Smut of Corn Smut and Protein by Atomic Force Microscopy

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Abstract
Understanding the surface morphology of cells is an important basis for further understanding of cell function and exploring life phenomena. On the one hand, AFM and optical microscopy were used to characterize the surface morphology of S. cerevisiae cells, and the effects of bacteriostatic agents, wilting and hygromycin on the surface morphology of S. cerevisiae cells were observed. Further analysis explained the mechanism of drug influence on cells; on the other hand, the interaction of bud-coating proteins was studied by AFM single molecular force spectrum. The morphological changes of the growth of the sphaerotheca fuliginous during the haploid period were observed by AFM, and the black powder bacteria in the air for a long time were observed. The morphology of the growth of the haploid tube of the black powder fungus and the surface morphology of the microbial cells of several tens of nanometers can be clearly observed by the AFM image. The effects of bacteriostatic agents, wilting and hygromycin on the cell morphology of S. cerevisiae were studied by AFM. The results showed that the rust-free spirit not only inhibited the growth of the black powder fungus, but also appeared many small pits on the surface of the black powder fungus.

Key words: Atomic Force Microscopy, Sphaerotheca Fuliginous, Surface Morphology, Bud Gestation Protein, Single Molecular Force Spectrum

1. Introduction
Corn black powder fungus, also known as maize black powder fungus, corn black mold gray grain valley, stick package, etc., is one of the common corn pathogenic fungi. Corn black powder disease is a local invasive disease caused by corn black powder fungus. Corn black powder bacteria are rich in chemical nutrients: 16 amino acids including nucleic acid, fumaric acid, glutamic acid, arginine, lysine and alanine. It also contains various nutrients such as glycolipids, monosaccharides, keratins, 2, 3 epoxy decene, wool polyols, ergosterol lipolytic enzymes and iron pigments. After inoculation, corn black powder can form black powder tumor on the surface of corn tissue. It has sweet taste when it is young and can be eaten. Regular consumption can prevent and treat liver system and gastrointestinal ulcers, and can help digestion and laxative. It can treat blood deficiency, lack of fluid or fever and qi and yin. Fresh crunch piles or old cooked dumpling powder can be used. Based on the nutrients of the genus Helicoverpa armigera, many research teams have conducted in-depth research on the composition and application of the smut. Zhang Jiaqi and others used electronic tongue-assisted detection technology to evaluate the taste of two corn varieties and three different stages of corn smut [1]. The results showed that the main tastes of the smut of corn smut were sweet, umami, sour and salty; the two corn cultivars had no significant effect on the sweetness and umami taste of the smut of corn smut, and the sweetness was observed during the harvesting period. The umami effect is significant. If corn sphagnum can be used rationally, it can turn waste into treasure, with far-reaching social, economic and environmental benefits. Li Shengyan pointed out that it has been shown that the pathogens and pests can induce the expression of TPS6 after damage to maize, which indicates that the TPS6 gene may be involved in the pest-resistant process of maize. The expression of TPS6 gene in maize inbred line B73 was detected by real-time quantitative PCR. Wang Zongfang and others suggested that it is best to use the fungicides triazolone, diniconazole, difenoconazole, flucarizole, propiconazole, thiram, etc. Before or during the onset of disease, select one of the 800~1000 times solution to spray the plant to reduce the incidence rate, interval 7~10d, and spray 2~3 times, which can effectively reduce the risk of reinfection. Lei Yuming used seed treatment and chemical spray to determine the efficacy of six pesticides and the safety of corn. The safe concentration of the six kinds of pesticide seeds is 0.10%~0.30%, and the optimal mass concentration of the medicament spray is 1250-2500
Based on atomic force microscopy (AFM) and analyzed the factors affecting the deposition process embedded real atomic force. The microscope reflects the research of lithium properties of electrode materials under electrochemical reaction conditions, indicating that AFM will further promote the research of lithium in tapping mode, the probe, and using a spherical probe micro cantilever model to calculate the atomic force microscope operation in mechanics. Liu Shihua used a silicon microsphere to adhere to a needleless micro cantilever to make a spherical powder and zinc oxide powder. AFM topography test was conducted. Song Cui and others proposed that the use of AFM to detect particle-cell interactions helps determine important parameters in the action process and explains the deeper mechanisms of particle application in drug delivery [5], immune response, and cell mechanics. Liu Shihua used a silicon microsphere to adhere to a needleless micro cantilever to make a spherical probe, and using a spherical probe micro cantilever model to calculate the atomic force microscope operation in tapping mode, the tip-sample distance is extremely small (micron) In the case of the grade, the effect of the membrane damping between the tip and the sample. Gao Xiang reviewed the latest application progress of AFM in lithium-ion battery research [6], including the morphology change, Nano-mechanical properties and electrical properties of electrode materials under electrochemical reaction conditions, indicating that AFM will further promote the research of lithium-ion batteries. Cheng Liqun proposed an optical path automatic adjustment method based on image processing and least squares method [7]. By installing a computer-controlled, two-dimensionally movable motor on the probe holder of the AFM system, the algorithm is used to achieve atomic force. The microscope reflected light path is automatically adjusted. Wang Xuming gave an overview of the application of atomic force microscopy in the field of mineral engineering research from the aspects of hydrobicity and surface electrical properties of minerals [8], analysis of interaction between mineral particles and bubbles and agents, and imaging of mineral surface microscopic morphology. Gao Qian et al. use two high-performance microcontrollers of ARM Cortex-M4 core for frequency demodulation and system control. The frequency demodulation is designed by software phase-locked loop. The control system adopts embedded real-time operating system CMSIS-RTOS. RTX performs task management and scheduling to ensure the real-time performance of the system. Liu Zenglei studied the field evaporation deposition processing method based on atomic force microscopy (AFM) and analyzed the factors affecting the deposition process [9].
general, the deposition process has good repeatability and controllability. Li Dan designed an AFM scanning imaging controller based on iterative learning algorithm [10]. By transforming the scanning motion in the horizontal plane into the path tracking control problem, the non-causal learning of the error information of the previous iteration period is carried out during the tracking process to ensure the fast convergence of the output along the iterative axis to obtain the ideal tracking performance.

