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Infection pathways of soft rot pathogens on *Amorphophallus konjac*

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With the rapid expansion of cultivated land, bacterial soft rot of konjac is more and more serious. The main pathogen is *Erwinia carotovora* subsp*. carotovora.* Some researchers think the natural openings and wounds are the main infection pathways on konjac. In this study, the infection pathways are investigated. From macro-observation, the soft-rot pathogens could not directly invade the intact corms, but it could invade the infected corms through the new rots. At the same time, bud scales, roots and wounded corms could be directly infected. From micro-observation, the epidermis of the corm had no stomas and lenticels, the stomas of the bud scales had no pathogens gathered around, and the pathogens broke down the infected roots and bud scales cells layer by layer. The results showed that soft rot pathogens invaded *Amorphophallus konjac* through the wounds and the growth of the organizations rather than the natural openings.

Key words: *Amorphophallus konjac,* bacterial soft rot, infection pathways.

INTRODUCTION

Erwinia carotovora subsp*. carotovora* is a phytopathogenic enterobacterium, responsible for the soft rot, blackleg, or stem rot in many economically important crops (Pérombelon and Kelman, 1980) . This soil borne facultative anaerobic pathogen causes maceration and rotting of parenchymatous tissue of all plant organs, eventually resulting in plant death (Pérombelon and Kelman, 1980; Wright, 1998). The pathogen is difficult to restrain be-cause of many reasons, such as absence of effective bactericides (Blom and Brown, 1999), genetic variability (Avrova et al., 2002; Gardan et al., 2003), wide host range, broad array of virulence factors (Pérombelon, 2002). The main weapon in the soft rot pathogen is the coordinated production of high levels of multiple exoenzymes, including pectinases, cellulases and proteases, which macerates the cell walls when it is inside the plant (Lam et al., 2001; Vlot et al., 2008; Beraha et al., 1974; Collmer and Keen, 1986; Willis et al., 1987). A whole plant may be transformed into a soft, watery, rotten mass within 3 - 5 days. *Amorphophallus konjac* is a perennial herbaceous species, mainly distributes throughout South-east Asia and Africa (Vasques et al., 2008). It enriches in glucomannan, which has been widely used in food, medicine, chemistry and agriculture industries. But the bacterial soft rot causes a heavy loss in konjac production every year. And sometimes this disease can destroy all the konjac in the whole infected filed (Xiu et al., 2006). For a long time, this disease has been recognized as the bottleneck of konjac industrial development.

Bacterial soft rot in *A. konjac* is caused by *E. carotovora* subsp*. Carotovora* (*E.c.c.*) and *Erwinia chrysanth-emi* (*E.ch.*) and *E.c.c.* is the main pathogenic species (Huang et al., 1999). At the same time, *E.c.c*. also infects the other crops very easily, such as Chinese cabbage, potato, Pinellia ternata et al*.* Wang et al. (1986) find that *Erwinia* bacterial invades Chinese cabbage by the roots. Zhao et al. (2000) prove that *Erwinia* bacterial invades potato from the wounds and the natural openings. As we know the infection pathways of soft rot pathogens on konjac have not been carefully studied. There is only the hypothesis from experience that natural

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openings and wounds may be the main pathways for *Erwinia* bacterial infection on konjac (Nguyen et al., 2002, Ban et al., 2009). So in order to find the approach to control the soft rot disease, it is the first step to understand its infection pathways in konjac.

MATERIALS AND METHODS

Soft rot pathogens

The culture of *E. carotovora* subsp. *carotovora* was isolated from an infected corm of konjac from Hubei province in China (Registry number FJ463871). The concentration of bacteria suspension was determined by ultraviolet (UV) spectrophotometry (Perkin Elmer Lambda Bio 20 UV/VIS) at 260 nm through three independent measurements and it was 10^7 CFU·mL^{-1}.

Plant materials

The healthy corm was provided by the Agricultural Science Academy of Enshi Autonomous Prefecture in China, which cultured in greenhouse. The surface of the corms was sterilized with 20% sodium hypochlorite for 5 min and then thoroughly rinsed with sterilized water. The corms of konjac were planted with sand in 15 cm diameter pots, which the pots and sand were sterilized at 121°C for 1 h before used. The dormancy breaking corms were classified into four types for infection pathway research. (1) The roots of 1 cm were wrapped with sterilized cotton soaked bacterial suspension for 1 h (Mount et al., 1982). (2) The bud scales of 1 cm were disposed as above (Mount et al., 1982). (3) The wounded corms were covered by sterilized cotton soaked bacterial suspension for 1 h (Mount et al., 1982). (4) The 2/3 bottom of the intact corms were immersed into bacterial suspension for 1 h. Then the treated corms were dried by sterilized filter paper and then they were all planted as mentioned before. Three repeats were applied. The environmental temperature was maintained at 28 ± 2°C under natural light conditions. All the tested materials were observed every day.

