

Using of bioinformatics and computer morphometry in study of *Fusarium* spp. causing potato dry rot

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Abstract

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Introduction. The purpose of research – to evaluate the possibility of using the methods of computer morphometry and bioinformatic analysis in studying of reactive changes in *Fusarium* spp. causing potato dry rot.

Materials and methods. Tubers of "Nevski" potato with *Fusarium* contamination signs were selected. Three areas of the damaged tuber potatoes were studied: the epicenter of contaminated plant tissue; peripheral area of contamination; plant tissue outside of contamination epicenter. Morphological research and computer morphometry of fixed preparations of microorganisms stained by 1,0% methylene blue solution were made under magnification x 400, x 1000.

Results and discussion. Macroconidium morphometric characteristics of *Fusarium* spp. (square, perimeter, length, number) were obtained by automatic image analyzer "Micros". The algorithm and the computer program based on morphometric analysis of *Fusarium* spp. macroconidium cells causing potato dry rot was created to study bioinformatic cell characteristics.

The following information characteristics of fungal cells have been calculated: H – information entropy characterizing the real structural diversity; H_{max} – the storage capacity characterizing the maximum structural time-diversity of biological system; R (%) – information redundancy coefficient characterizing the relative organization of biosystem.

The *Fusarium* macroconidium cells have demonstrated low values of H (0,002) compared to H_{max} (0,5). The values of R in *Fusarium* cells were 99,40% – 99,69% thus demonstrated a high degree of orderliness and relative organization in microbial cellss. Our research proves the maximum adaptation level of *Fusarium* cells as parasites in biocenosis with plant cells. It should be expected that in the case of the reaction of *Fusarium* cells on chemical or biological preparations the value of H should increase up to values of H_{max} , and the value of R will decrease. It's may be occurred as a result of disruption structural and functional integrity of *Fusarium* cell system.

Conclusions. The method of bioinformatic analysis of *Fusarium* spp. cells causing potato dry rot can be recommended as a testing system in development of new plant protecting preparations.

Introduction

Safety of food and agricultural products during production and turnover is a keystone in development of food market in many countries [1].

The potato is one of the leading agricultural crops in many countries. The total volume of its production in EU countries in 2013 amounted to about 60 million tons [2]. Despite the fact that the potato is a high yielding culture, total number of potato tubers has significantly reduced in some unfavourable years. The main reason of the potato harvest decrease is the damage of the potato tubers by bacterial and fungal infections such as *Rhizoctonia*, phoma rot, late blight, *Fusarium* and others [3].

Fusarium dry rot is a second most common plant disease after late blight. *Fusarium* spp. belongs to the division Ascomycota and causes many important diseases. These fungi may contaminate food products with mycotoxins, threatening to animal and human health, can cause a wide spectrum of human infections, especially in immune compromised patients (cancer) or HIV positive patients [4, 5].

The fungal genus of *Fusarium* spp. are wide spread in all regions of Northern Hemisphere and other regions [6, 7, 8]. *Fusarium sambucinum*, *Fusarium coeruleum*, *Fusarium solani* App.et. Wr., *Fusarium avenaceum* are the main potato pathogens (Figure1).



Figure1. Tuber contaminated by *Fusarium* spp. causing potato dry rot.

A new molecular method of PCR is widely used in science experiments together with traditional morphological and physiological research methods of *Fusarium* spp. [9, 10].

The following measures are used to prevent *Fusarium* plant contamination: mechanical cleaning of tubers, disinfection of storage containers, treatment storage period, keeping the optimum temperature, early diagnostics of tuber infection, chemical treatment of seed material [11, 12].

Biological protection of agricultural plants is one of the alternative methods to reduce soil damage against mineral fertilizers. Biologicals suppress the tissue pathogenic flora by their own microorganisms or plant biologically active extracts [13].

The chemical compound of plant tissue is a good growth medium for microorganisms. That's why the fungi of the genus *Fusarium* should be considered as one of the components of biological ecosystem.

New informative methods to characterize morphological and functional state of *Fusarium* cells are required in searching of chemical and biological plant protective means [14].

There is no data on using the bioinformatic modelling in study of morphofunctional state of *Fusarium* spp.

There are several articles on the bioinformatic status of human tissues [15]. It was demonstrated that the bioentropy reflects morphofunctional adaptive capacity of organism and is related to changes in bioinformatic status and development of pathological processes in tissues and organs [16, 17].

Our work was to explore the possibility of using bioinformatics and computer morphometry in studying the reactive changes of *Fusarium* spp. causing potato dry rot.