In order to solve the problem that the smut of corn smut is not detailed and the image is not clear [11], the different surface morphological structures of the haplotype cells of S. cerevisiae are observed by AFM. The AFM map can clearly observe the combination of smut, changes in the morphology of tube growth and surface morphology of tens of nanometers. AFM scanning was used to observe the different effects of bacteriostatic drugs, wilting and hygromycin on the morphological changes of the cells of sphaerotheca fuliginous, and the different action time and different concentration of rust and rust were studied. It influences surface topography. The interaction of bud-coating proteins was studied by AFM single-molecule force spectroscopy. The interaction forces were measured at different loading rates. The relationship between the binding force and the logarithm of loading rate was used to calculate the CotE protein. Dissociation rate is constant. To lie the foundation for the subsequent implementation of CotE controllable self-assembly in vitro.

2. Method

2.1. How AFM Works

The AFM is mainly composed of a laser detection system, a force sensor, and a displacement control system. The optical detection system consists of a prism, a mirror, a laser diode, and a four-quadrant photodiode detector. The force sensor consists of a flexible micro cantilever and a pointed tip that is attached to a rectangular substrate of the same material. The displacement control system consists of a piezoelectric ceramic tube and its control circuit. By applying an external voltage to the piezoelectric ceramic tube, it can be precisely moved in three directions of X, Y, and Z. Figure 1 shows the working principle of the atomic force microscope.

