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Research Article

Effect of plant lectins on *Ustilago maydis* in vitro

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Abstract. *Ustilago maydis* is an edible parasitic basidiomycete, which specifically infects corn (*Zea mays*) and teocintle (*Z. diploperennis*). To characterise the interaction between the basidiomycete and its host organism, we tested the effect of plant lectins with well-known sugar specificity on the growth and germination of *U. maydis* spores. Lectins specific for N-acetyl-D-galactosamine, such as those from *Dolichos biflorus* and *Phaseolus lunatus*, and the wheatgerm agglutinin specific for N-acetyl-D-glucosamine inhibited spore germination, but were ineffective in modifying *U. maydis* cell growth. The galactose-specific lectin from the corn coleoptyle inhibited both germination and cell growth, while the lectin concanavalin A (mannose/glucose specific) activated spore germination and growth. Our results suggest that specific saccharide-containing receptors participate in regulating the growth and maturation of *U. maydis* spores.

Key words. Lectin; plant-pathogen; cell wall; corn smut; *Ustilago maydis*; basidiomycte; carbohydrate.

Ustilago maydis belongs to the family of basidiomycetes, which includes several edible fungi. It is a parasite that induces tumours exclusively in *Zea mays* and *Zea diploperennis* [1, 2]. The fungal disease due to *U. maydis* is known as 'corn smut disease'. In Mexico, it is called 'Huitlacoche', a word derived from an ancient indigenous language, and it is considered a delicacy. The disease is characterised by large black tumours in leaves, stems, tassels and kernels. Symptoms include initial chlorosis or pigmentation due to anthocyanin production followed by gall formation [3]. *U. maydis* has two distinct life cycles: a haploid non-pathogenic and a dicaryotic pathogenic filamentous form. The fusion of two haploid cells with different alleles generates a dicaryotic filament present only in plants with tumours [3]. The fungus infects the plant by entering stomata and stigmas [4]. Nevertheless, the specific mechanisms of fungal recognition in the host are unknown at the present time.

Plant lectins are proteins with saccharide-binding properties, whose biological role in the plant is ill-defined [5]. Recent findings have indicated that lectins play a crucial role in symbiotic nitrogen fixation in legumes [6] and contribute to plant defence against fungal and insect attack [7]. Lectins from *Triticum vulgaris* (wheat germ agglutinin, WGA) [8–10], *Solanum tuberosum* [11], *Glycine max* [12] and *Urtica dioica* [13, 14] have been shown to inhibit the infectivity capacity of the fungus, suggesting active participation of carbohydrate residues as specific mediators of parasite-host recognition. The aim of the current work was to identify, with the aid of lectins with well-known sugar specificity, the participation of surface carbohydrate structures that could be implicated in the regulation of germination and development of the pathogenic fungus in vitro.

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Materials and methods

Lectins. The galactose-specific lectin from corn (*Z. mays*) coleoptile (CCL) was purified by affinity chromatography [15, 16]. Concanavalin A (Con A) from *Canavalia ensiformis*, specific for mannose/glucose (Man/Glc) was purified by affinity chromatography according to Agrawal and Goldstein [17]. *Phaseolus lunatus* Sylvester Baudet lectin (PLA), specific for N-acetyl-D-galactosamine (GalNAc) was isolated according to Feria et al. [18]. Lectins from *Dolichos biflorus* (DBA; GalNAc) and wheatgerm (WGA; *T. vulgaris*) specific for N-acetyl-D-glucosamine (GlcNAc), as well as all sugars used in this study were obtained from Sigma (St. Louis, Mo).

U. maydis. Spores were collected from tumour samples present in fresh corn kernels. The spores were washed extensively with sterile distilled water and inoculated (1×10^4 cells/ml) in small test tubes containing potato-corn culture medium (50 g potato, 50 g corn, 5 g sucrose, 250 ml water) [19].

Effect of lectins on *U. maydis* spore germination and fungal growth. Lectins were dissolved in phosphate-buffered saline (PBS; 0.01 M sodium phosphate, 0.14 M sodium chloride, pH 7.2), and sterilised by filtration through a sterile 0.22-µm filter and added to *U. maydis* cultures. Spore cultures (1×10^4 cells/ml) containing lectin at various concentrations (100–500 µg/ml) were cultured in the dark at room temperature (23 ± 3 °C) for different time periods. Aliquots of the cell cultures were obtained at various intervals from 6 to 54 h, and examined by light microscopy with an American Optical microscope (Model 60-63 American Optical Corp., Buffalo, N.Y.). Germinated spores were located and counted with the aid of a haemocytometer. Specificity of the interaction of lectins with fungal spores was determined in sugar inhibition assays by incubating the lectins for 30 min with their specific carbohydrates (at 15 mM concentration in PBS) prior to adding to the spore cultures.

