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RESEARCH ARTICLE

First report of a new potato disease caused by *Galactomyces candidum* F12 in China

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Abstract

Potato (*Solanum tuberosum* L.) is an important crop throughout the world. An uncharacterized disease has been observed on potato plants during the growing season and tubers during the storage period from Nileke County, Qitai County and other locations in Xinjiang, China. A particular fungus was consistently isolated from the infected potato plants and tubers. Based on its morphology, molecular characteristics, pathogenicity test and internal transcribed spacer (ITS) sequence, the pathogens was identified as *Galactomyces candidum* F12. Further study also showed that the hyphae and conidia of the pathogenic fungus grew faster as the temperature was 30°C, pH was 7, soluble starch was used as optimal carbon source and yeast powder as optimal nitrogen source. In addition, 12-h continuous illumination light was beneficial to the hyphal growth, while 24-h continuous illumination was beneficial to the sporulation of the strain at 30°C. To our knowledge, this is the first report of *Galactomyces candidum* causing leaf wilt and postharvest tuber rot on potato in China.

Keywords: potato, pathogenicity, growing season, postharvest, *Galactomyces candidum*, tuber rot, biological characteristics

1. Introduction

Potato (*Solanum tuberosum* L.), an annual crop belonging to the family Solanaceae, is planted in March, and harvested three months later. Because of its high nutritional value, it can be used as a staple food and a series of derivative products, and it has a high economic value. In recent years, the tubers have been threatened by diseases, affecting their yield and taste, especially diseases that seriously affect tubers after harvesting. The main diseases of potato in Xinjiang include potato black scurf (*Rhizoctonia solani*) (Wang *et al*. 2011), potato common scab (*Streptomyces acidiscabies* and *Streptomyces scabies*) (Du *et al*. 2010), and potato early blight (*Alternaria solani*) (Luo *et al*. 2018). However, the pathogenicity to the potato of *Galactomyces candidum* has not been reported. It has been isolated from the milk, stored feed, plant tissues, digestive tract of humans and other mammals. The morphological characteristics of *G. candidum* are intermediate between yeasts and molds. The original *G. candidum* was a mold, until 1983 (Barnett 1983). It is now included in the yeast group and can cause

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kimchi "raw flowers" and spoilage, tangerine rot, tomato acid rot and Brazilian red fruit rot (Rao *et al*. 2013; Yang *et al*. 2013; Chen 2015; Zhao *et al*. 2017; Zhou *et al*. 2018). In recent years, acid rot diseases of various plants, such as tomato, lemon, carrot, lychee, peach, grape, sweet potato, caused by *G*. *candidum* are difficult to control (Pritchard and Porte 1923; Wright *et al*. 1964; Mahmood 1970; Jamaluddin *et al*. 1975; Kuramoto and Yamada 1975; Wells 1977; Morris 1982; Moline 1984; Holmes and Clark 2002; Song *et al*. 2008; Cai *et al*. 2009; Yaghmour *et al*. 2012).

The disease caused by *G*. *candidum* on potato has not been reported in China until now. In this study, the pathogen was isolated and identified by treating the post-harvest potato from Nileke County of Xinjiang, China, and in order to provide a basis for future research, the biological characteristics of the pathogen were analyzed to determine its suitable growth conditions.

2. Materials and methods

2.1. Field symptoms of susceptible potatoes

The susceptible potato plants and tubers were found in Ulastai Village, Haulashai Town, Nilka County of Xinjiang in August 2018. The variety of the diseased potato was "Atlantic". The leaves were found to be yellow and wrinkled with wilted edges of the leaves (Fig. 1-A). The diseased tubers showed a large number of depressions and many black lesions, as well as cracks. Some lesions were easy to peel off (Fig. 1-B). The same variety was selected for the pathogenicity tests in the later experiments.

2.2. Isolation and purification of the pathogen

The 5 mm×5 mm skin and tuber tissue samples were cut out at the junction of the diseased site, disinfected with 75% ethanol for 5 min, rinsed with sterile water 3 times, and the surface moisture was removed by sterile dry filter paper. The tissues were placed on a sterile PDA, 3–4 pieces per plate, and incubated for 2 d at 30°C. Under sterile conditions, the white tipped hyphae that had grown around the diseased tissue were transferred to PDA with a sterile bamboo stick, and new colonies were cultured at 30°C. The above operation was repeated until pure colonies were obtained. Then the strains were cultured on a slanted surface at 30°C for 3–5 d, and stored at 4°C (Fang 2014).

