Biological Characteristics of the Causative Agent of Cotton Gommosis Xanthomonas Campestris Var Malvacearum

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Abstract: The following article is devoted to the study of morphological-cultural and pathogenic features of six strains of the causative agent of cotton gommosis - Xanthomonas campestris var malvacearum, isolated by us from cotton varieties Sultan and S–6524. The study of the fermentation of carbohydrates and alcohols of six strains of Xanthomonas campestris var malvacearum showed that all the strains we studied ferment carbohydrates and alcohols to form acid, have the ability to break down protein and peptone to form hydrogen sulfide, and cause diseases of cotton gommoses. The study of the morphology of Xanthomonas campestris var malvacearum showed that the causative agent of cotton gommosis has flagella, capsule, shell and nucleoids, and polymorphism of both the individuals and their nuclear apparatus is characteristic of diurnal cultures.

1 INTRODUCTION

As known that diseases of cotton significantly reduce its yield both in quantity and quality, worsen the textile properties of the fiber and the quality of the seeds sown. The threat to the harvest of raw cotton from numerous diseases constantly exists. Gommosis causes significant damage to cotton production.

Nowadays, cotton gommosis is registered in almost every country where cotton is grown. This disease is considered as a serious threat to cotton in India, Pakistan, China, the countries of the former Soviet Union, South America, and Australia.

Gommosis was first described by G.F. Atkinson in 1891. The first report on the appearance of cotton gommosis in Central Asia was made by R.R. Schreder in 1903.

Gommosis affects the aboveground organs of cotton cotyledons and real leaves, stipules, stems, bracts, flowers, boxes. The signs of the disease in all parts of the plant are basically the same. In the first stage of the disease, the affected parts are covered with oily round, angular or elongated spots, which then dry out, darken, become covered with dried mucus and die off.

In 1901, Erwin Smith established the bacterial origin of cotton gommosis and isolated it into a pure culture.

The author gave the first scientific name to the causative agent of cotton gommosis *Bacterium malvacearum*. Bergey (1936) et al called *Phytomonas malvacearum*. In 1949, M.A.Krasilnikov restored its former name *Pseudomonas malvacearum*, noting as a characteristic indicator of the genus *Pseudomonas* the polarly located flagella of non–spore-bearing rods, the presence of fluorescent pigment and some other properties, not considering it advisable to isolate phytopathogenic forms into a special genus - *Phytomonas*.

V.P.Israilskiy (1960) includes the causative agent of gommosis in the genus *Xanthomonas* isolated by Stoughton, which is characterized by abundant mucus formation, yellow color of colonies and monotrichial flagellation.

Many scientists have studied the biological properties of cotton gommosis (Askarova, 1960; Babayan, 1963; Wickens, 1961; Yogan, 1960; Jones, 1983; Grigoryants et al. 2009; Bobonazarov, 2012).

X. malvacearum according to W.H. Burkholder (1932) does not form acids on dextrose, sucrose and lactose.

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According to Nizametdinova (1968), the causative agent of cotton gommosis belongs to a weak acid-forming agent.

X. malvacearum as an acid-forming agent was described by N.A. Krasilnikov, Bergey, Israilskiy, Nizamitdinova.

Other researchers like, Smith, Yevis, Yachevskiy, Gorlenko think that, the causative agent of gommosis does not form acids on environment with sugars.

Data on the morphological-cultural properties of the causative agent of cotton gommosis is limited and contradictory.

Therefore, the literature data indicate that many researches have been devoted to the study of the morphological-cultural properties of the causative agent of cotton gommosis (Verderevsky, 1960; Babayan, 1963; Marupova, 1968; Sattarova, 1973; Shukri Mohamed El Gremi, 1990; Rashidov, 2003; Fallhzaden, Ahmadzaden, 2010; Gulmurodova, 2023).

However, the analysis of the literature materials indicates the need to continue research on the morphological-cultural characteristics of the pathogen causing cotton gommosisn world practice, it has been proven that in pneumatic diluents it is most acceptable to use landing discs rotating along a vertically longitudinal surface, and most companies producing pneumatic diluents produce diluents equipped with just such discs. The research paper considers the main parameters of the landing disc, which rotates on a vertically longitudinal surface, that is, on a horizontal axis located transversely to the direction of movement of the seal (Fattah, 1976; Babanazarov et al., 1993; Babayan, 1963; Verderevsky, 1960; Grigoryats et al., 2009; Beltyukova, 1968).

