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IN VITRO GERMINATION OF THE TELEUTOSPORE OF *USTILAGO CONSIMILIS* (SYDOW.)

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In vitro germination of the teleutospores of the fungus *Ustilago consimilis* (Sydow.) the causitive pathogen of the reed *Narenga porphyrocoma* (Bor.) was very difficult. The spores failed to germinate under normal condition. However, with the regulation of temperature, media and pH it was made possible to germinate the spores of this fungus.

Keywords : Media; Minerals; pH; Teleutospores; *Ustilogo consimilis.*

Introduction

Ustilago consimilis (Sydow.) is a causative fungus that brings about infection to the common reed, the *Narenga consimilis* (Bor.) bringing considerable damages of the host at the inflorescence tissues. The fungus creates hypertrophy at the inflorescence head which looks like spindle shape. When the infection is complete the entire inflorescence tissues are converted into black mass of spores. This black mass is a good protein source and consumed by the local people of Manipur with flavoured taste. In the present investigation attempts have been made to *in vitro* germination of the spores (Teleutospores) of the fungus in different physical parameters. It is reported that the *in vitro* germination of these teleutospores under normal condition is found difficult and in all attempts made so far it was found failure[1,2]. During this research programme the authors came to realise that temperature, pH, media, wetting and drying, and a mass spore effect, were the different physical parameters that are essential for the germination of fungal spores.

The present investigation was undertaken to study the physiology behind the *in vitro* germination of teleutospores of *Ustilago consimilis* in relation to ranges of pH and nutrient solution.

Materials and Methods

The teleutospores of *Ustilago consimilis* were collected from the hypertrophy growing in the experimental pots. In the present investigation two types of spores were selected depending upon the maturity of the spores.

i) *Premature spores* : At this stage spores were adhering closely to the columella of the host plant. The shooty mass of spores were covered by a thin white membraneous layer. The whole structures were again covered tightly by leaf sheaths.

ii) *Mature spores* : At this stage the tighly arraged leaf sheaths opened naturally. Spores were started blasting from the columella.

For the study of shape and colour some of these spores were being examined microscopically in a drop of distilled water by Ellis method[3]. Growth of the germ tubes were studied in martin's Rose-Bengal solution without agar.

One drop of autoclaved Rose-Bengal solution containing spores were put into a cavity slide and covered with slide cover to prevent excessive evaporation of the solution. Controls consists of sterilised

distilled water into separate cavity without Rose-Bengal solution and incubated at room temperture at 28±2°C. Growth of the germ tubes in two different nutrient solutions, viz., Solution 'B'- (KH_2PO_4, Peptone, glucose and Rose-Bengal), Solution 'C' - ($MgSO_4$, Peptone, Glucose, Rose-Bengal), comparing with Rose-Bengal (Solution A) within 24 hours, was also studied.

In the course of investigation germination capacity of the *Ustilago* teleutospores on the minimum and maximum pH values of the culture media was also investigated. pH of the cultured media (Rose-Bengal) was adjusted at pH 2 to pH 11 with the help of a pH meter using KOH and KCI before autoclaving with 15 minutes at 15 atms. It was then poured into the culture tubes and petri dishes (9 cm in dia) and smeared the spores uniformly in triplicate replication.

Results and Discussion

Seventy percent of the teleutospores are spherical to elliptical in shape (Fig. 1) started germination at room temperature

Table 1. Growth rate of teleutospore germ tubes of *Ustilago consimilis* in three different nutrient solutions within 24 hrs. at 28±2°C.

Sl. No	Solutions	Growth rate of germ tubes	
		Mature spore (MS)	Pre-mature spore (PMS)
1.	Solution - A	17.5 µm	Nil
2.	Solution - B	9.6 µm	Nil
3.	Solution - C	11.7 µm	Nil

Table 2. Germination intensity for teleutospore of *Ustilago consimilis* in different pH levels of Rose-Bengal media.