2.3. The pathogenicity test

The healthy potato tubers and the small tomato surfaces were cleaned and surface-sterilized with 70% ethanol solution then washed 3 times with sterile water. Three

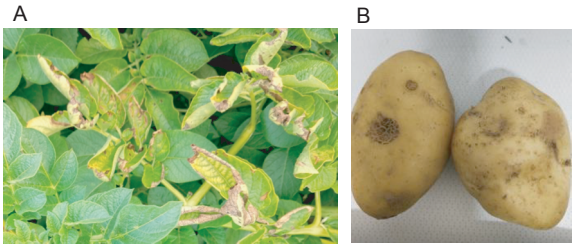

Fig. 1 Symptoms of leaves of susceptible plants (A) and tubers (B) of *Solanum tuberosum cv.* Atlantic in the field.

methods were used to test pathogenicity: i) Tuber inoculation method. The strains purified as above were tested by the acupuncture inoculation method and the drilling inoculum method to determine the pathogenicity of the fungus on potato and small tomato (Yang *et al*. 2013; Wang *et al*. 2014). ii) Radish slice and potato chip inoculation method. The strain cakes (5 mm×5 mm) were placed on the potato chips (5 cm×5 cm) and radish slices (5 cm×5 cm) in plates for 10 d at 30°C. iii) Seed inoculation method. The potato tubers were inoculated by acupuncture with the strains and were then planted in a pot, observed at regular intervals until the symptoms appeared, and then the same pathogen was isolated again. The sterile media cakes on the potato chips were used as controls.

2.4. The pathogen hyphae morphology and structure

The strains cakes were placed in the center of the PDA medium and incubated for 3–4 d in the dark at 30°C. The morphological characteristics of the pathogenic fungus were observed to determine its taxonomic identity according to the fungal identification manual (Wei 1982; Dong 2012; Liu 2014; Zhou *et al*. 2018).

2.5. DNA extraction, PCR, and sequencing of pathogenic fungus

Primers used for ITS sequence amplification were ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′), and the PCR reaction system used routine settings (Table 1). After purification and recovery, the PCR product was sent to Dingguo Changsheng (Xinjiang, China) Co. Ltd. for testing. The obtained sequences were aligned on UNITE and NCBI, and the homologous and morphologically similar strain sequences were selected. The phylogenetic tree was constructed by the Neighbor-Joining Method in MEGAV7.0. Developmental trees were tested using 1 000 replicates (White 1990; Thornton *et al*. 2010; Rashid *et al*. 2016).

Table 1 Primer design and PCR program

Item	Contents
Primer design	ITS1: 5′-TCCGTAGGTGAACCTGCGG-3′; ITS4: 5′-TCCTCCGCTTATTGATATGC-3′
Primer synthesis	Dingguo Changsheng (Xinjiang, China) Co., Ltd.
Target band	1 000 bp
PCR reaction system	2× Mix 25 μL, ITS1 (10 μmol L^{-1}) 1 μL, ITS4 (10 μmol L^{-1}) 1 μL, ddH_2O 50 μL, DNA template 1 μL
PCR program	Predenaturation at 94°C for 5 min; 30 cycles of 94°C for 30 s, 54°C for 30 s and 72°C for 1 min; and extension at 72°C for 10 min
Sequencing company	Dingguo Changsheng Co., Ltd.

2.6. Biological characteristics of the pathogenic fungus

The effects of carbon source, nitrogen source, temperature, light, and pH, on the mycelial growth and sporulation of *G. candidum* were studied (Yang *et al*. 2013; Mou *et al*. 2017).

Carbon source and nitrogen source The mycelium growth and sporulation on different carbon sources and nitrogen sources were determined by using Czapek's medium. There were five carbon sources tested, namely sucrose (Suc), maltose (Mal), soluble starch (Sol), glucose and mannitol (Glu), with no carbon source as a blank control (CK). There were five sources of nitrogen tested, namely KNO_3, $NaNO_3$, yeast powder (Yea), urea (Ure), and peptone (Pep), with no nitrogen source as a control (CK). Six treatments, three replications, and a 30°C constant temperature inversion culture were set up to study the mycelial growth and sporulation on the different carbon and nitrogen sources.

