

Research on Advance of Rice False Smut *Ustilaginoidea virens* (Cooke) Takah Worldwide:

Part II. Studies Progress on the Pathogen and Its Toxin of U. virens

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Abstract

In this part, the history of the study on RFS pathogen *U. virens* was reviewed, including the pathogen naming and the change process, morphological characteristics of *U. virens* and culture characters both of asexual and sexual stages, and mycelium, chlamyospore, conidiophore and sclerotium germination. Genetic diversity, pathogenicity, the strain-host interaction, host range of *U. virens* and its early detection were also discussed. The research of Ustiloxins of RFS, including biological activity, toxicity to plants and animal, the potential possibility utilization of Ustiloxins, for example use as screening agent for rice varieties resistance to RFS, and anticancer drugs.

Keywords: *U. virens*, pathogen classification, characteristics, ustiloxins

1. Introduction

It possesses great significance for realizing the occurrence, epidemiology, harm and the management of the diseases to study the biological characters and morphological characteristics of the pathogens which caused the diseases. Rice false smut was first found in 1878 and the caused pathogen was named *Ustilago virens*. The pathogen was finally named *Ustilaginoidea virens* (anamorph) after more than once alteration, and the sexual generation was *Villosiclava virens* (teleomorph). The biological characters and morphological characteristics of *U. virens* was discussed in this paper. The *U. virens* has genetic diversity, and the pathogenicity shows obvious difference of different source pathogen. There was strong interaction between the *U. virens* and the host rice varieties. The pathogen of RFS could infect various plants. The abilities of different *U. virens* producing toxin there are differences. The toxin produced by *U. virens* shows obvious poison and negative effect on both plants and animals, on the other hand, the toxins of *U. virens* potentially develop the beneficial preparation products.

2. Research Progress of RFS Pathogen

The Basidiomycota smut fungi have been intensively studied over the last century because of their threat to the yield and quality of major crop plants (Kronstad, 1996).

2.1 Naming and Classification of the RFS Pathogen

RFS was first recognized by Cooke from infected rice samples from India, and at the time was named by Cooke as *Ustilago virens*, as a species of *Ustilago* (Cooke, 1878). Then, Patouillard carried out independent study on RFS samples from Japan, and named it *Tilletia oryzae* Pat., a species of rice *Tilletia*. In 1895, Brefeld held that

the development and sporulation pattern of *Tilletia oryzae* Pat. was similar to an asexual stage of *Ustilaginoidea*, namely the sac fungi of *Ustilaginoidea*. Therefore, *U. virens* was transferred to the genus of *U. virens*, and its name was changed by Brefeld to *U. oryzae* (Pat.) (Padwick, 1950; Ou, 1985; Tanaka et al., 2008b). In 1934, Sakurai found that the sexual spores of the pathogen, the sporangia sclerotium produced by sclerotium germination and the fungus was attributed to the Ergot fungi genera of sac fungi known as *Claviceps virens* (che). However, it did not obtain a valid name for various reasons. The teleomorph of *U. virens* had been named *Claviceps virens* (Sakurai ex Nakata) and *Claviceps oryzae-sativae* (Hashioka) because its characteristics of teleomorph are similar to those of *Claviceps* (Hashioka, 1971). Due to the fact that the characteristics of the spores in the conidiospore stage of *Claviceps* and *U. virens* differed, the name has not been admitted in academy (Dodan et al., 1996). In 1988, Ahuja and Payak proposed that the genetic difference discrimination of family should not be based on the same sex in most cases, instead it should be based on the characteristics of conidial stage. They also suggested that *U. virens* (che) was the valid name of the pathogen *U. virens*, and *Claviceps oryzae sativae* was its alias (Ahuja et al., 1988). This name has gradually been accepted in academic circles, thus RFS was officially named as *U. virens*. Until 2008, RFS has been independent of *Claviceps* by Tanaka based on comparative research, and the sexual state was named as *Villosiclava virens* (Tanaka et al., 2008a, 2008b).

However, the molecular phylogenetic analysis, which based on both large subunit of the rRNA gene and acetaldehyde dehydrogenase gene sequences, revealed that members of *Ustilaginoideae* are distinct from teleomorph genera of *Clavicipitaceae* and should be recognized as a monophyletic group within *Hypocreales* (Bischoff et al., 2004; Tanaka et al., 2008b). As a result, it was suggested *Villosiclava virens* as the new name for the teleomorph of *U. virens* (Tanaka et al., 2008a), which was accepted and used in recent reports (Ashizawa et al., 2012; Fu et al., 2012; Tang et al., 2012).

