Retraction

Retraction: Study on Molecular Qualitative Detection Technology of Potato Scab Disease (IOP Conf. Ser.: Earth Environ. Sci. 769 022010)

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This article has been retracted by the authors following correspondence with IOP Publishing in which the authors claim the work is unreliable. The author's explanation follows:

"The material of the paper is bacteria, it is not be visible to the naked eyes, the material was contaminated by the drugs that out of date and got polluted, and it caused the serious mistake in the figure 2. The result of different strains of DNA by the primer in 3.3 was not match of our material. The figure 2 was the amplification results of different strains of DNA, and the fragment size of different strains were same in the result, but the result should not be same, so the result was wrong. When we continue to the study, we got new result about the figure 2. There was the amplification results of different strains of DNA by primer TXT in repeated experiment, and the result in the published article was wrong, the material was contaminated. We are afraid that other results were also incorrect in this paper."

IOP Publishing cannot verify this information as accurate, however in the interest of transparency and reproducibility, IOP Publishing agrees to retract this article. This notice will be updated if more information comes to light.

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Study on Molecular Qualitative Detection Technology of Potato Scab Disease

Wenjuan Chi¹,², Yinan Liu¹,*, Wenyu Liu¹ and Chenglong Li²

¹College of Life Engineering, Shenyang Institute of Technology, Fushun, 113122 China
²Shandong Provincial University Laboratory for Protected Horticulture, Weifang University of Science and Technology, Shouguang, 262700

*Corresponding author: yinanliu@situ.edu.cn

Abstract. The paper uses a method that combines biological characteristics and 16S rDNA sequence characteristics to analyze potato scab bacteria collected from China. The study found that the obtained universal detection primers B1/B2 showed good specificity for all pathogenic strains, and could stably expand the target band, while non-pathogenic strains had no bands. The paper uses the spore dilution method to verify that the established detection method has a sensitivity of 20 pg·μL⁻¹ for strain DNA, and the detection threshold for spores is about 4.0 CFU/μL. The genome amplification results of the test samples showed that the target bands were detected in the scab pathogen Streptomyces, diseased potato sample tissues, and diseased soil samples, but not the scab pathogen Streptomyces, healthy tuber tissues, non-sick soil samples and other strains. None of the bands of interest were expanded, indicating that the method has good specificity. Therefore, we can conclude that the qualitative detection method of potato scab bacteria can realize rapid detection of strains, diseased tissues and soil samples.

Keywords: Potato scab disease, molecular qualitative detection technology, specific primer.

1. Introduction
Potato scab disease is caused by Streptomyces spp. It is a widespread soil-borne disease worldwide. Affected by environmental and management factors, the occurrence of scab disease has become increasingly serious and has risen to the fourth major potato disease. Potato scab disease can produce small brown spots on the surface of potato tubers at the early stage of onset. In the later stage, the cell tissue of the diseased part is corked, the epidermis is rough, and gradually expands to form nearly round or irregular convex, concave or flat lesions, resulting in the appearance of the tuber. Decrease and increase in the proportion of small potatoes not only lead to a decrease in potato tuber starch content, but also affect the processing quality of potatoes and reduce their market competitiveness. A variety of pathogens have been reported to cause potato scab, including Streptomyces scabies, S. acidiscabies and S turgidiscabies, etc., and some new pathogenic species of Streptomyces have been discovered. For diseases caused by such a variety of pathogens, analyzing the biological
characteristics of each pathogen can provide information on the causes and pathogenic mechanisms of
different pathogens, and lay a foundation for in-depth research on diseases [1]. In order to understand
the types and distribution of the pathogenic bacteria of the disease, this article classified and identified
potato scab bacteria collected from different regions in China, in order to find out the pathogen
causing potato scab disease in China.

2. Materials and methods

2.1. Materials

2.1.1. The tested disease samples and potatoes. From January 2018 to October 2019, 29 samples of
diseased potato pieces with typical scab symptoms and 8 samples of soil from diseased plots were
collected and brought back to the laboratory for future use. The potato variety tested was Favorita, and
its virus-free test-tube seedlings came from the Key Laboratory of Agricultural Biotechnology [2].
After the virus-free test-tube seedlings were planted in pots, when the seedlings grew to about 10 cm,
plants with the same growth were selected as the inoculation materials.