Sl. No	pH of the culture media	Intensity of germination	
		Mature spore (MS)	Pre-mature spore (PMS)
1.	2	-	-
2.	3	-	-
3.	4	+	-
4.	5	+	-
5.	6	+	-
6.	7	+	-
7.	8	+	-
8.	9	+	-
9.	10	+	-
10.	11	-	-

(+ geminated; - not germinated)

Solution A - KH_2PO_4, $MgSO_4$, Peptone, Rose-Bengal, Distilled water.

Solution B - KH_2PO_4, Peptone, Glucose, Rose-Bengal, Distilled water.

Solution C - $MgSO_4$, Peptone, Glucose, Rose-Bengal, Distilled water.

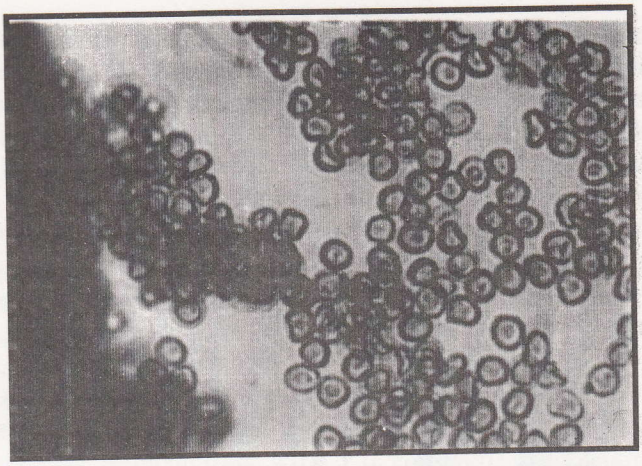

Fig. 1. Shape of teleutospores of *Ustilago consimilis.*

Fig. 2. Germination of teleutosporeswith promycelium of *Ustilago consimilis.*

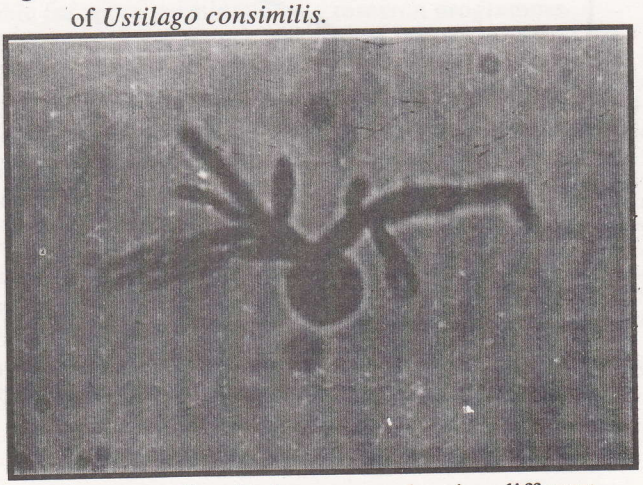

Fig. 3. Promycelia of teleutospore showing different branches in *Ustilago consimilis.*

Soln. A- Potassium dihydrogen phosphate + Magnesium sulphate + Peptone + Glucose + Rose Bengal.
Soln. B- Potassium dihydrogen phosphat + Peptone + Glucose + Rose Bengal.
Son. C- Magnesium Sulphate + Peptone + Glucose + Rose Bengal.

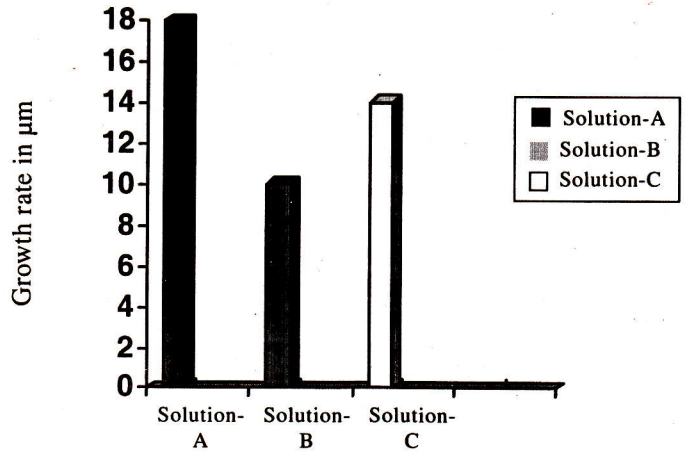

Fig. 4. Growth rate of the germ tubes of *Ustilago consimilis* teleutospores in three different nutrient solution in 24 hours.