Materials and methods

Tubers of "Nevski" potato grown in the Leningrad region of Russia were selected as an object to study. The potato "Nevsky" belongs to intensive type of varieties and well responds to fertilizer application. Investigated tubers were in storage for 3 months after the harvest in 2015, average storage temperature was + 18 °C, relative humidity was 75%.

Sampling from potato lot was carried out in accordance with ISO 7002:1986. Potatoes tubers with *Fusarium* contamination signs were selected. The changes of tuber with dry rot were: drying up, shrivelling and falling off the epithelial tissue.

The tuber was cut with a knife along the longitudinal axis through the stolon and examined the tuber flesh. The flesh looked like a dry rotten black mass with voids, filled with fluffy mycelium of the fungus – the epicenter of contaminated plant tissue.

Three areas of the damaged potato tuber were studied:

- the epicenter of contaminated potato tuber tissue;
- peripheral area of potato tuber tissue contamination;
- potato tuber tissue outside the epicenter of contamination.

The qualitative method has been used to detect the presence or absence of *Fusarium* sp. in potato tissues (ISO 7218–2011). Microbiological study of tuber tissues was carried out using "imprint method" ("contact method") by analogy of horizontal sampling techniques from surfaces using contact plates (ISO 18593:2006). A glass slide was applied for 5–10 seconds to the each areas of contaminated potato tissues. Samples of potatoe tissues were stained by 1% methylene blue solution [18]. Morphological research of microorganism fixed preparations was made on microscope "Micros" (Austria), model MS 100 (XP), under magnification x 400 or x 1000.

Morphometric macroconidium cell characteristics of *Fusarium* spp. (square, perimeter, length, number) were obtained by automatic image analyzer "Micros". The quantification of microorganisms was made in 10 view fields for each sample [20].

Morphologic identification of fungi of the genus *Fusarium* was determined by curvature of macroconidium, the shape of the apical cells. The species identification of *Fusarium* according to the morphological characteristics of their macro – and microconidia wasn't studied.

The following information characteristics of fungal cells have been calculated:

- H – information entropy characterizing the real structural diversity.
- H_{\max} – the storage capacity characterizing the maximum structural time-diversity of biological systems.
- R (%) – information redundancy coefficient that characterizing the true biosystem organization.

Statistical data processing was provided with program "STATGRAPHICS".

Results and discussion

The results of recent study of morphofunctional state of yeast and bacterial producers of biotech products by bioinformatics and morphometric analysis show the usefulness of these methods in research of the adaptive reactions to some microorganisms [15]. The research purpose was to determine the possibility of using bioinformatics and computer morphometry to the fungal pathogens of valuable agricultural crops, including *Fusarium* causing potato dry rot.

The results of microscopic study of potato tuber tissues contaminated by *Fusarium* spp. are shown on Figure2.

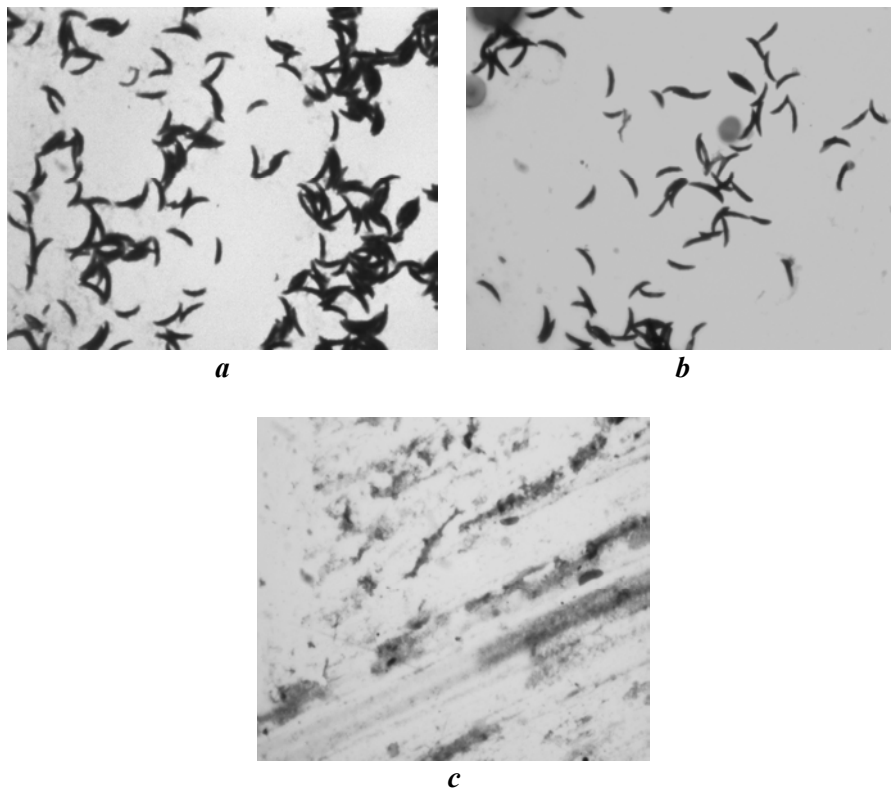


Figure 2. Conidia of the *Fusarium* spp. in potato tuber tissues:

- a – the epicenter of contaminated potato tuber tissue;
 - b – peripheral area of potato tuber tissue contamination;
 - c – potato tuber tissue outside the epicenter of contamination.
- Methylene blue. Magnification x 400.