It is known that in order to obtain higher crop yields, it is necessary to evenly distribute seeds throughout the field, that is, plant them evenly to the desired depth, ensuring a given range and distance to the node. In world practice, when using seeds prepared with high quality, seed sowing is used in a clear norm, one seed per slot.

In addition to the general requirements, specific planting requirements are developed according to the climate and soil of each area. In particular, the soil and climatic conditions of Uzbekistan are such that in the spring sowing period, after precipitation, in most cases tar appears in the soil, and seeds planted in one grain risk getting stuck under it, failing to split the resin.For this reason, when planting rotten seeds or other seed materials, it is advisable to sow them in a slot way, that is, laying 2-3 seeds in each slot (Gulmurodova & Sattarova, 2023; Gorlenko, 1966; Marupova, Sattarova, 1973; Rashidov et al., 2003; Gremi, 1990).

2 MATERIALS AND METHODS

The objects of the research were samples of diseased cotton plants of S-6524 and Sultan variety affected by gommosis, as well as isolates of isolated by us from diseased cotton plants.

Isolation of the causative agent of cotton gommosis was carried out from cotyledon leaves affected by gommosis according to the method of K.I. Beltyukova (1968).

A section of cotton leaf tissue affected by gommosis was placed for 2-3 minutes in 96% alcohol, which was then thoroughly washed with sterile water and placed in Petri dishes with potato–glucose agar.

Pure cultures of phytopathogens were obtained by three-times re-sowing of individual colonies on solid nutrient environment. The appearance and size of colonies were determined on various nutrient environment.

Cell morphology and size, Gram staining, were studied using light microscopy.

The cultural-morphological biochemical and physiological properties of microorganisms were studied according to generally accepted methods and tests described in the relevant manuals (Yegorov, 1976; Bergey S of terminative bacteriology1974).

The morphology of colonies and cells was studied on potato agar, meat– peptone broth and Ashby nitrogen-free medium. The shape and size of colonies and cells were determined. The colony structure was studied under a magnifying glass and a microscope.

The cultural properties were determined by the nature of growth on oblique potato agar, meat peptone broth, gelatin, potato slices.

In order to study the biochemical properties of the studied strains, *X.malvacearum* was sown on a color row containing glucose, sucrose, lactose, maltose, levulose, xylose, arabinose, mannitol, glycerin, dulcite, sorbitol, sallicin in an amount of 0.5%. The results were taken into account on days 3,5,7.

Gelatin was used to identify proteolytic properties. Sowing on gelatin was performed by injection of a loop. The culture tubes were left at room temperature and scanned 2-3 times a week.

The formation of ammonia was established by reaction with the Griss reagent. Indole was determined by the method of Morelli and Beltyukov (1968) using strips of filter paper impregnated with a saturated solution of oxalic acid. In the presence of indole, the indicator paper is colored red.

Nitrate reduction was determined by the reaction of the studied culture sown in a meat-peptone broth containing 0.1% potassium nitrate with Griss reagent.

To identify the ability of bacteria to hydrolyze starch, they were sown with strokes on meat – peptone agar containing soluble flagella was detected by staining a suspension of *Xanthomonas malvacearum* with silver nitrate according to the Nefedov method (Peshkov, 1855). The capsule was stained using the Anthony method (Peshkov, 1955).

The capsule was colored using the Anthony method. The smears are dried in air and stained without fixing in the cold with a 1% solution of crystal violet, the paint is washed off with a 2% solution of copper sulfate, dried and washed with water.

The coloring on nuclear elements was carried out as follows: the smears were dried in air, fixed with Carnois liquid for 15 minutes, placed in a wet chamber, a ribonuclease solution was poured on top of the smears at a concentration of 2 mg/ml and placed in a thermostat for 1 hour at t 37^0C. They were washed off under running tap water and painted according to Gimsa for 1 hour at 37^0C.

3 RESULTS AND DISCUSSION

We have isolated six strains of *Xanthomonas campestris var malvacearum* from cotton leaves of Sultan and S-6524 cotton varieties affected by gommosis.

The structure of *X.malvacearum* cells was studied by light microscopy. The method of prints from the surface of the agar was used for the work.

