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TOXINO FORMATIONS BY PHYTOPATHOGENIC BACTERIA

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TOXINO FORMATIONS BY PHYTOPATHOCENIC BACTERIA

M. S. Matishevska

In literature devoted to the study of parasite penetration conditions and the possibility of its development in vegetations, phytotoxins acquire greater attention. Considering the fact that parasitism of phytopathogenic bacteria is presented exclusively with facultative microorganisms and the characteristic property of this group is the ability to form toxic substances, questions will be filed immediately on the creation of toxines by representatives of these exciters of bacteriosis.

Regardless of the fact that toxines of phytopathogeneous bacteria are already being investigated for over 50 years, many questions to that time remain unanswered. Even in the very concepts of "toxines" in phytopathology individual scientists insert different contents (10, 18, 30, 39).

Furthermore, if phytopathologists under the name of "toxine" combine all substances which are formed by the exciter of sickness and under the effect of which occur the corresponding changes in the metabolism of substances of vegetation-boss, then, for example, in medical immunobiology, actual toxines are considered only these substances, which cause the formation of antitoxines.

Classification or any other grouping of phytotoxines is very hampered because of the little studies of same. However in last year toxicity of other components of cultural filtrate have considerably expanded the investigations, as well as general questions of toxinc formation in pathogeneous for vegetation fungi, bacteria.

Today tests are made to group toxines. And so, Gambogi (22) separated three groups of fungus phytotoxines and phytotoxines of bacterial origin, which cause various sickness symptoms in vegetations:

1. Toxines causing local action, e.g., exciter of "wild ambustion" tobacco (Pseudomonas tabacum).

2. Toxin, which eliminates fading Pseudomonas solanacearum.

3. Toxines, which cause hypertrophia or hyperplasia bacterium tumefaciens.

To obtain the toxic substances of sickness exciters we reared for a certain time on a rarely feeding medium, then through bacterial filters or by centrifuges was separated the fluid of the cultural filtrate from the mass of bacterial cells. When utilizing the above settled liquid was

exotoxin. In another case, the bacterial mass of the cells to obtain endotoxin was subjected to special treatment. A majority of the investigators think, that toxin of phytopathogeneous bacteria are thermostable.

Studying the nature of toxines, the first investigators turned attention to the presence in filtrates of an exciter (fungus) of organic acids (oxalic, citric, lactic and a series of other strong and weak acids). In time the filtrates inside revealed polysaccharides and albumens and products of its decomposition - amino acids.

The first one to study the effect of bacterial toxin on Van Khal' vegetation, and somewhat later with the same testa have occupied themselves Appel and Potter. These authors established, that in Bac subtilis, Bac. vulgatus (11) and winia phytophtora (1) are situated poisonous for vegetation substances, which were also called toxines. Potter (38) considered that the destruction of broccoli when stricken with Pseudomonas destructans is due to the diffusion of toxic substances of the exciter in the cells of vegetations after partial destruction of same with cytasis fermentation. This year Schuster (43) separated poisonous substances with Pseudomonas xanthochlora which settles with alcohol the filtrate from a culture of this microorganism. The author considered, that in this case, together with substances of enzyme type, are formed poisonous substances, which affect directly protoplasm.

Johnson and Murwin (37) were the first ones to show, that aureoles on leaves which originate during affection of same with Pseudomonas tobacum, depends upon the presence of toxines in the bacteriosis exciter. A filtrate from the culture of this bacteria during the spraying of a tobacco leaf gave the very same aureoles as when they were affected by the illness exciter. These discoveries were fully conformed in later investigations carried on by Clayton (28) who obtained a toxin - a native cultural filtrate with Pseudomonas tabacum. The author established, that the exciter of "wild ambustion" Ps. tabacum has a sharp exotoxin, which ruins chlorophyll, producing a characteristic symptom on vegetations "wild ambustion" of the leaf. The derived substance is thermo stable, rapidly loses its activity when lye is added to it for dilution.