![Figure 1. Atomic force microscope working principle diagram](image)

The basic principle of AFM is to analyze the surface properties of the sample by detecting the interaction between the sample and the tip. The detection probe uses a sensitive and elastic micro cantilever with a tip on one end and a fixed end on the other end to detect the sample to obtain surface topography or other surface properties. Usually the sample is placed on a piezoceramic platform. When the distance between the tip and the surface of the sample is close enough, an interaction force is generated between the tip and the sample, causing the micro cantilever to be deflected by force. When the laser illuminates the back surface of the micro cantilever, the micro cantilever shoots the laser beam to the photoelectric by reflection. On the detector, the difference in laser intensity received by the detector in different quadrants is proportional to the amount of deflection of the micro cantilever, and the amount of deflection of the micro cantilever acts as a direct measure of the interaction of the sample with the tip. At the same time, this deflection signal is recorded by the optical detection system, and the signal can be directly used to construct an AFM image or as a feedback signal [12]. The feedback system continuously adjusts the position of the tip or sample in the Z-axis direction based on changes in the detector voltage to maintain a constant force between the tip and the sample. By measuring the change of the detector voltage corresponding to the scanning position of the sample, a three-dimensional topographic image of
the atomic level of the sample surface is obtained [13]. Some AFMs use a piezoceramic tube to control the tip movement and the sample to be tested remains stationary.

2.2. AFM-Based Single Molecule Force Spectrum

When the modified probe is far from the surface of the sample, the tip of the needle is not attracted by the sample molecules and is in a relaxed state. At this time, the ordinate has no transition of the optical detection signal, that is, a weak response signal, and the recorded scanning signal is the baseline. When the tip of the needle approaches the sample until it contacts the surface, the micro cantilever deflects due to the repulsive force between the modified probe and the surface of the sample. When the modified probe is in contact with the sample, the molecules on the sample are combined with the tip modifier molecule by specific interaction, physical adsorption or chemical bonding. When the piezoelectric ceramic tube is moved away from the probe, the molecules that bond the modified tip to the sample are stretched, causing the micro tip of the tip to bend. The strong signal on the force curve is generated, and when the probe retreats to a certain extent, that is, the elastic force of the cantilever exceeds the binding force of the binding molecule, the weaker part of the binding molecule will fall off in turn or the breakage causes the two to separate quickly. The degree of bending of the micro cantilever rapidly causes a large deflection, and then returns to the original relaxed state.

This process is performed on the force curve to quickly return to zero for the force value. This force value can be attributed to the magnitude of the dissociation force of the tip-modified molecule to the surface of the sample or the binding strength of the specific interaction. When each single-molecular force spectrum measurement cycle (approximation-retraction) is completed, the deflection signal is recorded by the optical detection system to obtain a force-distance curve. The force value $F$ of the measured data in the AFM is calculated by the formula:

$$F = k \cdot x$$

It is calculated that $k$ is the tip elastic constant; $x$ is the degree of bending. In a single molecular force spectrum, the magnitude of the dissociation force of the receptor/ligand interaction depends not only on the strength of the receptor/ligand interaction, but also on the loading rate of the probe. Where the loading rate: and the stretching rate has the following relationship

$$r = v \cdot k_{eff}$$

Where $k_{eff}$ is the effective elastic constant, the dissociation kinetics of the ligand/receptor complex can be obtained by measuring the dissociation force at different loading rates. The dissociation force $f_u$ is linear with the log rate of the probe loading rate plant:

$$f_u = \left( k_n T / x_n \right) \ln \left( r x / \left( k_n T k_{eff} \right) \right)$$

Wherein $k_{off}$ is the dissociation rate constant of the complex at zero force; $k_n$ is the Boltzmann constant; $T$ is the thermodynamic temperature; $x$. The energy barrier required for the separation of the relative equilibrium position. The strength of the intermolecular force, the dissociation constant, and the like are obtained by statistical calculation.

2.3. AFM Probe and Sample Fixation

(1) AFM probe

One of the core components of the AFM is the microprobe. An AFM probe consists of three parts: a substrate, a micro cantilever, and a tip. The micro cantilever is usually fabricated by photolithography of silicon (Si) or silicon nitride (Si3N4). The back of the cantilever is coated with a layer of metal to achieve reflection. For microprobes, the following characteristics are required: low force elastic coefficient; high force resonance frequency; short force elastic coefficient; high reflectivity on the back of the micro cantilever; and sharp tip as possible. Figure 2 is a scanning electron micrograph of the needle tip. With the use of the AFM probe, the probe needs to be cleaned to keep the tip surface clean and to ensure image quality [14].