Optical microscope

The infected and health bud scales, roots and epidermis of the corms were fixed in FAA solution [formalin/acetic acid/ethanol (70%) in the ratio of 5:5:90(v/v/v)] for 36 h and dehydrated in a set of ethanol–xyloland embedded in paraffin wax (Hu et al., 2008). Sections (8 m) were cut using a rotary microtome, stained with crystal violet and then covered by the cover slips with a drop of neutral balsam before examination under an Olympus CH microscope and photo by digital camera.

Scanning electron microscopy

The epidermis of the corms and infected bud scales were prepared as described by Bae et al. (1997) and examined by scanning electron microscopy (SEM) (Hitachi S100, Japan) . The morphological characters of pathogen were observed by SEM (QUANTA200, Netherlands). SEM preparations were performed as described by Yoon et al. (2003).

RESULTS AND DISCUSSION

The bud scales, rootsand wounds treated by the bacterial suspension were soft rotten after 24 h (Figures 1a - c).

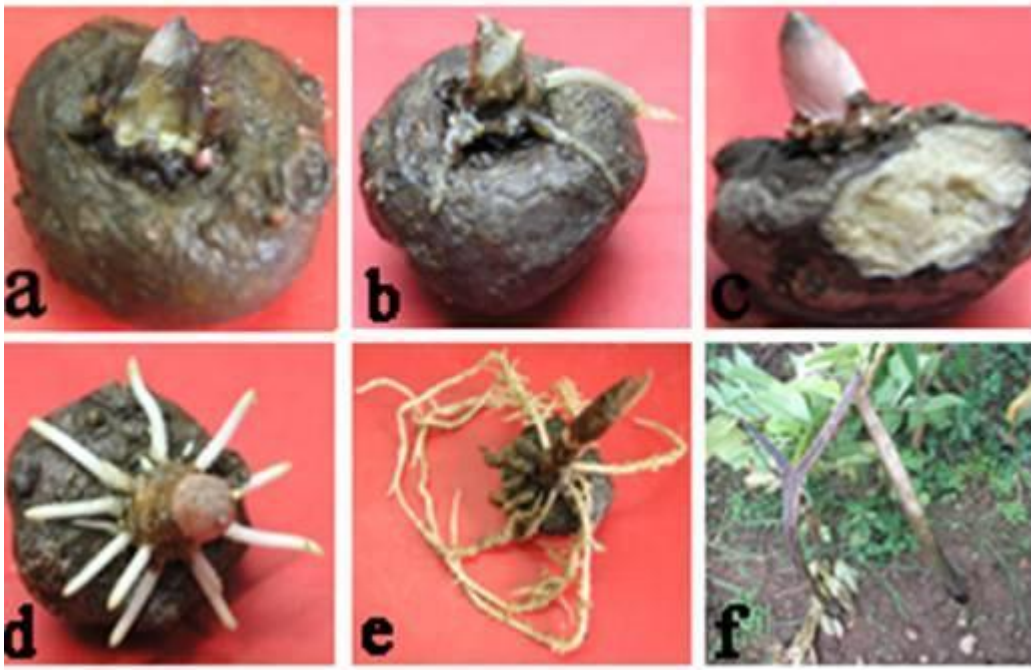
The corms, which the bottom 2/3 were immersed in bacterial suspension, seemed healthy in 6d (Figure 1d), some roots showed the infected symptom after 15 days and the buds were growing (Figure 1e), then the leafstalks performed the typical soft-rot symptoms in the late growth (Figure 1f). According to these results we deduced that the pathogens could not directly invade the dormant intact corms, but infect the konjac through the exuberant and young organs roots, bud scales and also wounds.

Through light microscopic observation, the healthy root and bud scale cells were well arranged (Figures 2a and 2c), but the infected samples showed a serious collapse of cell, which were broken from the external to internal cell by pathogen (Figures 2b and 2d). No stomata and lenticel on epidermis of konjac corm were observed under SEM (Figures 2e and 2f). No pathogens gathered around the stomata of the infected bud scales (Figure 2g). The pathogens were rod-shaped (Figure 2h). All these results suggested that the soft rot pathogen might infect konjac by other means rather than its natural openings.