Temperature Seven different temperature treatments were used to study the mycelial growth and sporulation: 26°C, 28°C, 30°C, 32°C, 34°C, 36°C and 40°C, and each treatment had three replicates (Yang *et al*. 2013).

Light illumination To study the mycelial growth and sporulation in different light conditions, three light treatments were set, which were continuous illumination, continuous darkness and alternating 12 h light and 12 h dark separately. Each treatment had three replicates and was incubated at 30°C.

pH The ten treatments were set as follows: pH3, pH4, pH5, pH6, pH7, pH8, pH9, pH10, pH11 and pH12, at 30°C constant temperature culture, with three replicates for each treatment.

The above data were analyzed by statistical software (SPSS 17.0) for differential significance analysis (SSR), and were analyzed and mapped with Excel Software.

3. Results and discussion

3.1. Separation and purification of the pathogen

The 17 strains purified on the PDA, NA and Gause's medium were named as B1, B2, F1, F2, etc. The purified strains were transferred to the PDA slant culture, and preserved in a refrigerator at 4°C for subsequent experiments.

3.2. Pathogenicity test of the strain F12

There were easily peeled lesions and the tissue hardened inside the tuber after 15 d (Fig. 2-B) compared to the control potato, though there were no water-stained lesions on the tuber surface. Inoculation with fungus cake by drilling produced a brown big hole and grayish white hypanthium in the tuber (Fig. 2-C). The leaves of the potato plants became yellow with wrinkled black edges in the pot experiment after planting the inoculated tuber seed (Fig. 2-D). The strain F12 showed slightly rotted potato chips but no symptoms on carrot slices after 10 d (Fig. 2-E). The studies shown that *Geotrichum candidum* can cause acid rot in carrot, which is not consistent with the results of this study. The reasons may be related to the time of inoculation, the species of radish and the species of strain (Horita and Hatta 2015). Although there was no change in the surface of the tomato, black spots and softened tissues could be found in the tomato after inoculation with F12 by vertical needling with the sterile toothpick, and the strain F12 was isolated again from the softened tissue (Fig. 2-F).

Geotrichum candidum is a filamentous fungus mostly used in the dairy industry for cheese ripening and flavoring. Another study reported that *G. candidum* can cause acid rot of tangerine, tomato, Brazilian red fruit (*Eugenia uniflora* L.), lemon, grape, and litchi. The symtomptoms were internal spoilage, surface water-stained lesions, and tissue-softening (Mahmood 1970; Song *et al*. 2008; Cai *et al*. 2009; Yang *et al*. 2013; Zhao *et al*. 2017; Zhou *et al*. 2018).

Huang (2018) reported that potato ring rot, its symptom is the tubers's skin brown. And the tail part of the tuber shrinks and dents. The vascular bundle ring of the plant turns yellowish brown, and yellow sputum in the ring rot are also different. Three potato tubers inoculated were found to be common symptom. It was initially shown that pathogenic white mold was one of the potato pathogensin in Nileke County of Xinjiang, China. The occurrence of this