Fungal growth was also determined by measuring the turbidity in microplates as follows. Aliquots of spores (800 cells/80 µl), under sterile conditions, were seeded in 96-well microplates (Nuaire, New Hampshire) in corn-potato culture medium, and incubated in the dark at room temperature (23 ± 3 °C) for different time periods. The volume of the spore cultures was adjusted to 120 µl by adding PBS. The plates were examined for fungal growth by assessing the increase in the turbidity of the culture medium [20] at $OD_{405\ nm}$ in an ELISA reader (Stat Fax-2100 Awareness, Mountain View, Calif.). Spore aliquots (80 µl) were incubated with Con A (200 µg/20 µl) and CCL (65 µg/20 µl) in the conditions indicated. The effect of lectins on spore germination and fungal growth was obtained by comparing the $OD_{405\ nm}$ of spore growth with those aliquots incubated in the presence of the lectins, at different concentrations. The $OD_{405\ nm}$ readings representing *U. maydis* growth curves were plotted against time. Control experiments were performed using lectins, previously incubated with 15 mM of their specific sugar, in each independent assay. The ELISA reader was calibrated to 0, using the corn-potato culture medium containing lectin at each tested concentration.

Protein concentration. Protein concentration was determined according to the Bradford procedure [21] using Coomassie blue G-250 (Sigma) and bovine serum albumin (Sigma) as a standard.

Statistics. Statistics were calculated using the one-way Dunnet multiple-comparisons test.

Results

Effect of lectins on germinated spores. Germination time was determined by light microscopy. After 24 h culture, *U. maydis* showed the characteristic emerging spore germ tips (fig. 1b). In the presence of 100 µg CCL, the number of germinated spores decreased by 20% (figs 1a, 2). At higher concentrations of CCL, the number of germinated spores also increased again, and at 500 µg CCL, the percentage of germinated spores was similar to that in the control without lectin or of spores treated with lectin incubated previously with 15 mM Gal. Addi-

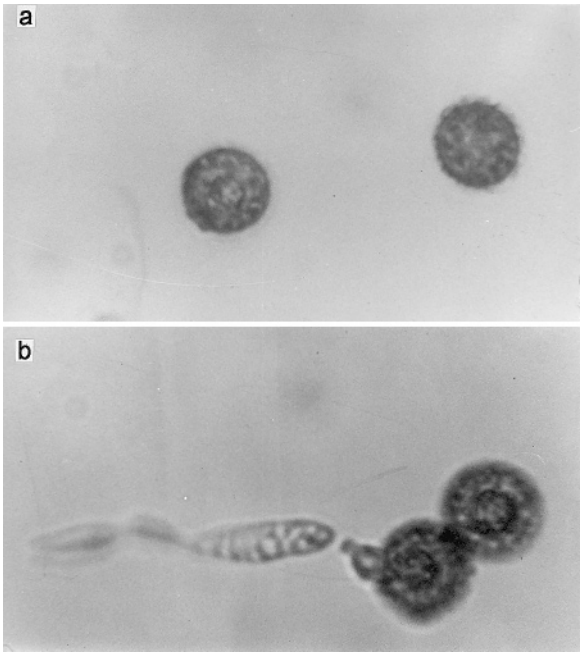

Figure 1. *Ustilago maydis* spore germination under light microscopy. Spores (1×10^4 cells/ml) with 100 µg/ml CCL (*a*) or Con A (*b*) were cultured in the dark at room temperature (23 ± 3 °C) for 24 h. Positive germination was determined by the presence of emerging spore tips. × 80.

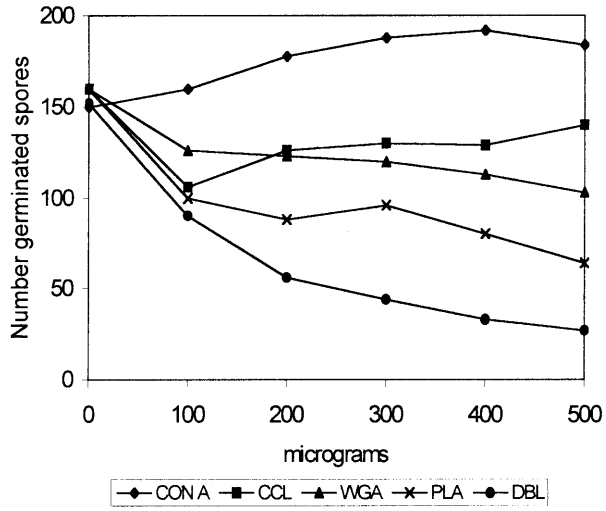

Figure 2. Effect of lectins on the rate of *U. maydis* spore germination. Lectins from Con A (◆), corn coleoptyle (■) (CCL), wheatgerm agglutinin (▲) (WGA), *Phaseolus lunatus* (x) (PLA) and *Dolichos biflorus* (●) (DBL) at different protein concentrations in 100 µl PBS were added to 1×10^4 spores and cultured at 23 ± 3 °C for 24 h. Positive germination was determined by light microscopy by identifying emerging culture tips in 200 cells from separate cultures. Data are the means of three independent experiments.

