Guide for isolation and identification methods of *Ustilaginoidea virens* causing false smut of rice

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Introduction

False smut of rice is caused by the flower infecting fungus, *Ustilaginoidea virens*. The disease is characterized by the appearance of round, orange colour powdery mass spores which later turn to dark smut balls. For a long time, the disease remains neglected as it was considered a minor disease. However, presently the disease is causing devastation and alarmingly increasing its spread in different rice growing countries of the world. Pure culture of pathogen is the foundation on which many studies exclusively rely upon. The pathogen is slow growing in culture media and takes about >30 days to cover a Petri dish. Hence, the pathogen unlike many other fungal pathogens, is difficult to isolate in pure culture form. Nevertheless, meticulous and right method will bring successful isolation and identification of this elusive pathogen. Therefore, in this short note a guide to the researchers on false smut isolation and identification methods of the pathogen is elucidated for achieving success.

Smut ball

Fresh smut balls should be used for isolation of the pathogen as such samples produce better results as compared to the old samples (Fig.1).

Fig.1.False smut of rice

Isolation Method

Media preparation

- Potato sucrose agar (PSA)is the medium of choice for artificial culture of *Ustilaginoidea virens*.
- The incubation temperature of 27°C and hydrogen ion concentration (pH) of 6.0 should be maintained during culture of the pathogen.
- Add streptomycin @100 ppm into the media to avoid bacterial contamination at lukewarm stage before pouring into Petri-dish.

Surface sterilization

1. Dip the smut ball in 1% sodium hypochlorite solution for 1 minute using sterilized forceps followed by 70% ethanol wash for 1 minute.
2. Wash three times with sterilized distilled water.
3. Dry the smut ball between two sterilized filter papers.

Inoculation and incubation

4. Tease out the outer powdery mass of spores into tiny pieces using a sterilized blade and inoculate two or three pieces ontoa PSA medium.
5. Wrap the Petri-dish using a parafilm to avoid air contamination and incubate at 27±2°C for about 3-5 days.
6. To get the pure culture of the fungus, use hyphal tip method for sub-culturing the fungus in media slants/Petri-plates.

Observation

- Observe the inoculated plates for tiny mycelial growth after 5 days of inoculation.
- Subculture the fungus to another petri dish/slants to avoid contamination from other unwanted microbes.

Identification methods of *Ustilaginoidea virens*

Morphological method

- The mycelium of the fungus is creamy white, flat or raised with slight undulations, compact and leathery.
- Yellowish chlamydospores usually develop on the mycelium (Fig.2 & 3).

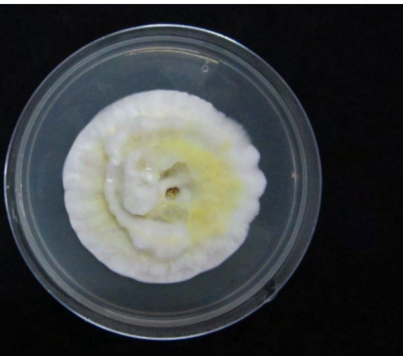

Fig.2. A mycelium of *U. virens* at 30 DAI

Fig.3. Yellowish chlamydospores appear on the mycelium of *U. virens* (Image courtesy, Baite and Sharma 2015).

- The conidia are spherical, hyaline and warty (Fig.4).
- The conidia appear echinulated under a Scanning electron microscope (SEM) (Fig.5).

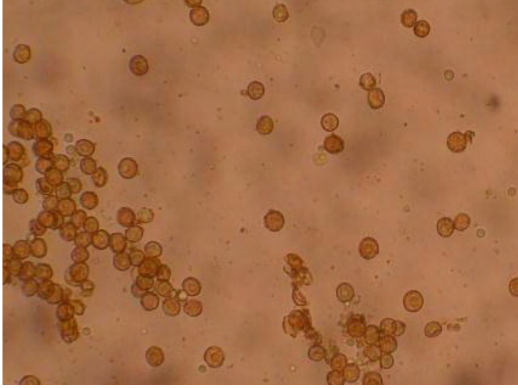

Fig.4. Conidia of *Ustilaginoidea virens* as seen under a compound microscope

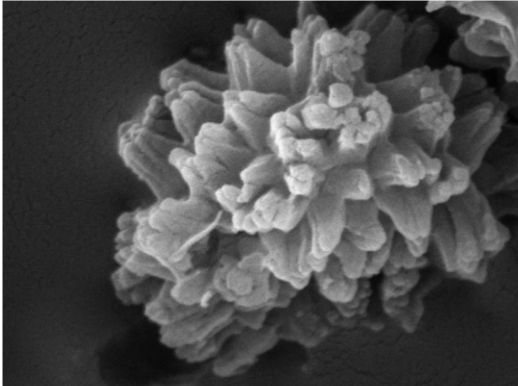

Fig.5. Conidia of *Ustilaginoidea virens* showing spines as seen under Scanning electron microscope (SEM) at 34.83 KX

Molecular method

Validation of the candidate sequences should be performed on the basis of ITS regions sequencing and analysis. The process involves the following steps;

1. DNA extraction
2. PCR test using the ITS1 and 4 primers
3. Presence of amplified band
4. Sequencing of the PCR products through outsourcing
5. Sequence analysis and identification through BLAST search in NCBI
6. A similarity of >90% identity would certainly confirm the identity of the fungus in question.

Pathogenicity test

This is an optional but important test because of low success rate. The fungus should be able to produce false smut disease upon inoculation into a susceptible genotype by fulfilling all conditions of disease development (Fig.6).

Fig.6. Inoculation of *U. virens* on rice.

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