A new type of *U. virens* strain was isolated from white false smut balls. The pathogenicity test and the analysis results of isoenzymes and RAPD demonstrated that the taxonomy of albino strains was independent of *U. virens*. Whether or not it can establish its new position as a species still requires further research (Wang et al., 1998, 2008b; Jecmen et al., 2015).

2.2 Morphological Characteristics of Pathogens

The *U. virens* forms smut ball on the rice panicle, the symptoms (smut ball) produced by *U. virens* are visible after flowering only (Biswas, 2001), its color changes from cream white, yellow to dark green or dark brown with the passing of time, and this is the conidia pedestal. The spore pedestal section is divided into three layers: the outer layer of yellow green is mature chlamydospores, the middle orange layer is hypha and spore, and the inner white or pale yellow layer is radial hyphae and spores that are in the process of being formed (Ou, 1985; Lee et al., 1992; Biswas, 2001). The morphological characteristics of *U. virens* include the asexual stage and sexual stage.

2.2.1 Morphological Characters in Asexual Stages

The vegetative state of *U. virens* includes mycelia, chlamydospores and conidia. Chlamydospores are conidiospores with thick walls, round or ovular in shape, with a size of $4.5-7.8 \times 4.5-7.0 \mu\text{m}$, and yellow to dark brown in color. The cell wall is thick, and on the surface there is a large amount of verruca (Zhang, 1988). The chlamydospore on the white false smut of RFS balls is spherical, colorless and transparent, and the outer wall is smooth (Verma et al., 1988; Wang et al., 1997). Under appropriate conditions, chlamydospore germinates and produces a germ tube, and the germ tube forms dissepiment and differentiates into conidiophores. The tips of the conidiophores produce secondary conidium (Zhang, 1988). Conidiospores are thin-walled spores, ovoid or oblong in shape, with a size of $2.6-8.0 \times 2.0-5.0 \mu\text{m}$, have single cells, colorless and transparent, and have a smooth appearance (Mulder et al., 1971; Zhang et al., 2003a, 2003b, 2003c).

2.2.2 Morphological Characters in Sexual Stages

The sexual stage of RFS mainly includes the formation of stroma by sclerotium germination, ascus and ascospore. The fungus can form sclerotium on rice diseased grain. Sclerotium is black, hard, falls off easily, fusiform, horseshoe shape and various shapes, and has irregular sizes (length of 2-20 mm). The newly grown stroma is usually yellow in color, and the color turns black green after reaching maturity. The monolayer in stroma has many perithecia, the perithecium is ovular or pear-shaped, with a front opening, and the size is $357.5 \times 247.0 \mu\text{m}$, containing about 300 asci. The asci have a long cylindrical shape, colorless and transparent, and have a smooth surface, with a size of $130-234 \times 3.12-5.2 \mu\text{m}$ and 8 ascospores within. The ascospore is colorless unit cells, linear, easily broken, with a size of $52-176.8 \times 0.52-1.04 \mu\text{m}$ (Zhang et al., 2003a, 2003b).

2.3 Culture Characteristics of *U. virens*

2.3.1 Isolation of Pathogenes

The pure isolate of RFS pathogen *U. virens* was acquired for the first time in 1895 by Brefeld. In 1975, Sharma & Joshi isolated conidiospores from a fresh sclerotium on a yeast PDA medium (Wang et al., 1990; Zhou et al., 1999; Ji, 2001; Chen, 2004). Thereafter, the isolation technology of *U. virens* has been further developed and improved. At present, the main methods of separation of *U. virens* include the tissue, sclerotia, RFS ball isolation method and chlamyospore suspension method.