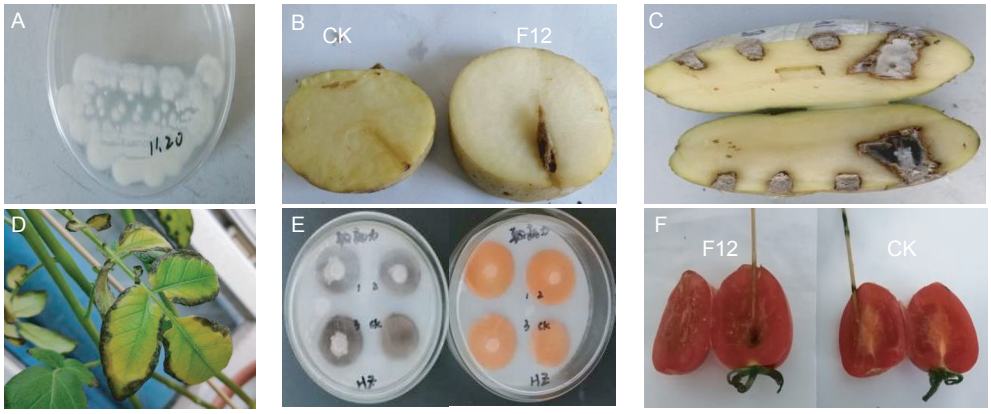

Fig. 2 Pathogenicity of the strain F12 (infected sample) from the potato *Solanum tuberosum cv.* Atlantic. A, colony morphology of strain F12 on PDA medium. B, acupuncture inoculation method. C, drilling inoculum method. D, leaf symptoms in the pot experiment. E, effect of strain F12 on potato chips and carrot slices (left, potato; right, carrot). F, verification test on tomato. CK, control check.

strain may be related to the agricultural production conditions such as climate in Nilek region of Xinjiang.

3.3. Morphology of the strain F12

The colonies of the strain F12 were flat and round, and milky white. Its surface is short, velvety and nearly powdery, and the back substrate is colorless. The average diameter of the strain F12 colonies reached 47 mm, and the spore amount reached 6×10^8 mL^{-1} on PDA after culture for 5 d at 30°C. The mycelium is fluffy and powdery, radiating, and the edges are light and clear (Fig. 3-A). The hyphae were bifurcated with a horizontal septum, colorless, hyphal morphology as shown in Fig. 3-B, and when mature the hyphae broke into single or chained, long tube-shaped, obtuse terminal ganglia spores (Fig. 3-C). This strain showed a typical morphology of *Galactomyces* (Minter 2001; Dong 2012).

3.4. Molecular identification

The ITS sequence of strain F12 had 100% similarity with *G. candidum* strain UOA/HCPF 9994^T (GQ376093.1) using the UNITE and NCBI. Meanwhile, the eight species with similar morphology and internal transcribed spacer (ITS) sequences were selected to construct a phylogenetic tree adopting the proximity neighboring method of MEGAV7.0 (Fig. 4). Based on these criteria, the F12 strain was identified as *G. candidum* F12. Fig. 4 shows the amplified electropherogram of strain F12.

3.5. Biological characteristics of strain F12

Strain F12 could grow in the temperature range of 26–35°C (Fig. 5), and the optimal temperature was 30°C for mycelial growth and sporulation. When the temperature was higher than 40°C, neither pathogenic mycelia nor spores grew.

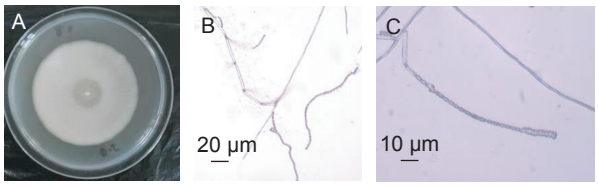

Fig. 3 Morphological characteristics of *Solanum tuberosum* F12 (infected sample). A, colony morphology of strain F12 on PDA medium after 5 d. B, mycelial form of strain F12 (20×). C, spore morphology of strain F12 (40×).

Alternating 12-h light and 12-h dark is conducive to the growth of mycelia, and the full light is beneficial to the spore production of the pathogen (Fig. 6). Strain F12 can grow and sporulate in the range of pH 4–12. The mycelium of the strain stopped growing at the pH 3. And the mycelia grew best at the pH 7 (Fig. 7). When pH is 6, *G. candidum* of *Cerasus humilis* was suitable for growth. This may be related to the different host, the suitable growth pH of the potato soil is 5–8.

The results showed that soluble starch and yeast powder were the best carbon source and nitrogen source, respectively, as the mycelial growth rate was faster and more spores were produced (Figs. 8 and 9). Those results also supported the reports of Gao *et al*. (2012), which found that glucose, fructose, beef extract, and yeast powder are suitable for the growth of *G. candidum*.