2.1.2. Main instruments and reagents. KYKY-2800 scanning electron microscope, SCB-12 ion
sputtering instrument. Yeast extract, Tryptone and Peptone were purchased from Oxiod, and the other
reagents were domestically pure.

2.2. Observation of morphological characteristics
Test strains were inoculated on oat solid medium (OMA), cultured on the insert at 28°C for 5 days,
then the glass insert was pulled out, fixed with 2.5% glutaraldehyde for 1.5h, washed with phosphate
buffer for 15-20min, and repeated 3 times. Then fix with 1% osmium acid at 4°C for 2h, wash with
phosphate buffer for 15-20min, repeat 3 times. Then ethanol gradient dehydration 50% - 75% - 85% -
95% - 100% (1) - 100% (2) 10 minutes each, after drying, ion sputtering gold plating. Observe the
morphological characteristics of the spores and spores of the colony under a scanning electron
microscope. At the same time, take the colonies cultured for 15 days and observe the colony colour
and pigment production under an optical microscope.

2.3. Determination of physiological and biochemical characteristics of pathogenic bacteria
Add the test carbon sources arabinose, rhamnose, raffinose, fructose, xylose, sucrose, mannose,
maltose, and glucose to the carbon source utilization basic medium, and add them at a concentration of
1%, and cultivate on the carbon source utilization basis the base is the control. Cultivate the
pathogenic bacteria identified in 1.2.1 on various carbon source media for 7 days and observe the
growth of the strains. If the strains can grow normally and can be cultured for 3 consecutive
generations, the carbon source can be used [3]. The test nitrogen sources L-methionine, histidine, and
L-hydroxyproline were added to the nitrogen source utilization basal medium. After the basal medium
was autoclaved, filtered and sterilized amino acids were added to a final concentration of 1%, and the
nitrogen source utilization basal medium was used as a control. Cultivate the above-mentioned
pathogenic bacteria on various nitrogen source media for 7 days and observe the growth of the strain.
If the strain can grow normally and can be cultured continuously for 3 generations, it is considered that
the nitrogen source can be used. At the same time, the test strains were cultured on tyrosine agar
medium for 7 days to detect the production of melanin.

2.4. Strain genomic DNA extraction
Each Streptomyces strain was cultured in TSB liquid medium (30g trypticase soy peptone broth
dissolved in 1L of water, autoclaved) for mycelial culture, and the genome was extracted according to
the method in "Practical Streptomyces Genetics". The genomes of other strains were extracted by
CTAB (hexadecyl trimethyl ammonium bromide) method [4].
2.5. Primer design
According to the gene sequences of txtA, txtB, txtC (P450), txtD (nos) in the registered toxin synthesis gene cluster of scab bacteria registered in NCBI GenBank, primer premier5.0 software was used to design primers, and the primers were delivered to Shanghai Shenggong Bio Engineering Technology Service Co., Ltd. Synthesis.

2.6. Screening and sequence determination of specific fragments
According to the amplification results of different primers, select stable bands, recover them with a DNA recovery and purification kit, clone them into pGEM (R) - T Easy vector and transform into E. coli DH5α, pick positive clones, and extract the plasmids. Handed over to Beijing Zhongke Xilin Biotechnology Co., Ltd. for sequence determination [5]. After sequence proofreading, perform Blastn comparison analysis in GenBank to verify the fragments and select the best amplified fragments as detection probes.

3. Results
3.1. Observation results of strain morphological characteristics
Through the observation of the morphological characteristics of potato scab bacteria in different regions, it was found that the culture characteristics of different pathogens were quite different. The colonies produced spiral or flexible spore chains, the spiral spore chains were tight or loose, the spores of the strains were gray or white, and soluble pigments were produced or not [6]. The spore surfaces of all tested strains were smooth (Figure 1). Among them, the aerial hyphae of strains G4-1 (Figure 1-A) and G9 (Figure 1-B) are yellow, the intranasal hyphae are yellow-brown, and the spore size is about 11μm×5μm, smooth and cylindrical. The aerial hyphae of strain SYNT3 (Figure 1-C) are yellow, the intranasal hyphae are reddish brown, and the spore size is about 10μm×3μm, smooth and cylindrical; strain NLG4-1 (Figure 1-D) The aerial hyphae of the strain GBH1 (Figure 1-E) are yellow, the intranasal hyphae are brown, and the spore size is about 8μm×4μm, smooth and oval; the aerial hyphae of strain GBH1 (Figure 1-E) are yellow, and the intranasal hyphae it is reddish brown, with a spore size of about 8μm×5μm, not smooth and short columnar; the aerial hyphae of strain SYN13 (Figure 1-F) are white, the basal hyphae are white, and the spore size is about 10μm×6μm, smooth and cylindrical.