2.3.2 Mycelium Culture

The mycelium growth rate is related to environmental conditions and medium types. The temperature range of mycelial growth is 10-37 °C, and the optimum temperature range is 26-28 °C. The pH range is 3-10, and the most suitable pH is 5-7. Light demonstrates no significant effect on mycelia growth. The growth rates of *U. virens* in different media are different. The optimum carbon source of mycelial growth is sucrose, followed in order by maltose, glucose and starch; the optimum nitrogen source is L-asparagine; inorganic salt is a mixture of disodium hydrogen phosphate and magnesium sulfate (Lu et al., 1996; Zhang et al., 2003a; Pan et al., 2007a; Wang et al., 2012a). The optimum nitrogen and carbon source for different virulent strains different (Wang et al., 2013). The growth speed of different growing stage of *U. virens* is different at various media. The growth of *U. virens* in PDA and PSA media is slow, while large amounts of sclerotia may be produced in the PDYP medium (Zhou et al., 1999).

The sporulation ability and pigmentation of *U. virens* are positively correlated with pathogenicity, and the strain growth rate is negatively correlated with pathogenicity (Wang et al., 2013). In the same kind of solid medium, the colony morphology and color of *U. virens* strain in the initial stage are similar, but different characteristics appear after one month of culturing (Zhou et al., 1999).

2.3.3 Chlamyospore Culture

The production of chlamyospores of *U. virens* is correlated with sporulation ability and medium type. Some *U. virens* strains have sporulation ability, and some strains cannot produce spores (Verma et al., 1988; Cheng et al., 1996). An oatmeal liquid medium is more conducive to *U. virens* produced chlamyospores than in the liquid media of PS and PD (Zhou et al., 1999).

The life span of chlamyospore is quite long, as it can survive for more than 19 months under dry conditions (Lv et al., 1994). The optimum temperature for chlamyospore germination is 28 °C, and the optimum pH value is 5.8-6.3 (Lu et al., 1996). Some nutrients could improve the germination rate, proper order is 2% sucrose > 2% maltose > rice washing water > 2% millet sprout liquid. The rice tissue liquid in different parts is also conducive to spore germination, and the effect of pollen was the best. Under suitable temperature, the germination rates of yellow chlamyospore in water and rice pollen exceed 80% and 90% after culturing for 5-6 h, and the dark green chlamyospores were only 10% and 35%, respectively (Liu et al., 1989). pH value showed a significant effect on the germination and sporulation of chlamyospore, Neutral partial acid was conducive to germination and sporulation of chlamyospores, but peracid or paralkaline (pH 3.0 and pH 10.0) obviously inhibited sporulation and spore germination (Wang et al., 1998). Regarding the effects of light on the germination of chlamyospores, there are several different views. Wang (1988) and Liu et al. (1989) held that sunlight, fluorescent lamp, UV lamp irradiation had no significant effects on chlamyospore germination, but they could inhibit the formation of microspores (Wang, 1988; Liu et al., 1989). However, Lu et al. (1996) demonstrated that light had a stimulating effect on the germination of spores.

2.3.4 Conidiophore Culture

Conidia production and germination of *U. virens* are closely related to strains and culture conditions. Some strains can produce conidia, while some cannot. The same *U. virens* was cultured in four types of media for 144 h, and the medium with the most sporulation quantity was PS, followed in order by PD, YPPD and PW (Wang et al., 1998). *U. virens* was cultured in a liquid medium for 7-9 d at a temperature of 26 °C, and the cultured mycelium was placed into the plate medium for culturing for 3-5 d in the dark, after which a large number of spores was produced (Fujita et al., 1989). A large number of conidia were also produced by shaking the culture of mycelia in a PS medium for more than 7 d (Lu et al., 1996).

Potato, glucose and rice juice solid medium are the most suitable media for mycelium growth, while potato and dextrose broth are the most suitable for mycelial growth and sporulation (Lv et al., 2009). *U. virens* was cultured in PSB medium at altered temperatures of 22-29 °C and a constant temperature of 28 °C under natural lighting conditions for shaking culture for 12 d, and the lowest sporulation quantity reached 6.3×10^7 /mL, followed by

PDB medium, at 1.1×10^6 /mL (He et al., 2011). The conidia germination temperature was 22-31 °C, and the optimum temperature was 28 °C, the optimal pH was 6-7. The PSA media was most suitable for germination (Zhang et al., 2003a, 2003b). *U. virens* was cultured in a PSB for 9 d, and the conidia concentration reached 7.2×10^7 /mL (He et al., 2011). More than 12 months can survive if *U. virens* was periodically transferred in paraffin oil storage, which was known as suitable method for the storage of *U. virens*.