X.malvacearum has mobility, there was used the method to clarify the nature of flagellation. As a result of the staining of *X.malvacearum* to identify flagella, it was found that *Xanthomonas malvacearum* has monotrichial flagella.

There was also conducted research on staining for nuclear elements with pretreatment with RNA – aza. Smears prints were prepared through 2, 6, 10, 12, 14, and 24 hours after sowing on the surface of potato agar.

To make completely sure that the well–colored granules and strands located in the center of *X.malvacearum* cells are nucleoids and have a DNA nature, the preparations were treated before staining (azur – eosin) first with RNA aza and then with DNA aza. As a result of such treatment, preparations consisting of cells devoid of any colored structures were obtained.

In four-hour cultures, the length of individuals increases. In most individuals, the nucleotides are in the stage of division. There are cells with three nucleotides.

Starting from six-hour cultures, the polymorphism characteristic of *X. malvacearum* manifests itself, reaching its greatest development in 24 hour-cultures. Giant individuals (4-9 mmc) with strands of nuclear matter appear in the smears from the seals.

In the studied isolates, the cells of *Xanthomonas campestris var malvacearum* are small in shape, slightly tapering towards the end, movable rods 1.5 mk long, 0.5 mk wide, with rounded ends, nonporous, gram-negative, forming a capsule. Some strains have cells 9 microns long in 18-hour or daily cultures.. The presence of long chains of cells indicates a significant polymorphism of the microorganism.

The optimal temperature for growing *X. malvacearum* culture in laboratory conditions is 20-30^0C. The morphology of young cells (6-10 hour) cultures varies when growing on different nutrient media.

On potato agar, the culture of *Xanthomonas campestris var malvacearum* has the appearance of slender sticks. On meat–peptone agar, the cell contours are vague, indistinct, the cells are irregularly shaped, short and strongly thickened. On Ashby's nitrogen-free environment, the sticks are large and slender. The measurement of bacteria grown on various media gave the following results: on meat–peptone agar -1,9-2,5/0, 8-1,0, 8-1,0 mk, on potato 1,25 – 1,5 /0,5 – 0,8 mk, on pepton – 1,3 -1,5/0,9 – 1,0 mk, on Ashby – 1,8 /0.3 – 1 mk.

On sterile potato slices with a small amount of water, the growth of *X.malvacearum* is moistly convex, shiny, pale yellow, waxy yellow with growth, a brown plaque forms, the potatoes darken.

The growth on oblique potato agar is good, strong, spreading, convex, the growth surface is smooth, moist, the edges are even, the pigment is yellow.

The development of *X.malvacearum* cultures on meat-peptone broth causes uniform turbidity of the medium. At the bottom of a small slimy sediment, turbidity rises when shaken. A loose, granular film forms on the surface of the meat-peptone broth.

The morphology of colonies of *X.malvacearum* was also studied on various media. On potato agar in Petri dishes, *X.malvacearum* bacteria form round, flat, soft, pale yellow colonies with smooth edges, which darken on 5-7 days. The diameter of adult colonies is 1-10 mm. In transmitted light, colonies have a homogeneous structure, sometimes

concentric, radial, and spindle-shaped. On days 7-10, a seal forms in the center of the colonies. On meat-peptone agar, colonies are rounded, pale yellow, darken with age. On the Ashby mineral medium, the colonies are small, transparent, shiny, the edges of the colonies are smooth in the transmitted light and have a granular structure.

When all strains of *X.malvacearum* were sieved on potato agar to obtain individual colonies, 4 types of colonies were found: 1. Smooth, round, shiny, convex colonies, yellowish–green, with smooth edges, in the transmitted light there is a seal in the center; 2. Slightly convex, shiny, slimy, greenish–yellow, the edges are smooth, transparent, concentric circles are visible in the transmitted light; 3. Large, flat, shiny grayish-yellow, the edges are transparent, structures in the form of grains are visible in passing light, which are located radically along the edge and acquire a fusiform shape; 4. Convex, round, slimy, bright yellow, smooth edges, homogeneous in passing light.

Type I and II colonies are most characteristic of the causative agent of cotton gommosis *Xanthomonas campestris var malvacearum* and contains 80%.

At this point, the morphological properties of the colonies were preserved, and no further dissociation was observed. According to the literature, *Xanthomonas malvacearum* forms acid on sugars, peptonizes and coagulates milk, and hydrolyzes starch (Gorlenko, 1961; Babayan, 1963).

