	Amount of plant	Amount of disease	Incidence %	Amount of plant	Amount of disease	Incidence %	
3.3	95	95	100.00	83	74	89.16	93	77	82.80	90.65
6.7	84	67	79.76	88	83	94.32	81	71	87.65	87.24
10.0	136	93	68.38	144	89	61.81	142	94	65.10	65.09
Positive CK	111	96	86.49	116	110	94.83	132	120	90.91	90.74

3.2.2 Control effect of different amount CAF

Test place: Field of continuous cropping Sanqi in Experimental area of Wenshan Sanqi Institute

Test acreage: 220 m^2 for each repeat Test object: second germ chit

Test treatment: 1=CAF 15kg/ 666m^2 (Chinese mu); 2=CAF 25kg/mu; 3=CAF 35kg/mu; 4=positive control (oxadixyl mancozeb 0.5kg/mu+ 40 % Benzimidazole 120g/mu)

Test repeat: 6 times for each treatment

Fertilizing method: CAF as base fertilizer was applied in the germchit hole, and mixed with soil, when transplant; Topdressing once at May 20

Test duration: fertilization at Jan 10; Topdressing at May 20; Observe result at August 26, 2014

Influence of different amount CAF to survival rate Sanqi plant is showed in Table 4. Survival plants in 15, 25 and 35kg CAF were 111, 121, 130% of positive control respectively, up to prominence based on the Statistics using DPS (Data Processing System). It must be pointed that comparison with pesticides of 18 units from home and abroad in the same test field, effective of CAF was the best. Growth vigour of plant using CAF was robust.

Table 4. Survival rate of different amount CAF (Amount of survival plants/6m2)

Treatment	Six repeats of test plot (survival plant amount)						Average	%	Relative prominence	
	Ⅰ	Ⅱ	Ⅲ	Ⅳ	Ⅴ	Ⅵ			prominence at 5%	prominence at 1%
15kg/mu	233	363	215	356	246	311	287	111	Ab	A
25kg/mu	283	271	389	291	283	363	313	121	Ab	A
35kg/mu	281	411	297	333	349	343	336	130	A	A
Positive CK	332	314	247	236	213	204	258	100	B	A

3.2.3 Control effect of 20kg/mu CAF--Test 2

Test place: Field of continuous cropping Sanqi in Pingba village

Test acreage: 620 m^2

Test treatment: CAF 20kg 666m^2 (Chinese mu); Positive control (oxadixyl mancozeb 0.5kg/mu+ 40 % Benzimidazole 120g/mu)

CAF using method and duration: CAF was used in seeding hole, and mixed with soil at Jan 8; Observe result at August 18, 2014

The results on the yield test-2 of 620 in a yield of continuous cropping Sanqi in Pingba village are showed in Table 5 and Fig. 1-1, 1-2. The survival plants in 6 sampling points were average 162 (94.69%). The positive control was only 119 (69.33%). In general, the expectancy value is >90%. Main root of the plants using 20kg/mu CAF was thicker, fibre was more abundant, colour of leaf was deep green, and plant was higher and robust.

Table 5 Efficacy of 20kg/mu CAF on seedling survival rate (plants/m2)

Test plot		**Planting amount**	**Survival plant amount**	**Survival plant %**
Test plot	1	166	161	96.99
	2	168	161	95.83
	3	174	171	98.28
	4	182	177	97.25
	5	172	157	91.28
	6	165	146	88.48
Average		173	162	**94.69**
Positive CK	1	165	116	70.30
	2	174	85	48.85
	3	166	158	95.18
	4	181	114	62.98
Average		172	119	**69.33**

Fig. 1-1 CAF 20kg/mu

Fig. 1-2 positive CK

Another test was carried out in a serious disease yield (180m^2). The germchits with pathogens were transplanted in the yield. 20kg/mu CAF were used when transplant at Jan 10, 2014. Test result was observed at August 28. 118 plants/m^2 were survival in 20kg/mu CAF hole. But only 27 plants in positive control hole. The former is 4.4 times of the letter.