2.4 Sclerotium Germination

The temperature, humidity and illumination could affect the germination of sclerotium. The germination of *U. virens* sclerotia must undergo a period of dormancy. After winter dormancy, the sclerotium is more conducive to producing sporophores and ascospores. Whether the collected sclerotia germinate and produce sporophores, 12 h of light is needed (Dong et al., 1989). Sclerotia do not germinate after wintering under dry conditions, while at moist conditions and the temperature is 26-28 °C it could germinate. The dormant period of sclerotia can reach up to more than 6-7 months when the average temperature is below 20 °C, while the average temperature is above 27 °C, the dormant period is 3-6 weeks (Liao, 1994).

2.5 Genetic Diversity of *U. virens*

Information about the genetic diversity and population structure of *U. Virens* is essential for rice breeding and efficient control of the RFS (Sun et al., 2013).

Strains isolated from different regions or different rice varieties are distinguishable in genetic diversity and in virulence to rice. 110 isolates of *U. virens* isolated from Liaoning and Beijing of north China were analyzed by using amplified fragment length polymorphism (AFLP) markers. The isolates can be divided into three groups according to the genetic distance and the isolates from the same region can be placed into one group (Zhou et al., 2008). The coefficient of strains from Liaoning and Beijing was 0.92 and 0.55, respectively. There was no specific DNA pattern for the isolates from the same rice varieties, and there was no co-relation between the clusters based on genetic similarity coefficient and variety origin of isolates (Pan et al., 2007b). 59 isolates of *U. virens* what isolated from three rice varieties of hybrid in Sichuan province of west China could be classified into six groups based on their virulence to rice varieties (Lu et al., 2009).

The rDNA-ITS fragment of *U. virens* was amplified by using ITS4 and ITS5, and the electrophoresis band of PCR. The sequencing analysis showed that the rDNA-ITS sequences of 35 strains came from different parts of China were completely consistent, i.e. the homology was 100%. The sequence alignment results showed that the ITS homology of 35 strains and the strains collected from Zhejiang, Liaoning, Yunnan provinces, and Japanese (AB116645 and AB105954) were all 100%, indicating that the ITS sequences of *U. virens* from different geographical origins or ecological zones were highly homologous or completely consistent (Zhou et al., 2003). RAPD technology was used to analyze the population genetic structure of 55 strains from nine regions of eight provinces of China and one strain in Japan. The results exhibited that for the strains from different geographical origins was difficult to divide their geographical lineages, and the degree of differentiation of diversity of *U. virens* was relatively low (Zhou et al., 2004; Pan et al., 2006; Wang et al., 2009).

However, some studies suggested that *U. virens* exhibited rich DNA polymorphism and genetic diversity. The strains from different years and different regions had significant genetic differences, and the genetic grouping of RFS in different located was related to geographic origin (Zhou et al., 2008; Zhang et al., 2009). Yang et al. (2011) illustrated that *U. virens* in Fujian province had a rich genetic diversity, and the change range of genetic distance was between 0.02 and 0.67. The genetic diversity level of the strains isolated from western Fujian province was the highest ($PPB = 76.43$, $H = 0.2212$, $I = 0.3383$), and the genetic diversity of the isolate group from late rice ($PPB = 91.08$, $H = 0.2402$, $I = 0.3655$) was higher than that of early rice populations ($PPB = 63.06$, $H = 0.1892$, $I = 0.2870$). It was deemed to the geographic origin of isolates, rice varieties and their growing season are the main factors affecting the genetic diversity of *U. virens* in Fujian province, which may play an important role in the genetic variation and occurrence and prevalence of RFS.

The biological method and RAPD-PCR technology were used to analyze the mycelial growth rate, conidia production, spore germination rate and genetic diversity of 84 strains from 11 provinces (municipalities) in China. Based on the mycelium growth rate, the strains can be divided into two types of fast and slow, accounting for 58.33% and 41.67%, respectively. According to the sporulation ability and conidia germination ability, the isolates can be divided into three types of strong, medium and weak. Isolates both from the same and different regions showed different variations, and the variation degree of the strain groups of inland areas was significantly higher than that in the coastal areas (Wang et al., 2012b).