4. Conclusion

In this study, a new disease on potato caused by the

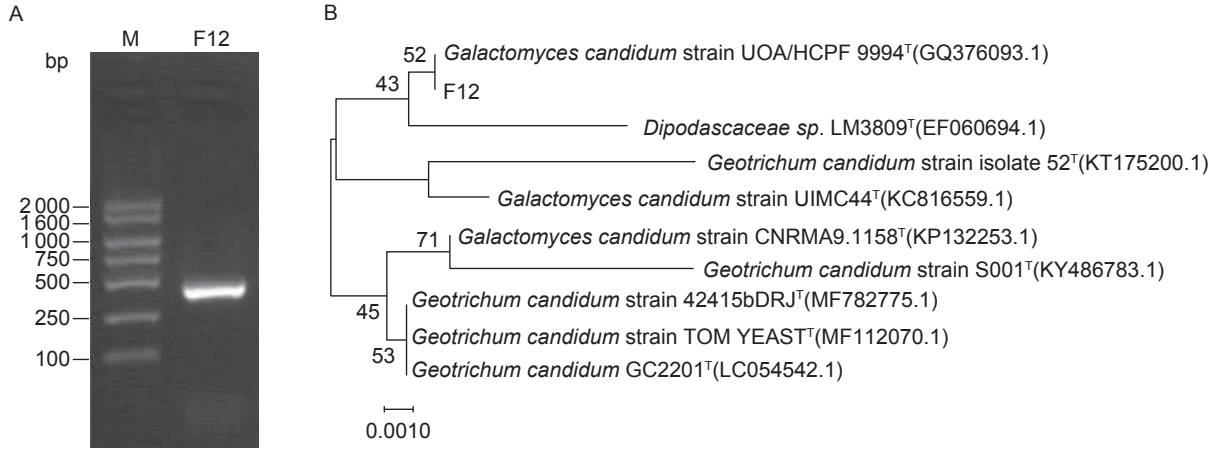

Fig. 4 PCR amplified electropherogram (A) of the internal transcribed spacer (ITS) region and the phylogenetic tree (B) of *Galactomyces candidum* F12 (infected sample). M, marker.

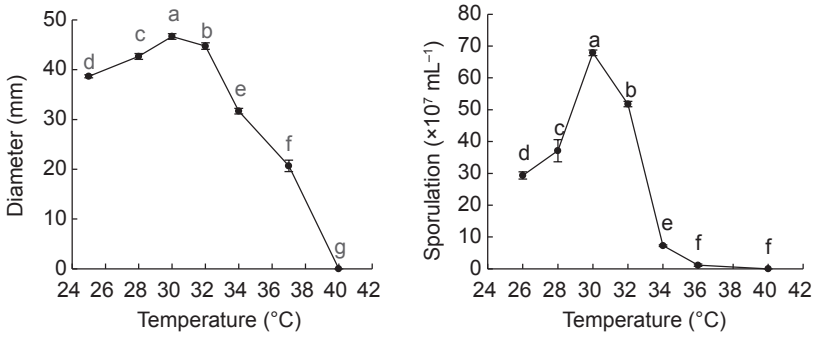

Fig. 5 Effects of temperature on mycelium growth and sporulation of *Galactomyces candidum* F12. Different lowercase letters indicate significant differences (P<0.05). Data are mean±SD (n=3).

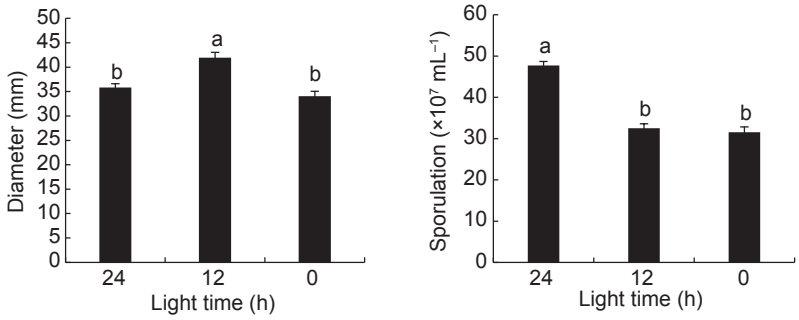

Fig. 6 Effects of light on mycelial growth and sporulation of *Galactomyces* candidum F12. Different lowercase letters indicate significant differences (P<0.05). Data are mean±SD (n=3).