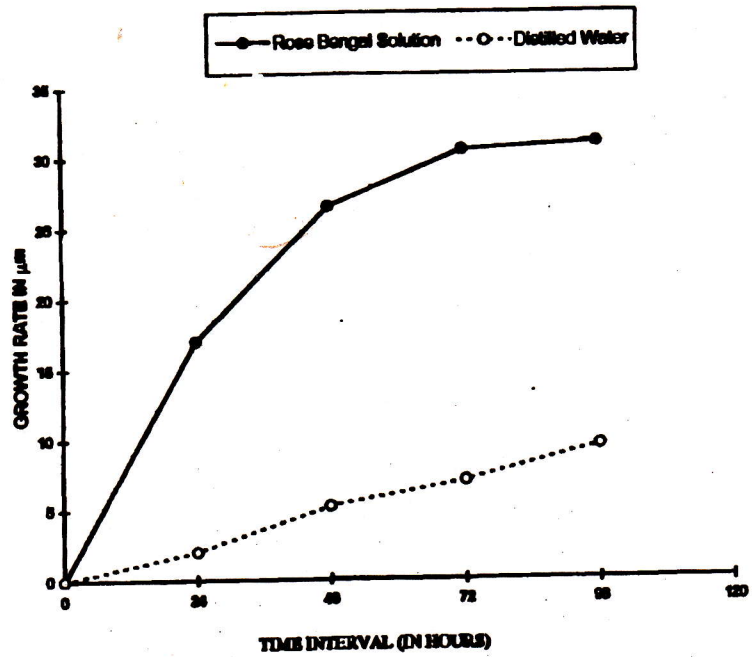

Fig. 5. Growth of the germ tubes of *Ustilago consimilis* teleutospores in 96 hours.

(28±2°C) and relative humidity (80-90%) in Martin's Rose-Bengal solution. Premature spores did not germinate at all. Germination was delayed in distilled water but after 48 hrs germination proceeded with low percentage and growing rate of the germ tubes.

Germination of the teleutospores started with the formation of slit like opening at one end due to crack in the spore wall. The germ tubes started producing into the opening. The germ tubes got elongated and formed 2-6 septate containing promycelium with vacuolated cytoplasm, which may be branched or unbranched (Fig. 2, 3). Each spore porduced 1-3 germ tubes. The length of the germ tubes increased in length upto 96 hours after that their growth ceased and the septate basidium started fragmentation at the respective septums. The growth rates of germ tubes of *Ustilago consimilis* teleutospores in different media are shown in Figs. 4, 5.

The fungus can grow vigorously between pH 4-10. The teleutospores was unable to grow below and above those levels. Spores of most rust fungi germinate with limits at pH 3 and pH8. Munjal[5] also reported that pH range of 4-9 as a favorable for spore germination. But the present findings do not agree with it. The pH value may alter the internal conditions of the spore by changing the protoplasmic contents and increasing the permeability of spore wall to water and nutrients[6] (Table2).

Germination of the *Ustilago consimilis* teleutospores in relation to mineral nutrients shows that solution (A) having mineral nutrients of KH_2PO_4, $MgSO_4$, Peptone and glucose showed the maximum growth of germ tubes over solution 'B' and 'C'. The results suggest that potassium, phosphorus, sulphate, magnesium, peptone and glucose are needed for the active germination and growths of germ tubes of *Ustilago teleutospores*. When there is deficiency in any one of the above nutrients, the growth of the fungus gets retarded. Cochrane[4] reported that fungi have relatively large requirements of P, K, S and Mg. (Table 1).

From the above findings it can be suggested that premature spores of *U. consimilis* did not germinate at all due to lack of morphological and physiological maturity. Mature spores can germinate within 24 hours and can readily grow between pH 4-10. In the nutrient solutions the germ tubes show growth upto 96 hours, after that fragmentation of the germ tubes started at the respective septa for vegetative propagation (Fig. 2, 3).

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