The DNA genetic diversities of 60 *U. virens* strains from six *indica* rice area in Sichuan province were investigated by means of ERIC-PCR fingerprint technology with UPGMA cluster analysis and similarity analysis. At the similarity level of 0.75, the tested strains were divided into 11 genetic types. The genetic similarity of *U. virens* from the same area is higher, while from different regions showed different degrees of variation. The correlation between the varieties and genetic differences of *U. virens* was low (Zhang et al., 2009).

2.6 Pathogenicity of *U. Virens*

Forty-six single spore of *U. virens* isolates were employed to inoculate three rice varieties of “Yue 938”, “Huai 9508” and “Wuyunjing 3”, which show different resistance level to RFS, to study the differentiation of the pathogenicity of *U. virens*. The response of different resistance rice varieties showed different on the same strain; similarly, the pathogenicity of different isolates to the same variety also showed significant differences, suggesting that the pathogenic differentiation of the strains of *U. virens* is significant (Chen et al., 2009; Pan et al., 2012; Yin et al., 2014).

2.7 Strain-Host Interaction

There were different viewpoints regarding whether there is interaction between the *U. virens* strains and rice varieties among different researchers. Zhang et al. (2003b) and Lu (2013) held that there were specific and significant interaction phenomena between rice varieties and strains of *U. virens*, the reasons are: (1) The pathogenicity differences of different *U. virens* strains on the same rice variety can generally be divided into three strain types of weak, moderate and strong virulence; (2) different rice varieties had different resistance to the same strain, which can be divided into the four types of moderate resistance (MR), moderate susceptible (MS), susceptible (S) and high susceptible (HS).

Jiang (2014) held that different rice varieties, showed significant difference in resistance to RFS, and there were significant pathogenicity difference among 25 *U. virens* strains. The relationship of *U. virens* strains and rice varieties could be divided into weak interaction and strong interaction, of which the weak interaction accounted for 91.3%, and the strong interaction was 8.7% (Yin et al. 2014). For example, the variety of Hui 9 was immune to strain GD1001 of *U. virens*, and the variety Jinyou 207 was susceptible to GD1001; in addition, the variety Hui 9 was susceptible to strain GZ1001 of *U. virens*, while the variety Jinyou 207 was immune to strain GZ1001. It indicates that there is strong interaction of rice varieties and *U. virens* strains of RFS (Pan et al., 2012). However, according to the Zhou et al. (2004) and Pan et al. (2006, 2007b) preliminarily concluded that there was no specific interaction between rice varieties and *U. virens*.

2.8 Host Range of *U. virens*

There has been no report on the host range of *U. virens* by artificial study, but the survey found that *U. virens* not only infected rice, it also infected corn and some weeds in fields, such as *Digitaria marginata*, *Panicum trypheron* and wild rice (Shetty et al., 1987; Abbas et al., 2000). It was found that there was a similar RFS pathogen on dry grass, and the two pathogens cross inoculations could lead to pathopoiesis of each other from rice and dry grass (Shetty et al., 1987). Atia (2004) reported that the weeds of barnyard grass (*Echinochloa crusgalli*) and cogongrass (*Imperata cylindrica*) in Egypt could be infected by *U. virens*. It has been reported in China that there were similar cases of RFS in *Sporobolus fertilis* (Steud.) (Li et al., 1986), and weeds with similar symptoms also discovered in paddy field weeds in many other locations (Hu et al., 2012).

2.9 Molecular Detection of *U. virens*

The advent of genetic transformation and several techniques have opened the possibilities for studying the interactions between plant and pathogen, including agrobacterium mediated transformation (Zhang et al., 2006) and electroporation (Tanaka et al., 2011) have been developed for the transformation of *U. virens*. A recent study utilized a transgenic strain expressing green fluorescent protein gene (GFP) (Ashizawa et al., 2012) to observe the initial infection of rice panicles before heading.

Zhou et al. (2003) designed specific primers and established a method for detection of *U. virens* with nested PCR, by using the sequences intraspecific conservative characteristics of the rDNA-ITS of ribosomal internal transcribed spacer of *U. virens*. It was found that there was attachment or infection of *U. virens* on the auricle of flag leaves at early reproductive growth stage of rice; at the same time, the *U. virens* could also be detected in the flag leaf ear of rice early reproduction growth and duckweed in the field (Zhou et al., 2006). Ashizawa et al. (2005) detected *U. virens* in the inoculated and non-inoculated rice at the booting stage, indicating that the *U. virens* spores could naturally intrude into the spike bud outer rice husk, and could attach to or infect young glume, thus suggesting that early and late booting stages of rice was an period of vailidity for *U. virens* conidia

infection (Chen et al., 2013). The establishment and application of these technologies laid a solid foundation for the in-depth study the regularity of *U. virens* infection, as well as rapid and accurate detection and prediction of RFS (Zhou, 2004).