These tests indicate that 20kg/mu CAF was effective for disease prevention and control of Sanqi, can reduce markedly incidence of diseases.

3.2.4 Influence of different amount CAF to Sanqi yield

Test content is the same with 2.2.2, except more one time of opdressing 20kg/mu CAF at August 20. Yield of Sanqi root was determined at January 10, 2015 (Table 6). The results indicate that using 15, 25 and 35kg /mu of CAF can increase 11, 21 and 30% of Sanqi root yield based on DPS, and up to prominence level. It is interest, these percentage are the same with the survival plant percentage. It is entirely coincidence.

Table 5 Effects of different use level of CAF on yield of Sanqi root

Treatment	Test plot (repeat)（Fresh weight kg/6m2）						Average %	relative prominence		
	I	II	III	IV	V	VI		5%	%	1%
15kg/mu	3.5	5.4	3.2	5.3	3.7	4.7	**4.3000**	Ab	**111**	A
25kg/mu	4.2	4.1	5.8	4.4	4.2	5.4	**4.6833**	Ab	**121**	A
35kg/mu	4.2	6.2	4.5	5.0	5.2	5.1	**5.0333**	A	**130**	A
Positive CK	5.0	4.7	3.7	3.5	3.2	3.1	**3.8667**	B	**100**	A

4. Discussion

In order to analyze the toxicity of the actinomycete strains, each group of 20 mice were fed with the fermentation broth with 36^8/ml viable actinomycetes of T005 (from *Elephas maximus* feces) and T019 (Sanqi serious disease yield) respectively, weigh body weight, observed the toxic reaction after feeding 3, 7, 14 days, and dissected the internal organs of the mice after 14 days. The results shown, the two actinomycete strains were totally nontoxic to mice.

Bioactive metabolites of 3 strains consisted of CAF were analyzed. 47 compounds were isolated and identified from T005, and 13 compounds of them were new. 22 compounds from T009, and 2 were new. 17 compounds from T019, and 3 were new. Known Geldanamycins, Adenosines, Cyclopeptides, Benzenedicarboxylic acids, Nonadecanoic acids, Heptadecanoic acids, Hexopyranosides, Triethanolamines, Actinomycins, Coscomycins, and Sannastitins etc. were isolated and identified from fermentation broths of the three strains.

Survival (planting) of actinomycete strains used in soil were determined. The soil samples of fertilized CAF and positive control were collected respectively after fertilizing for 5 months. The actinomycetes and fungi in the samples were counted, isolated and identified. 242×10^5 and 52.4×10^5/g dry wt of actinomycetes and fungi were existed respectively in the yield fertilized CAF, and all of 5 strains of the CAF were recovered. But only 32.4 and 85.7×10^5/g dry wt of actinomycetes and fungi in the yield of positive control were detected. No antibiotics were detected in these soil samples.

In 2015, the same CAF was used in cultivation of grape, tobacco, wheat and Chinese yam, can reduce disease occurs, and increased the production range of the crops about 10 to 13%.

Above-mentioned research results indicate that the microbial fertilizer prepared with composite actinomycetes can produce constantly and trace (cannot be determined out) various antibiotics to inhibit the pathogens, improve the microbial ecological system, and increase the yield of Sanqi in continue crop area. 25 to 35kg/mu as base fertilizer, and topdressing twice (each time 20kg/mu) in May and August respectively were relative suitable. In our view, the CAF had some advantages as following. First, comparison with single pesticide or microbe fertilizer, CAF made the pathogens to be not easy to produce the resistance, and was amicable and safe to environment. Second, production cost of CAF was lower, and did not need expensive equipment, and had not any waste in the production process. Therefore the CAF will be developed into an effective, safe, and cheap microbial fertilizer.

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