Figure 1. The morphological characteristics of different potato scab bacteria.

3.2. Physiological and biochemical characteristics of pathogenic bacteria
Among the 6 pathogenic strains, strains G4-1, G9 and SYN13 cannot use fructose and xylose as a single carbon source; strain SYNT3 cannot use raffinose as a single carbon source; all tested carbon sources can be used as a single carbon source [7]. The strains NLG4-1 and GBH2 are used. All three amino acids tested can be used as a single nitrogen source by the six strains. Except for strain NLG4-1, the other 5 strains can produce melanin on tyrosine agar medium (Table 1).
| Test index   | G4-1 | G9-1 | SY13 | GBH2 | NLG4-1 | SYNT3 |
|-------------|------|------|------|------|--------|-------|
| Carbon source |      |      |      |      |        |       |
| L-arabinose  | +    | +    | +    | +    | +      | +     |
| Rhamnose    | +    | +    | +    | +    | +      | +     |
| Raffinose   | +    | +    | +    | +    | +      | +     |
| D-fructose  | -    | -    | -    | -    | +      | +     |
| D-xylose    | -    | -    | -    | -    | +      | +     |
| Sucrose     | +    | +    | +    | +    | +      | +     |
| Mannose     | +    | +    | +    | +    | +      | +     |
| Maltose     | +    | +    | +    | +    | +      | +     |
| Glucose     | +    | +    | +    | +    | +      | +     |
| Nitrogen source |      |      |      |      |        |       |
| L-methionine| +    | +    | +    | +    | +      | +     |
| L-histidine | +    | +    | +    | +    | +      | +     |
| L-hydroxyproline | +    | +    | +    | +    | +      | +     |
| Tyrosine-agar| +    | +    | +    | +    | +      | +     |

### 3.3. Results of strain genome extraction and primer amplification

The OD260/280 values of the genomes extracted for each sample are all 1.8-2.0, indicating that the quality is good and can be used for downstream test operations. After comparing and analyzing the Tm values of the primers used in this study, 55°C was selected as the annealing temperature to amplify the genomes of different scab bacteria. Each strain successfully amplified A, B, and D gene fragments, and the size was consistent with the expected fragments. Primer C fragment amplification results were first excluded due to poor results (Figure 2). After the obtained fragments were recovered, cloned, sequenced, and proofread, the results of BLASTn analysis in NCBI showed that the nucleotide sequences of A and B fragments are identical to those of the registered S. turgidiscabies, S. scabies and S. acidiscabies strains in GenBank [8]. The similarity of each gene sequence is greater than 99%, which proves that the obtained fragments are all target fragments. The D fragment was found to be not the desired target fragment after sequencing, and it was also excluded.

![Figure 2](image.png)  
**Figure 2.** The amplification results of different strains of DNA by each primer.