A series method of high sensitivity to detect the pathogen of RFS in rice plants and soil have been developed recently, known as PCR-based (Zhou et al., 2003), nested PCR (Zhou et al., 2006) and the “real-time PCR” method (Ashizawa et al., 2010). We can use these methods to detect less than 50 fg DNA of *U. virens*, the equivalent of eight chlamydo spores in a gram of soil. The real-time PCR assay for the soil samples was at least 100-fold more sensitive than the conventional and nested-PCR assays tested. It may be a useful tool for optimization of disease control strategies (Ashizawa et al., 2010).

3. Ustiloxins

RFS not only caused a reduction of rice yield, increased empty grains and broken rice, decreased milled rice rate and quality of rice, but also had harm to plants and animals due to the toxins produced by *U. virens*. The *U. virens* could produce large amounts of mycotoxins, the ustilotoxins (more than 100 mg kg⁻¹ false smut balls) which inhibit cell division in animals and plants and thus frequently cause animal poisoning (Koiso et al., 1998; Nakamura et al., 1994; Li et al., 1995). The toxicity produced by different *U. virens* strains was quite different, the toxicity of toxin produced by white strain of *U. virens* was stronger than that of ordinary (black) strain (Bai et al., 1997). The *U. virens* of RFS is poisonous when the incidence exceeds certain degree and the grain should not be fed to animal. The chlamydo spores and conidia also contaminate the rice grains and straws with their antimetabolic cyclic peptides (known as ustiloxin), which are poisonous to both humans and animals (Koiso et al., 1994).

3.1 Research of Ustiloxins

In the early 20th century, it was found that *U. virens* extract was toxic to rabbits and other animals. In 1933-1937, Yabuta isolated a pigment from the ether extract of *U. virens* for the first time, called Ustilaginoidin. The structure of Ustilaginoidin and its homologues were ascertained (Shibata et al., 1963; Tsuchita et al., 1987), and found that the mechanism of the action of *U. virens* was different from the mechanism of plant toxins. In the 1950s, Chinese pathologist pointed out that *U. virens* contained toxic pigment C₉H₆O₇. Further study found that the toxin was a kind of alkaloid compound (Deng, 1989; Ma et al., 2001). Japanese scholars found that the Ustiloxins of *U. virens* was a cyclic peptide, a kind of anti-eukaryotic cell mitosis, including a 13-ring, in which there is an ether bond (Koiso et al., 1992).

Up to now, it has been found that there are two kinds of secondary metabolites of *U. virens*, one is colored fat soluble substance called “Ustilaginoidins”, belongs to naphtho-pyrones; the other one is a water-soluble colorless substance called “Ustiloxins”, also known as ustilazin, which is a cyclic peptide. It believed that the RFS toxin was produced by the chlamydo spores of *U. virens* and the false smut (Jiang et al., 2010). There were six kinds of toxins had been isolated from *U. virens* till now, namely Ustiloxin A, B, C, D, F and E, and their molecular formulas were C₂₈H₄₃N₅O₁₂S, C₂₆H₃₉N₅O₁₂S, C₂₃H₃₄N₄O₁₀S, C₂₃H₃₄N₄O₈ and C₂₁H₃₀N₄O₈, respectively. Due to the fact that the isolated quantity of Ustiloxin E was too less to conduct an experiment, its structure and molecular formula were not clear (Kosio et al., 1994, 1998).

3.2 Biological Activity of the Toxins of *U. virens*

3.2.1 Toxicity to Plants

Crude toxin of *U. virens* had strong inhibition effects on the germination of rice, wheat and maize seeds, as well as the growth of radicles and plumules. The inhibitory effect on the radicle growth is stronger than that of embryo growth and seed germination (Bai et al., 1997; Tian et al., 2000; Gao et al., 2013). Rice seeds were treated with the toxins of *U. virens*, the seeds germination of resistant varieties could be inhibited, on the contrary, the seeds germination of susceptible varieties were promoted. This suggested that there is a correlation between the inhibition ability of ustiloxins on rice seed germination and the resistance level of rice varieties (Gao et al., 2013). Ustiloxins could inhibit the mitosis of garlic root tip cells, but it did not inhibit cell elongation (Chen et al., 2004). Abbas et al. (2014) demonstrated that the extract of *U. virens* from Arkansas, USA, had almost no effect on rice seed germination, but it did exhibit toxicity to duckweed.