700
Figure 2. Scanning electron micrograph of the needle tip

(2) Fixation of the sample on the substrate or tip
When the interaction between the samples to be tested and the substrate or tip is weak, a donor (or acceptor) functional group can be modified on the sample to modify the corresponding acceptor (or donor) on the substrate and tip. By this interaction with a receptor, the molecular segment forms a bridging structure between the tip and the substrate, or the corresponding non-damaging binder is modified on the target sample or substrate to stably stabilize the target sample onto the substrate. As shown in Figure 3. Hinterdorfe et al. have improved the method of immobilizing proteins by linking a protein to a protein via a polymer PEG polymer chain, which not only increases the spatial freedom of antibodies and antigenic proteins, but also distinguishes needle tip and force from a force-distance curve to some extent. The non-specific interaction of the substrate becomes one of the commonly used methods for immobilizing proteins. Figure 3 is a needle tip modified NHS-PEG18-aldehyde chain.

Figure 3. Tip modification diagram

3. Experiment

3.1. Reagents and Main Equipment Used in the Experiment

(1) Main reagents used in the experiment

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(2) The main instruments used in the experiment
Table 2. Main instruments and specifications

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<td>Beaker Bottle</td>
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3.2. Preparation and Experimental Conditions of Corn Black Powder

(1) Black powder bacteria culture treatment

The corn black powder fungus was provided by the Jilin Academy of Agricultural Sciences. The strain was added to the liquid culture solution and shaken in a rotary shaker for about 12 hours (28 °C); the black powder of different morphology was obtained. 100 μg/ml of hygromycin was added to the bacterial strain of the black powder fungus haploid strain, and the drug was cultured for 8 hours under the conditions of sufficient nutrition and shaking. Obtain black powder fungus after hygromycin treatment. The smut of haploid bacteria haploid strain was divided into 5 equal portions, and added with 1μg/ml, 2μg/ml, 4μg/ml, 8μg/ml and 10μg/ml, respectively, under the condition of sufficient nutrition and shaker. Incubate for 8 hours. Obtain black powdery fungi after different concentrations of rust and rust. The mellow bacteria haploid strain was divided into 3 equal portions, and 4 μg/ml of rust was added separately, and cultured for 2, 4, and 8 hours under sufficient nutrient and shaker conditions. Obtain the black powder fungus after the rust and rust effect for different time.

(2) Substrate treatment

The slides were cut to the appropriate size for the AFM scan sample substrate, and the glass slides were soaked with absolute ethanol for one hour, rinsed with Milli-Q water and then with piranha solution (H2SO4: H2O2 = 3:1 (v/v) ) Soak in the surface for 1 hour by ultrasonic soaking, remove it, rinse it with Milli-Q water, blow it dry with nitrogen, stuff it into the centrifuge tube for sealing or drop 2 μL of Cell-Tak cell binding solution on the slide, place it about After 2 hours, it was washed 3 times with 70% ethanol, and stuffed into a centrifuge tube for sealing. Drying a 1.2 cm x 1.2 cm square coverslip was washed in the same manner, placed in a clean biological Petri dish, and sealed for use as a sample substrate for an optical microscope.

3.3. Modification of Sample Substrate and AFM Probe

(1) Modification of the sample substrate

Phosphate buffer solution PBS (pH 7.5): 0.1225g KH2PO4 + 1.3535g N2HPO4 + 0 · 106g KCl +4.0908NaCl constant volume 500ml configuration, and then filter the insoluble matter with a filter. The maximum use period is one month, and it is stored in the refrigerator at 4 °C. NaCNBH3 solution: 32mg NaCNBH3 + 50μL of 100mol / L NaOH and 450μL H2O Chromic acid solution: Dissolve 5g-10gK2Cr2O7 in a small amount of hot water. After cooling, slowly add 100mL concentrated sulfuric acid, stir, cool and then inject into the desiccator to seal.