The study of the fermentation of some carbohydrates and alcohols showed that the studied strains of *Xanthomonas malvacearum* slightly differ in their ability to ferment individual carbohydrates from the control strain. As a control strain, we used the data given in the determinant by N.A.Krasilnikov, Bergey.

The control strain forms acid without gas on glucose, sucrose, lactose, galactose, xylose, raffinose, glycerin, does not ferment arabinose, rhamnose, dulcite and mannitol, coagulates and peptonizes milk, hydrolyzes starch, indole and hydrogen sulfide does not form.

The studied strains of *Xanthomonas malvacearum*, with the exception of strains 2 and 5, do not form acid on xylose. Only strains 1 and 3 form acid on maltose, which brings them closer to the control one. Strain 3 does not ferment sucrose (Table 1).

As a result of studying the proteolytic activity, it was found that the studied strains of *Xanthomonas malvacearum* have the ability to cleave protein and peptone with the release of hydrogen sulfide (Table 2), and some strains (1, 2, 3) intensively secrete hydrogen sulfide, others (5, 6) have a weak reaction to the formation of indole in all strains of *Xanthomonas campestris var malvacearum* is negative.

Due to N.A. Krasilnikov, M.V. Gorlenko, V.P. Israelsky, the causative agent of cotton gommosis does not emit hydrogen sulfide and indole.

Xanthomonas malvacearum cultures slowly dilute the gelatin, which remains transparent. Strains 1, 2, 3 dilute gelatins more strongly than strains 5 and 6 (tab 2).

All researched strains of *Xanthomonas malvacearum* have proteolytic activity and saccharolytic properties. *Xanthomonas malvacearum* strain peptonizes and coagulates milk with the exception of the fifth strain, which only peptonizes milk. The rate and intensity of hydrolysis in all strains of *Xanthomonas malvacearum* is different; strains (1, 2, 3) hydrolyzed starch already on the 3rd day, and (5 and 6) – on the 7th day.

In order to identify reducing properties, a litmus serum was used, on which cultures develop in different ways. Some strains (1, 2, 3, 4 and 5) alkalize the medium, others (2 and 6) do not change it (tab 2).

Microorganisms are able to restore nitrates. However, according to N.A.Krasilnikov (1949), *Xanthomonas malvacearum* does not restore nitrates. According to the data, only strains 2 and 4 are capable of reducing nitrates to nitrites.

In Clark's medium, no strain of *Xanthomonas malvacearum* secretes acetyl–methyl–carbinol.

On the Klodnisky–Peshkov medium, all strains grew throughout the entire thickness of the medium. Consequently, *Xanthomonas malvacearum* is a facultative anaerobe.

The studied strains of *Xanthomonas malvacearum* do not hydrolyze fats, i.e. they are not capable of forming lipase.

Cultures obtained from different types of colonies differ in cultural and physiological properties.

Strains differing in colony morphology differ from the main strains in some cultural-physiological features (Table 3).

In cultures isolated from colonies of types II and IV (strains 3/II, 3 IV), proteolytic activity is more pronounced than in the main strain 3.

In cultures isolated from colonies of types I and III (strains 2 I and 2 III), proteolytic activity turned out to be weaker than that of the main strain 2. In addition to this, strains 2 (I) and 2 (III), unlike the main one, do not dilute gelatin and do not form acid on xylose.

Table 1: Fermentation of carbon sources by various strains of *Xanthomonas campestris var malvacearum.*

Strains	Omelyanskiy medium											
	Glucose	Sucrose	Lactose	Maltose	Levulose	Xylose	Mannitol	Glycerin	Arabinose	Dulcite	Sorbitol	Salicin
1	+	+	+	+	+	-	-	+	-	-	-	-
2	+	+	+	-	+	+	-	+	-	-	-	-
3	+	-	+	+	+	-	-	+	-	-	-	-
4	+	+	+	-	+	-	-	+	-	-	-	-
5	+	+	+	-	+	+	-	+	-	-	-	-
6	+	+	+	-	+	+	-	+	-	-	-	-
Control	+	+	+	+	+	+	-	+	+	-	-	-

Note: + existence of acid, - absence of acid

Table 2: Reducing and proteolytic properties of *Xanthomonas campestris var malvacearum.n*