Galactomyces spp. was reported in Xinjiang. The strain F12 was also isolated in other areas of Xinjiang, and it existed in the growth period and post-harvest period. Based on its morphology, pathogenicity test and ITS sequence analysis, the fungal pathogen of potato tuber-rot disease was identified as *G. candidum* F12. And it was the pathogeny of the acid rot disease on tomato by the tomato fruit puncture incubation. To our knowledge, this is the first report of potato tuber-rot disease caused by *G. candidum* F12 in China. The biological characteristics of *G. candidum* F12 were also studied. Further studies on this pathogen are needed, including its host-specificity and ecological characteristics,

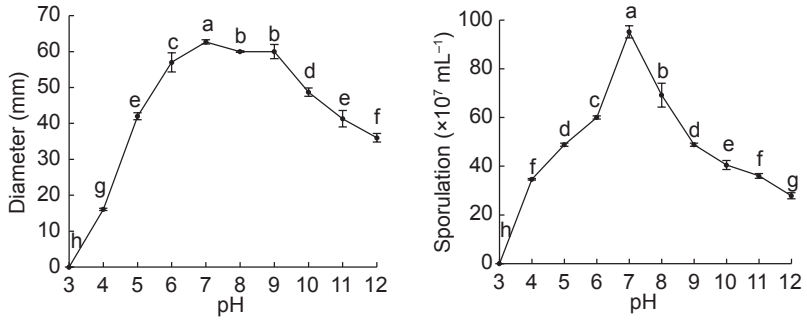

Fig. 7 Effects of pH on mycelial growth and sporulation of *Galactomyces candidum* F12. Different lowercase letters indicate significant differences (P<0.05). Data are mean±SD (n=3).

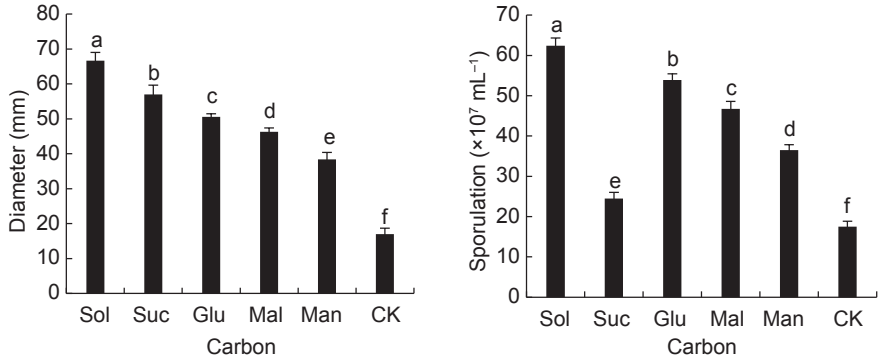

Fig. 8 Effects of carbon source on mycelial growth and sporulation of *Galactomyces candidum* F12. Sol, soluble starch; Suc, sucrose; Glu, glucose and mannitol; Mal, maltose; CK, no carbon source as a control. Different lowercase letters indicate significant differences (P<0.05). Data are mean±SD (n=3).

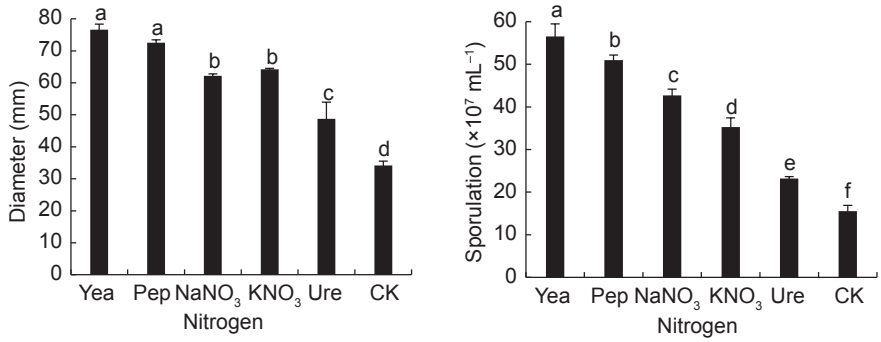

Fig. 9 Effects of nitrogen source on mycelial growth and sporulation of *Galactomyces candidum* F12. Yea, yeast powder; Pep, peptone; Ure, urea; CK, no nitrogen source as a control. Different lowercase letters indicate significant differences (P<0.05). Data are mean±SD (n=3).

as well as its relevant management strategies.

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