Clayton (28) called attention to the fact, that under certain conditions when a leaf is affected with Ps. tabacum are formed necroses and without characteristic aureole. The author ties this phenomenon with the loss of culture exciter of the ability to form toxin, To an analogous conclusion, somehwat later, come also Braun (2, 3). He established, that the exciter of "wild OPIK ambustion" - Ps. tabacum and muffling spotty Ps. angulatum however good plants grew well on media with corresponding sources of carbon and nitrogen, which indicates their identity. Since Ps. tabacum forms

toxin and causes characteristic aureoles on leaves, Ps. angulatum has no such properties and when affecting vegetation no aureoles on the plants are formed. The idea of the author is, and he assumes that the difference between symptoms exist between these two bacterias. Furthermore, Braun established, that during the re-sewing of cultures within the duration of a certain time on artificial media the exciter can lose the ability to form toxines. When vegetations are affected by such a culture the outer view of the sickness symptoms was similar to the symptoms when the leaf was infected by Ps. angulatum, which forms no toxins. Braun and co-workers paid much attention to the obtaiment of chemically pure toxic substance, they examine its properties and the effect on the metabolism of plants. He studies a cultural filtrate, handed by special processers, because the obtained substance can no longer be taken as a native liquid of the culture filtrate. It was sufficiently toxic and retarded the growth of chlorella. Somehwat later Braun and co-workers (45, 46) obtained in a complicated way a chemically pure toxic. This substance is hygroscopic and unstable. At room temperature and pH 8.2 within 15 minutes, its activity decreases to perhaps double; in larger alkaline solutions the inactivation is even faster. It was established, that toxin of the "wild opik" is a derivative of a new α -amino acid; it can give a lactone of alpha-lactylamino-hydroxy-e-pimelic acid. The effect of toxin Ps. tabacum (5) is connected with the fact that it disturbs the methionine exchange of vegetation cells. The delay in chlorella growth is fully liquidated when adding l-methionine into the medium.

into the medium.

When leaf plates of kidney beans are affected by Pseudomonas phaseolicola, in the early stages spots are formed with characteristic aureoles, which are especially remembered when they are observed against the light. Zaumeyer (21) considers the appearance of such aureoles around fine necrotic spots as due to the effect of exciter toxin. In this connection for a certain interest, the credit goes to Scocc (40). The author established, that the appearance of these aureoles around fine noncrotic spots is actually due to the effect of the exciter. In this connection is given a certain interest by Scocc (40). The author established the toxin formations Ps. phaseolicola is recorded on the 10-14 day if the exciter is grown at a temperature of from 10 to 15°. He turned attention to the fact, that the typical illness system fillet appears only when the bean is innoculated with the exciter, basically at 20°C and lower. This temperature is an agreement with the temperature, optimum for the formation of toxin in pure culture.

K. G. Beltyukova (6), studying the ability of forming toxic substances of exciters of snap bean bacteriosis (Xanthomonas phaseoli, X. phaseoli var. fuscans, Pseudomonas vignae, Ps. Medicaginis var. phaseolicola) and certain other (Pseudomonas pisi and Corynebacterium michigananse), established that snap bean seeds, treated in a culture suspension or with an aqueous solution of culture filtrate of the exciter, when it is grown on potato agar reduced the complexity in comparison with control. The negative action of toxic substances was designated also at the development of the

root system of sprouts. During immersion in a filtrate of cultured liquid of above cut down snap beans was noticed a quite rapid fading of same (within 30-45 min). The filtrate of culture liquid Xanthomonas vesicatoria, obtained during the growing of the exciter of black bacterial spottiness on different nourishments or nourishing media it affected negatively respiration and peroxidases of tomato sprouts (31).

V. P. Izrail'skiy considers, that formation of toxin is characteristic of many phytopathogenous bacteria (Erwinia phytnophthora, Pseudomonas xanthochlorum, Ps. solanacearum, Bac. subtilis, Bac. Vulgatus. Ps. tabacum).

We have discussed toxin formation in bacteriosis exciters, the illness symptoms of which appear in spotty form.

For reasons of fading of stricken vegetations exist two theories. According to one (41, 42) the phenomena are caused by mechanical plugging of vessels with parasite cells, which do develop.

This theory is supported by many authors, And so, Melhus, Muncia and Ho (33) when explaining the question on the causes of fading carried out direct fluometric measurements of the behavior of the ill of an apple tree root affected by Bact. tumefaciens, cabbage, infected by Fusarium conglutans and Alfa, the fading cause of which was not proven. Leclerg and Durral (25) also utilized the fluoride method to designate the behavior of water in Alfa-Alfa, stricken with Corynebacterium (Phytomonas) insidiosum. In both cases the behavior of the stricken parts of the vegetations was much

lower, then of nonaffected (healthy) vegetations. The authors come to a conclusion, that fading is caused by partial or full plugging of vessels. Meer (32) considers, that fading of tobacco and tomatoes, infected by Ps. solanacearum, takes place as result of damaging the root tissues, formation of bodies and gum in vessels and the plugging of vessels with bacteria and it is not bound with the toxic act of the parasite on the living elements of the bosses cells. The same idea is maintained also by Greve (23) who made histological investigations on potatoes and tomatoes, stricken with the very same exciter.