Experimental preparation:

The glassware is washed successively with detergent, tap water, and soaked in chromic acid washing solution overnight. The next day, it is washed with a large amount of water and Milli-Q water to ensure that the surface of the instrument is clean and the instrument is placed in an oven for drying. The dialysis bag clips, magnets and tweezers were sequentially boiled with 1% sodium dodecyl sulfate, ethanol bath, and Q-water bath for 30 minutes. Before the AFM test, the liquid pool was ultrasonically washed three times with Milli-Q water for 2-3 minutes each time, and another suitable size of mica was prepared as the bottom of the liquid pool, which was used when it was used.

(2) PEG-Linker modification of silicon wafer

Add 100 μL of chloroform to a glass vial of a bifunctional cross-linked chain PEG-Linker (about 3.3 mg/ml, NHS-PEG18-aldehyde, polyethylene glycol with oxime imine and aldehyde functional groups, respectively)
And 5 μL of triethylamine, the silanized silicon wafer was washed twice with chloroform, and the modified silicon wafer was immersed in a PEG-Linker solution for 2 hours. The amino group on the silicon wafer was reacted with hydrazine on the PEG-Linker to crosslink the PEG-Linker onto the silicon wafer. The reacted silicon wafer was washed with chloroform for 3 times, then gently blown dry with hydrogen, and placed in a desiccator to pass hydrogen gas for use. Figure 5 is a chemical modification of the AFM tip.

4. Results and Discussion

4.1. AFM Map before and after the Action of Corn Black Powder

In the experiment, the black powder fungus haploid before the action of the drug was used as a comparative reference, and the bacteria maintained the original body shape without dehydration or serious damage. Figure 4 is a diagram showing the surface topography and deflection diagram of the haploid of black powdery fungus without drug treatment. It can be seen from the figure that the body shape of the black powdery fungus without drug action is rounded and has a certain height. It can be seen from the D deflection diagram in Figure 4 that the surface is not very smooth and has some convex or irregular texture. The roughness RMS was used to characterize the surface roughness RMS of the cells in the range of 2 micrometers. The sample selection of n>20 shows that the average roughness of Figure 5 are 6E+01-12E+01nm.

![Figure 4](image1.png)

**Figure 4.** Surface topography and deflection diagram of black powdery bacteria without drug treatment

After 8 hours of hygromycin incubation, the black powder fungus was characterized by AFM. It can be seen from Figure 5 that the halophyte haplotype stopped growing after the addition of hygromycin, and the size of the body before the addition of the drug did not change much. However, compared with the blank sample, the surface of the black powder fungus became very smooth after the action of the drug, and it can be clearly seen from the small range map that the surface of the bacterial body has no pits, collapses, or texture. This may be due to the specific effects of hygromycin on the cell wall of S. cerevisiae.

![Figure 5](image2.png)

**Figure 5.** Roughness of a 2 micron surface topography of black powdered bacteria without drug treatment

4.2. Morphological Changes of Corn Black Powdery Bacteria after Different Concentrations of Drugs

The experimental fixed culture time examined the effects of different drug concentrations on the black powder fungus. The black powder fungus was treated with 1μg/ml, 2μg/ml, 4μg/ml, 8μg/ml and 10μg/ml rust rust for 8 hours, and the black powder bacteria were characterized by AFM contact mode in air. It was found that as shown in Figure 6, a lot of pits appeared on the surface of the bacteria after the action of the rust and rust, and these pits were randomly distributed on the surface of the cells.
Figure 6. Deflection diagram of different concentrations of rust and rust on black powdery mildew

With the increase of the concentration of rust and rust, the number of surface pits of smut is also increasing. As shown in Figure 7, it can be seen that the number of phlegm pits increases with the concentration of rust.