3.2.2 Toxicity to Animal

Ustiloxins of *U. virens* is a kind of cyclic peptide that resistant to mitosis of eukaryotic cells (Koiso et al., 1994), and it has a wide range of biological activity on animal cells. The active mechanism of Ustiloxins is the inhibition of mitosis of animal and plant cells (Nakamura et al., 1992; Ludueña et al., 1994; Li et al., 1995). The liver cells and renal tubular cells of mice *in vitro* were rapid necrosis after one-time injection with Ustiloxins

(Koiso et al., 1994). It also suppressed the cell mitosis or caused abnormal mitosis, which was similar to the symptoms expressed with colchicine. Ustiloxins A and B could inhibit the mitosis of a variety of human tumor cells, it is stable to heat, and the toxicity is not destroyed by heating at 100 °C for 30 min (Chen et al., 2004).

Crude toxins of *U. virens* can caused acute, occasional necrosis of hepatocytes and renal tubular cells, followed by increased number of mitotic figures with occasional multinuclear giant cells. Erosions and ulceration of the forestomach and atrophy of the thymus were observed a week later (Nakamura et al., 1994). Feeding rabbits, chickens, mice and other animals with rice grains mixed with RFS can cause lesions of the liver, kidney and other internal organs (Shang et al., 1985; Nakamura et al., 1993; Bai et al., 1997). The pathological change of the animals' organs and/or death were caused after feeding rice grains contaminated by RFS for 35-84 d. The mortality rate of the rock roosters was 37.5%, and the lethal dose was 0.14-0.17 g RFS grains daily consumption of per kg of animal body weight, which could lead to an inability in the laying hens to lay eggs, as well as ovarian atrophy (Leng, 1984; Gao, 1992).

Feeding pigs with the feedstuff mix with 0.5% infected rice grains of RFS, it could slowed down the growth of the pigs, decreased the pigs' weight gain rate, and pathological changes of multiple organs, such as liver, kidney and spleen and other diseases were caused. It also affected sow's reproductive performance, such as ovarian hyperemia, hemorrhage; decreased the litter size, the weaning litter weight and the survival rate of piglets (Shang et al., 1985). At the same time, the phenomena of stillbirth and/or mummification of fetal and fetal malformation were also present (Huang et al., 2002). Ducks fed with rice containing 5% of RFS grains could cause hepatomegaly (Huang et al., 2002; Wang et al., 2008a). A typical example was from Shexue Township of Guizhou Province, from 1999 to 2002, 1914 livestock and poultry appeared a kind of disease with the main symptoms of diarrhea, fever, salivation, vomiting, central nervous excitement or paralysis, shortness of breath, and rapid heartbeat. The animals often died of severe dehydration and exhaustion, and the death rate reached 71.12%. It was diagnosed as feeding infected rice grains of RFS and resulting in toxin poisoning (Wu, 2004).

3.3 Utilization of Ustiloxins

3.3.1 Use as Resistance Screening Agent

Rice seeds of resistance varieties were treated with toxin of *U. virens*, the seeds germination could be inhibited; on the contrary, the seeds germination rate of susceptible varieties were promoted. It provided a simple and efficient method for the identification the resistance of rice varieties to false smut (Ma et al., 2007; Gao et al., 2013). The crude toxins of RFS were employed as selection pressure to screen rice resistance mutants to RFS, and the resistance of various rice varieties at the cellular level was consistent with that of the rice in fields. It suggested that it is feasible to select disease resistant mutants with the crude toxins of *U. virens* as the selection pressure.

3.3.2 Anticancer Drugs

Due to the Ustiloxin A and B can inhibit the mitosis of a variety of human tumor cells (Kosio et al., 1994), it is possible to develop the fungal toxin of false smut into cancer targeted therapy drug by using modern molecular biological technique and gene engineering technology.

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