### 3.4. Results of the lowest detection threshold of primers

In the thesis, the spore suspension of the pathogenic bacteria was gradually diluted, and then counted by the plate counting method. A plate with a relatively obvious single colony was taken for counting, and the concentration of the spore suspension of each strain to be tested was measured, and the spore amount of each sample CPS-1 is $2.5 \times 10^{10}$CFU/mL, CPS-2 is $7.0 \times 10^{10}$CFU/mL, CPS-3 is $2.2 \times 10^{10}$CFU/mL, and CPS-4 is $6.5 \times 10^{10}$CFU/mL (Figure 3). DNA was extracted from 0.2mL spore suspension of each strain, and the concentrations of the extracted genome were determined to be CPS-1: 201.7ng·μL\(^{-1}\), CPS-2: 367.7ng·μL\(^{-1}\), CPS-3: 218.0ng·μL\(^{-1}\), CPS-4: 324.6ng·μL\(^{-1}\).
4. Discussion
Potato scab is a serious soil-borne disease that has occurred in recent years. At present, there are more than 20 kinds of streptomycetes that cause potato scab in the world. Potato scab disease is mainly spread through diseased seed potatoes and soil. The inter-regional transportation of diseased seed potatoes is the main external cause leading to the wide spread of different kinds of pathogenic bacteria of scab. The pathogenicity island (PAI) of Streptomyces scab-related genes can be horizontally transferred to other species of Streptomyces to produce new pathogens, leading to the emergence of new pathogens, which may be an increase in the types of pathogens the root cause [9]. In the process of pathogen identification, there may be regional differences in various biological characteristics, so it is necessary to choose as many biological indicators as possible for systematic analysis. At present, Streptomyces scab, S. acidiscabies, S. europaeiscabiei and S. turgidiscabies are widely distributed. Streptomyces species are greatly affected by the planting environment and climatic factors. Only by clarifying the types of local scab pathogens can we formulate targeted disease prevention and control measures. The diversity of pathogenic bacteria has increased the difficulty of in-depth research and prevention of potato scab disease. Therefore, in production, it is necessary to strictly prevent the cross-regional transfer and planting of scab-infected seed potatoes, control the spread of new pathogens, and continue to develop potato scab chains in different regions. Monitoring of molds and genetic diversity studies, analysis of the common and independent characteristics and causes of formation of the Streptomyces scab population, in-depth analysis of the PAI structure of different pathogenic species, analysis of gene functions, and systematic research on the pathogenic mechanism of the disease, which is a disease Lay the theoretical foundation for prevention and control of.

5. Conclusion
Based on the cluster analysis results of the biological characteristics of potato scab bacteria in different regions of China, it was found that the pathogens were mainly composed of S. scabies, S. galilaeus, S. bobili and other populations. Comparing the results of this study with the results of the 16S rDNA sequence cluster analysis of the strains used in the previous work, it is found that there are certain differences between the two, indicating that the current identification of potato scab pathogens cannot rely solely on one method, and still need to combine the organisms of the strains. Polyphasic classification and identification of scientific characteristics and molecular genetic characteristics.

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References
[1] Kobayashi, Y. O., Kobayashi, A., Soejima, H., & Takenaka, S. Enhanced Suppressive Effect of Antagonistic Streptomycetes sp. WoRs-501 on Potato Scab in Conjunction with Other Control Methods. Japan Agricultural Research Quarterly: JARQ, 51 (3) (2017) 251-257.
[2] Karagoz, K., Dadasoglu, F., Mohammadi, P., & Kotan, R. Screening bacterial antagonists to common scab disease. JAPS, Journal of Animal and Plant Sciences, 28 (4) (2018) 1068-1073.
[3] Song, Y., Xiang, J., Shen, Y., Wang, J., Liu, Q., & Yin, K. Screening and identification of Bacillus strain BU108 and bio-control effects on potato scab. Journal of Zhejiang A&F University, 35 (4) (2018) 757-764.

[4] Yu, L., She, X., Lan, G., Tang, Y., Li, Z., Deng, M., & He, Z. Identification of pathogen causing sweet potato scab in Guangdong. Journal of Southern Agriculture, 51 (3) (2020) 579-585.

[5] Bagheri, A. Situation of Potato Common Scab Disease in Iran. University of Yasouj Journals System Plant Pathology Science, 6 (1) (2017) 47-56.

[6] Clarke, C. R., Kramer, C. G., Kotha, R. R., Wanner, L. A., Luthria, D. L., & Kramer, M. Cultivar resistance to common scab disease of potato is dependent on the pathogen species. Phytopathology, 109 (9) (2019) 1544-1554.

[7] Yang, X., Li, L., Fan, W., He, H., Tang, Z., & Tan, G. Physiological mechanism of inducers to resistance inducing of potato scab. Journal of Southern Agriculture, 49 (6) (2018) 1111-1117.

[8] Natsume, M., Tashiro, N., Doi, A., Nishi, Y., & Kawaide, H. Effects of concanamycins produced by Streptomyces scabies on lesion type of common scab of potato. Journal of General Plant Pathology, 83 (2) (2017) 78-82.

[9] Braun, S., Gevens, A., Charkowski, A., Allen, C., & Jansky, S. Potato common scab: A review of the causal pathogens, management practices, varietal resistance screening methods, and host resistance. American Journal of Potato Research, 94 (4) (2017) 283-296.