In accordance with a different theory, which has been first proposed by Hutschinson (20) fading of plants is due to the toxic action of the parasite. The author supports his idea by the fact, that the injection of an aqueous residue solution, obtained in culture filtrate Ps. solanacearum, causes fading of tobacco. In this way, the author maintains the idea, that parasitic bacteria emit a poisonous substance (or substances), which, when approaching the tissues of the boss poison the protoplast. There is also an idea (36) that fading is promoted by toxin, which is formed in the infected plants, and the plugging of vessels - this is a secondary cause of fading. The data by Harris (12) also indicate the presence of toxic substances in the filtrate, which accumulate during the growing of exciter culture on meat peptone bullion. In the investigations by the author, the filtrates obtained from Erwinia tracheiphila and Ps. stewarty cause fading

of cucumber and corn cuts. In control (water works water) fading was not observed. Control unsown media (meat peptone bullion) caused during the investigation fading of cucumber cuts but only in one case. In addition fadings were caused by nonautoclave as well as autoclave filtrates, which points toward the thermostability of the toxic substance and its nonfermental nature. Together with the designation of filtrate toxicity, the author investigated the rate of conductivity of water through cucumber blades, infected with the above mentioned bacterioses. It was established, that the conductivity of water through the damaged blades of cucumbers decreases by 82%, and of corn - by 74% in comparison to healthy blades.

On the basis of experimental data the author comes to the conclusion, that these microorganisms form toxic filtrates, because of the presence of the latter does not bring the possibility of plant perdition, due to the fact of the plugging up of the vessels.

It should be stated, that even to this time testing the causes of fading are not solved yet. The discussional nature of the theory of fading favored the expansion of investigations in this direction. In recent years the theory of mechanical plugging of vessels found a somewhat different explanation, in accordance with which stricken vegetations fade away as result of vessel plugging not only by bacterial mass, as with high molecular substances (of greatest polysaccharide nature), which are formed by the exciter.

In connection with this interest is caused by investigations (19, 29

and others) devoted to the study of nature of fading when the plants are stricken by Ps. solanacearum.

In the investigations by Kunz (29) during the settling of the culture filtrate Ps. solanacearum with alcohol were obtained two fractions. The first fraction - polypeptide complex (bacterial mucus), which as result of physical effect causes plugging up of water conductive tissues of plants. The second fraction - toxic looking like a poison for plasma, which causes fadings, affecting the plasma membrane. Kunz also assumes, that bacterial mucus is reformed into toxic substance during the action of ferment of higher plants.

Husain and Kelman (19) on the basis of their investigations arrive at a conclusion, that mucus, which is formed by Ps. solanacearum, plays a considerable role in the fading of damaged vegetation. They established a very important phenomenon - culture filtrates of a highly pathogeneous stem caused fadings of tomato cuts, while the filtrate of a weakly pathogeneous stem caused no fading at all. These phenomena are used by the authors to connect same with the presence of mucus around the cells of a highly pathogeneous stem, and the absence of mucus around cells of a weakly pathogeneous stem. Analysis of the residue, obtained during the settling of culture liquid of a highly pathogeneous stem, shows, that this substance is by its nature a polysaccharide with glucose as its main component. The author are making assumptions, that virulent stems of bacteria after their penetration into the plant multiplies there rapidly and form a mucus, as result of which vessel pluggings occur and the influx of water through

the damaged tissue is gradually closed up. But the investigators remark, that it is impossible in this to find a single cause for fading the stricken vegetation. This thought is confirmed by data of histological investigations of faded plants in which the vessels were not always fully covered by a mass of bacterial cells. Furthermore, in other cases when infecting vegetations with weakly virulent stems, which have no mucus, the vessels of plants were filled with a mass of bacteria, although these plants showed no outer fading symptoms. Hendrick (13) investigating 16 types of phytopathogeneous bacteria of the types Bacterium, Corynebacteriu, Erwinia, Pseudomonas, Xanthomonas, established, that they all form a highly toxic exudate on media with saccharose or glucose as a carbon source. An analysis with the aid of chromatography on paper showed, that exudates are found in saccharous substances glucose, mannose, fructose, ramnose, xylose, rybose and galacturonic acid. Tomato and sunflower plants, mixed in acqueous solution of the exudate, faded away rapidly as result of mechanical plugging of the vessel system with polysaccharadis of greater molecular weight.