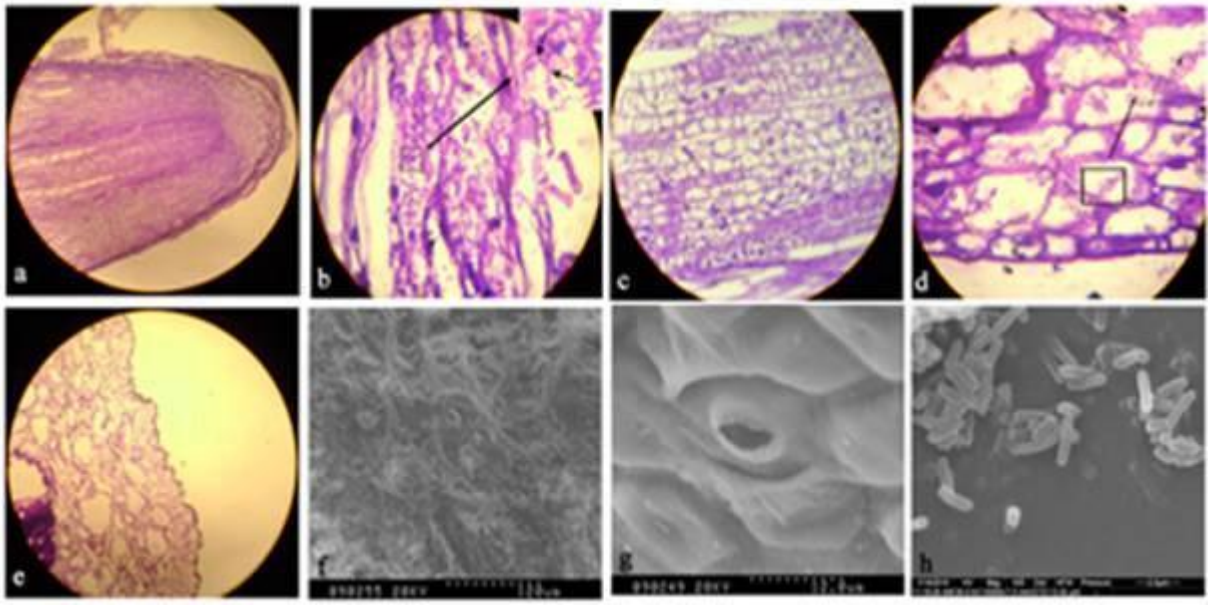
In this paper, the invading pathways on konjac of soft rot pathogens were explored for the first time, which first entered the plant from the growing organs or wounds. In this study micro-observation proved that there were no pores and no stomas in the epidermis of konjac corm. So obviously the pathogen could not infect the corm from the natural opening as it did on potato (Zhao et al., 2000). It was probably because the multiple exoenzymes excreted by soft rot pathogen could not macerate the protective layer of konjac corms but easily decomposed the cells of young organs.

The roots were the first sprouted organ and also could expand in a broader area when konjac corm was growing. Because the pathogens were carried by corms and soil, roots might have a large opportunity to meet the pathogen. On the other hand, roots had a mucilaginous covering and suitable for pathogen growth, so the root enriched pathogens and bacteria populations increased to achieve the requirements of intrusion (Goto et al., 1987). Through the research, we suggested that the root should be the mainly infection pathway. Once the bacteria entered the plant through root, it could spread to all the other parts. The infection could also happen first, on the aerial parts of plants. The rotten plant spread the pathogen into the soil and aggravated the disease in konjac field. This might be the reason why the soft rot disease could sometimes destroy the whole harvest.

This study made clear that *E.C.C* invade konjac actually through the young organs (especially the roots) or wounds not the natural openings. This result was of great significance for the further study of the pathogenesis, breeding for disease resistance and so on. It was a preliminary study for the pathway that soft rot pathogens invaded, and the process of the pathogen invading the intercellular spaces and histopathological



Figures 1a-f. Macro-observation the infection pathways of soft rot pathogens on *A. konjac* (a) Bud scale wrapped with cotton soaked bacterial suspension for 1h, the surface of it showed rot after 24 h, (b) Roots wrapped with cotton soaked bacterial suspension for 1 h, some roots completely decomposed after 24 h, (c) Wound tissue covered by cotton soaked bacterial suspension for 1h and became rot after 24 h, (d) The corm at the bottom 2/3 was immersed in bacterial suspension for 1 h and it stayed well after 6d, (e) The corm in d grew continually and half of the roots got rotted after 15 days, (f) The corm in e grew continually and the petiole rot.



Figures 2a-g. Micro-observation of soft rot pathogens' infection pathways on *A. konjac,* (a) Healthy roots × 100, (b) The tissue of infected roots was completely dissociated, and the soft rot pathogens existed intercellular spaces × 1000, (c) Healthy bud scales × 100, **(**d) The tissue of infected bud scales was completely dissociated × 1000, (e) The epidermis of corm showed no stoma and lenticel under the optical microscope × 100, (f) The epidermis of corm showed no stoma and lenticel under SEM, (g) No pathogens gathered around stomata on the infected bud scales under SEM, (h) *E. carotovora* subsp. *carotovora* grew on LB agar for 18 h at 37°C under SEM, showing rod-shaped (0.6 - 1.0 × 2.0 - 2.6 m).

mechanism were still needed to further study.

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