Strains	Reducing properties		Proteolytic properties		
	Nitrate recovery	Reduction of litmus serum	Release of hydrogen sulfide	Dilution of gelatin	Starch hydrolysis
1	+	AF	++++	+++	+++
2	-	AF	++++	+++	+++
3	-	AF	++++	+++	+++
4	+	AF	+++	++	++
5	-	AF	++	+	+
6	-	AF	++	++	++
Control	N/A	N/A	-	++	++
Note	AF - change of the medium towards alkali formation + - intensity of proteolytic properties, starch hydrolysis				

Table 3: Cultural-biochemical properties of colonies differing in morphology *Xanthomonas malvacearum.*

Strains	Omelyanskiy medium										Meat peptone broth	Litmus serum	Milk	Gelatin	Hydrogen sulfide
	Glucose	Sucrose	Lactose	Maltose	Mannitol	Dulcite	Sorbitol	Mannose	Xylose	Glycerin					
3	+	-	+	+	-	-	-	+	-	-	Wall film mud sludge	AF	Peptonizes	++	+
3 (II)	+	-	+	-	-	-	-	+	-	-	Small film of mud	AF	Peptonizes	+++	++
3 (IV)	+	-	+	-	-	-	-	+	-	-	Small film of mud	AF	Peptonizes and coagulates	+++	+++
2	+	+	+	-	-	-	-	+	-	-	Small film of mud	-	Peptonizes	++	+++
2 (I)	+	+	+	-	-	-	-	+	-	-	Muddy film	-	Coagulates, peptonizes	-	+
2 (III)	+	+	+	+	-	-	-	+	-	-	Mud is a small film	-	Peptonizes and coagulates	-	+

Note: AF - alkali formation + - presence of hydrogen sulfide acid, reduction of nitrates, dilution of gelatin.

The main criterion for the belonging of unknown crops to the species *Xanthomonas campestris var malvacearum* is their pathogenicity to cotton.

Regarding to this, we studied the ability of *Xanthomonas malvacearum* strains isolated by us to cause the incidence of cotton gommosis. For this purpose, artificial contamination of Sultan and S-6524 cotton seeds was carried out.

The results of artificial infection of cotton seeds with the studied strains of *X. malvacearum* showed that all strains cause cotton disease with gommosis, but not to the same extent (Peshkov, 1955; Vinay, 2013; Fallahzadeh & Ahmadzaden, 2010; Eholvel & Kurundkar, 2007; Yogan, 1960; Hillocks, 1992; Jnnes, 1992; Wickens, 1961; Saidova et. al., 2023).

The disease occurred after seven days, from the beginning of the experiment. The clearest results were observed in 15-day-old seedlings.

Strains 1, 2, 3 strongly affected Sultan cotton. The cotyledon leaves were completely covered with large oily spots.

Strains 5 and 6 were weakly affected. 2-3 small oily spots appeared on cotyledon leaves.

The difference in the degree of pathogenicity was also manifested in varieties S-6524.

Strains 1, 2, 3 affected the studied varieties more strongly, more intensively, and 5, 6 weaker.

Consequently, all the studied strains isolated from cotton leaves belong to the species *X. malvacearum*, because with artificial infection of seeds, symptoms of cotton gommosis appeared on cotyledon leaves.

4 CONCLUSIONS

Based on the experiments of the conducted studies, it can be concluded that all cultures isolated from cotton leaves affected by gommosis are representatives of the species *Xanthomonas campestris var. malvacearum*, since they are similar to the species in terms of the main cultural morphological, physiological and pathogenic signs. *Xanthomonas malvacearum* described in the determinants of Bergey and N.A. Krasilnikov. While studying the cytology of *Xanthomonas malvacearum*, we found at the level of light microscopy that *Xanthomonas malvacearum* has flagella, capsule and isolated nucleoids.

X. malvacearum cells aged 18-24 hours are characterized by the presence of polymorphism, both of the individuals themselves and their nucleoids. Strains differing in morphology of the colony differ from the main strains in some cultural-morphological features. It's worthy to note that some of the differences we have established between *X. malvacearum* strains may be strain-specific features rather than species-specific. To identify the causative agent of cotton gommosis, we need to conduct additional studies at the molecular level. Research in this direction will continue.

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