An analogous idea is also maintained by the group of scientists, who investigated the nature of bacterial exudate Xanthomonas phaseoli (24). Polysaccharaide, obtained during the deposition of exitation exudate setties by brown spottiness with acetone, form an opalescent, viscous solution causing reverse fading of tomato cuts, son flower and French beans. The authors make a conclusion, that the cause of the fading is mechanical plugging of vascular tissues of the stalk with

Highly important investigations of toxino formations in bacteria, which cause swellings of plants, have been carried out. Many authors express various assumptions on the reason of forming swelling of plants during the penetration of bact. tumefaciens. Studying the composition of the bacterial filter, together with the presence in it of acids - acids of formicdesoxyribonucleinic, growth substances of the type of indolacetic acetic acid they are also found in toxino similar substance. And so it was established (7, 8, 34) that endotoxic bact. tumefaciens - is a polysaccharide polipeptide lipoidal complex, which causes typical swellings when infecting a sunflower. This toxin can be decomposed, with hydrolyzing weak acid, into polysaccharade and plypeptide - pipoidal complexes. Levin and Chergoss (26) took from Bact. tumefaciens three fractions of toxic substances. In this case only the polysaccharides fraction was toxic and when introduced into the plant it formed tissue necrosis.

Within a series of years Hofgson, Riker and Peterson (14-17) have studied the ability of various high molecular substances of polysaccharide type of vegetable origin, to synthesize artificially also the formed Bact. tumefaciens, in order to form vegetation fading. From the culture filtrate of this exciter was obtained a substance which consists of a great number of polysaccharides. It was established, that the basic component, which causes fading of plants, is glucose. In their investigations the authors assume the existence of a direct dependence between molecular weight and degree of plant fading. The assumption that these highly toxic substances are harmful because of the fact, that the transpiranion system is mechanically

plugged up. But, together with this are made assumptions for the diffusion possibility of these substances and actively affects same. Feder and Ark (44) obtained from Bact. (Agrobacterium) tumefaciens, X. phaseolicoli and Erwinia carotovara. It was established, that the active component of endotoxine, which causes fading is not polypeptide-lipoid fraction, but the polysaccharide component. The authors assume, that the cause for fading is the polysaccharide and the mechanism of this process depends to a large extent upon their molecular weight.

Studying the nature of toxines of phytopathogeneous bacteria, many authors (5, 6, 27, 37 and others) turned attention to the nonspecificity of their action. And so, filtrate of culture fluid of an exciter of a wild opik leave of tobacco (Ps. tobacum) has caused characteristics spots also on other plants which belong to various families (cucumbers, French beans, eggplants etc). The toxine of this exciter retarded the growth of chlorella (5). The nonspecificity of the toxic action is also confirmed in the Bel' tyukova (6) investigations, in which the filtrate of culture liquid of the pea bacteriosis exciter - Ps. Pisi and bacterial cancer of tomatoes - Corynebacterium michiganense cause rapid fading of cut French bean leaves, which for the mentioned exciters is not a vegetation boss.

The nonspecificity of the toxic substance of the culture filtrate coincides also during the separation from it of the polysaccharide fraction. These conditions are confirmed by the Feder and Ark (44) investigations, in which endotoxine of two bacteria, which in natural conditions cause no fadings

(Erwinia carotovora and Xanthomonas phaseoli) with their basic properties are similar to polysaccharide, obtained from Bact. tumefaciens. This toxin has also caused fading of sunflower and tomatoes. The very same results were also obtained for polysaccharide fractions from X. phaseoli, which caused fading of French beans and tomatoes (24). On the basis of the mentioned data, the authors reach a conclusion as to the fact, that the obtained polysaccharide fractions of toxin are nonspecific neither for the very parasites nor for the plants-bosses.

Literature data confirm the fact that phytopathogenic bacteria do form toxic substances. For some of them the nature is established. These derivatives of components amino acids, belong to the polysaccharide complex. Studying the nature of toxines, their content is made difficult by the fact, that up until that time literature on toxin formation of phytopathogenic bacteria does weakly explain these questions on what is the effect of the composition of living media, pH media, temperature, at which the culture grows and a series of other factors are involved in it.

It should be pointed out, that the effect of phytopathogeneous bacteria on plants is highly complicated and, as a rule, is due to the action not just of anyone parasitic metabolism component, but is the result of simultaneous and multi actions of various metabolism elements of the exciter and plants, the surrounding medium through which the great role belongs to bacterial toxines.

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