![Deflection diagram of different concentrations of rust and rust on black powdery mildew](image)

Figure 7. Relationship between the concentration of rust and the number of holes in the surface pit of black powder

From the above experiments, it can be concluded that in the culture process of the black powder fungus, the rust is added, and after the culture is continued for 8 hours, small pits appear on the surface of the bacteria. It is found by statistics that the number of pits appearing on the surface of the bacteria body increases with the increase of the concentration of the rust. The average depth of the pits on the surface of the cells did not change much with the increase of the concentration of the rust, and the average width of the pits increased with the increase of the concentration of the rust.

4.3. Study on the Interaction between the Buds and Coat Proteins CotE

By studying the interaction between the cloning coat proteins CotE and CotE by atomic force single-molecule force spectroscopy, it was found that CotE molecules can recognize each other. Figure 8 is a typical force for the recognition of a CotE-containing substrate with a tip of a CotE protein. The distance curve, in the retraction curve of the force-distance curve, can be seen to show the mutant peak. In order to obtain statistically calculated values, the probability density of the dissociation force is constructed from a force-distance return curve between 1000 CotEs. Figure 8 shows the probability of density distribution of the dissociation force. When the cotE is modified on the tip, a binding probability of approximately 27.2% is measured (solid line in the figure). The maximum value in the Gaussian distribution force curve is the most likely CotE and CotE dissociation force at a certain loading rate. When the single molecule force spectrum measures the dissociation force of the bound protein molecule [15], the dissociation force and the tip point the retraction speed (loading rate) is related. When the loading rate is 3160pN/s, the interaction force is about 49.4pN.
In order to demonstrate that the cleavage peak of the dissociation process is indeed caused by the special interaction of CotE and CotE, the regression curve in a typical force-distance curve is performed by parallel experiments on a tip that only modifies the bifunctional cross-linking chain without linking the CotE protein. The middle mutant signal disappear Figure 8. At the same time, from the calculation of the statistical calculation of the regression curve of 1000 force-distance, the combined rate is reduced to 3.4%. This proves that when the CotE is modified on the tip, the peak in the retreat curve is indeed caused by the weak bond of the CotE and CotE. The experiment clearly demonstrated the existence of mutual binding or specific interaction between CotE and CotE.

5. Conclusions

In this paper, the surface morphology of the cells of S. Cerevisiae was characterized by AFM and light microscopy, and the effects of bacteriostatic wilting and hygromycin on the surface morphology of S. Cerevisiae cells were observed. The analysis explains the mechanism of the effect of the drug on the cells. The AFM single-molecule force spectrum technique was used to observe the CotE protein interaction between the buds and the conclusions were obtained:

(1) Using AFM and light microscopy to observe the different morphologies of the growth of S. Cerevisiae, it can be clearly observed that with the growth of the smut of the sphagnum, the morphological changes of the cytoplasm include short rods and branches, filamentous and so on. The surface morphology of the tens of nanometers can be observed. When the black powder fungus is placed in the air for a long time, it may cause large wrinkles on the surface of the bacteria due to the dehydration of the cytoplasm or vacuole of the cells. At the same time, it can be understood from the small-scale topography that the surface of the cells is in the shape of a granular protrusion.

(2) The mechanism of action of rust and hygromycin on smut is different, which leads to different surface morphology changes. The rust-free spirit not only inhibits the growth of the black powder fungus, but also causes many small pits on the surface of the black powder fungus. It is observed that the number and size of the pits on the surface of the fungus increase with the concentration of the drug and the incubation time. The corresponding is increase and increase. However, hygromycin made the surface of the black powder bacteria smoother, the roughness was reduced, and no pits appeared.

(3) The cotE coat has a weak interaction between the molecules of cotE protein. When the loading rate is 3160pNs-1, the interaction force is about 49.4pN, and within the loading range of 715.95pNs-1 to 19980pNs-1, between CotE the interaction force is linear with the logarithm of the loading rate. The dissociation rate constant of CotE and CotE is calculated to be 0.146s⁻¹, which can establish the kinetic equation and calculate the solution at different loading rates, separation.

References