tion of 100 µg WGA, PLA and DBA lectins to the spore cultures reduced the capacity of the spores to germinate by 30%. This effect was observed after 24 h incubation (fig. 2), but the inhibition persisted for the 54 h of culture. In all cases, the lectin effect was inhibited by the addition of 15 mM of its specific sugar, GlcNAc for WGA, and GalNAc for PLA and DBA. Con A, at 100 µg, activated spore germination by 30% compared with no untreated cultures (fig. 2). As with the other lectins, the effect of Con A was inhibited by the addition of 15 mM of its specific sugar, α-methyl-mannoside.

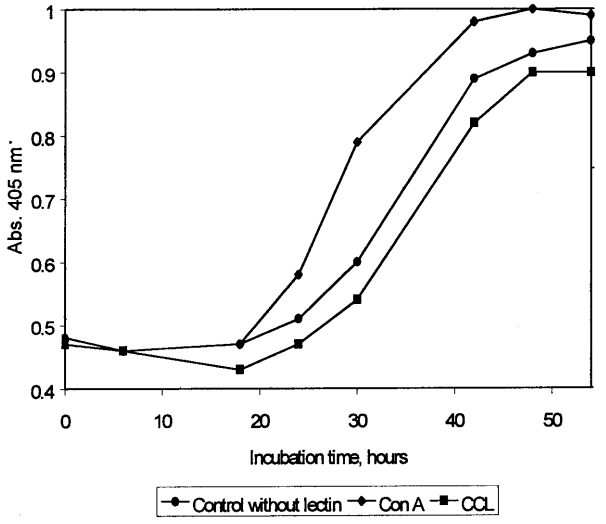

Figure 3. Effect of CCL and Con A on fungal growth in the presence of 65 µg of CCL (■) or 200 µg Con A (◆). Control without lectin or sugar (●). The OD values were obtained at 405 nm at room temperature (23 ± 3 °C) in potato-corn medium (see Materials and methods). The Con A and CCL effects on *U. maydis* growth differed significantly ($p < 0.05$) (Dunnet multiple-comparisons test).

Effect of lectins on *U. maydis* growth in vitro. Culture growth was measured in microplates by determinining the $OD_{405\ nm}$ of the preparations. Incubation of *U. maydis* with WGA, DBA or PLA did not modify culture growth. CCL induced a slight inhibition at 20 h incubation, which lasted for the duration of the experiment (54 h) (fig. 3). The addition of 200 µg Con A to *U. maydis* microcultures induced, after a 20-h lag period, culture activation (fig. 3). This effect was abolished when Con A had been previously incubated with α-methyl-mannoside. A significantly different effect on *U. maydis* growth was obtained between Con A and CCL ($p < 0.05$) (fig. 3).

Discussion

U. maydis is a basidiomycete which parasitises and induces tumours specifically in *Zea* species (i.e. *Z. mays* and *Z. diploperennis*) [1, 2], suggesting the presence of specific signals or receptors which allow it to recognize and infect its specific host. In this work, we studied, with the aid of lectins, the possible participation of sugar receptors as determinants of fungal specificity.

Some lectins added to viable and germinating *U. maydis* induced modifications in the normal growth of the fungus. The lectins from wheatgerm, specific for GlcNAc, and from *P. lunatus and D. biflorus*, specific for GalNAc, were able to inhibit spore germination. These results appear similar to those of previous studies describing the effects of these lectins on filamentous fungi [8, 9]. CCL is a Gal-specific lectin [22] able to inhibit both spore germination and fungal growth. These effects can be inhibited with Gal [22]. Since *U. maydis* is a parasitic fungi that exclusively affects the genus *Zea*, the interaction with a corn constitutive lectin suggests a potential physiopathological association.

Antifungal assays have recently shown that growth of germinated spores of the fungi *Botrytis cinerea*, *Trichoderma viride* and *Colletotrichum lindemuthianum* is inhibited by stinging nettle isolectin I at a specific phase of fungal growth [14]. The effect is temporal, suggesting that the fungi have developed an adaptation mechanism.

Analyses of the chemical composition of *U. maydis* cell walls indicate that mannose, glucose, xylose and galactose are present in various complex structures [23]. These carbohydrates may well become active as lectin receptors.

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