The maximum concentration of the *Fusarium* macroconidium cells was determined in the epicenter of contaminated potato tuber tissue (Figure 2-a). The amount of the *Fusarium* macroconidium cells decreased in the peripheral area of contamination (Figure 2-b). The *Fusarium* cells (only microconidium) were found in the plant tissue outside the epicenter of contamination in a single amount (Figure2-c).

The macroconidium of *Fusarium* spp. were from fusiform to lanceolate, mostly with a visible three partitions. Cells in the central part are typically square. The apical cell is slightly elongated, gradually and evenly tapering, and somewhat curved (Figure 3).

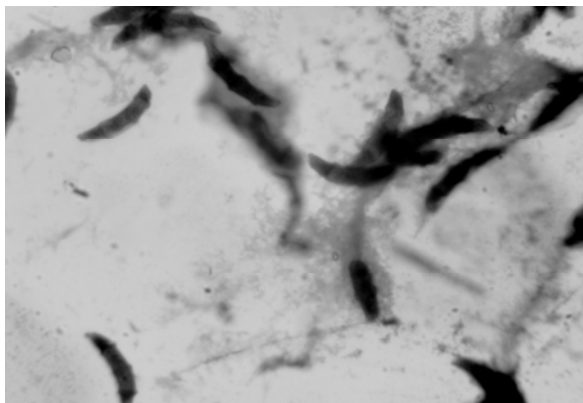


Figure 3. Macroconidium of *Fusarium* spp. causing potato dry rot. Methylene blue. Magnification x 1000.

The results of morphometric measurements of the *Fusarium* macroconidium cells in the epicenter of contaminated potato tuber tissue were: the area size was $30084,64 \mu\text{m}^2 \pm 0,02$, the cell perimeter – $996,15 \mu\text{m} \pm 0,01$, the cell length – $416,31 \mu\text{m} \pm 0,02$.

The results of morphometric measurements of the *Fusarium* macroconidium cells in the peripheral area of potato tuber tissue contamination were: the area size was $25670,66 \mu\text{m}^2 \pm 0,03$, the cell perimeter – $1010,54 \mu\text{m} \pm 0,01$, the cell length – $452,67 \mu\text{m} \pm 0,04$.

The morphometric measurements of *Fusarium* microconidium cells in the plant tissue outside the epicenter of contamination weren't to carry.

The results obtained show that *Fusarium* cells have their individual morphometric characteristics in two zones of contamination. The area size of *Fusarium* cells in the epicenter of contaminated potato tuber tissue was 1,7 times larger than in the peripheral area of potato tuber tissue contamination.

Integral criteria of the morphofunctional state of *Fusarium* cells causing potato dry rot were obtained by using the basic concepts of thermodynamics and informatics: entropy H as a function of system state, the information redundancy [15].

The algorithm and the computer program based on morphometric analysis of *Fusarium* spp. were created to calculate cell characteristics such as information entropy (H) and storage capacity (H_{max}). Due to values of H and H_{max} , we've calculated the values of the information redundancy coefficient ($R, \%$) that characterizes a measure of the biological system reliability, i.e. its ability to resist external and internal effect factors.

The data of H , H_{max} and R for *Fusarium* cells causing potato dry rot are presented in Table 1.

Table 1
Informational characteristics of *Fusarium* cells causing potato dry rot

Num	Area of potato tuber tissue	Value of information entropy (H)	Value of storage capacity (H_{max}).	Value of information redundancy coefficient (R, %)
1	The epicenter of contaminated potato tuber tissue	0,002	0,5	99,69
2	The peripheral area of potato tuber tissue contamination	0,002	0,5	99,4
3	The potato tuber tissue outside the epicenter of contamination	-	-	-

These data demonstrate that microscopic fungi cells have some differences in information characteristics of two areas of contaminated plant tissues.

The destruction of potato tuber tissue was maximally expressed in the epicenter of contamination. The redundancy coefficient R of *Fusarium* cells in this zone was 99,69%. The redundancy coefficient R of *Fusarium* cells in peripheral area of potato tuber tissue contamination was slightly reduced to 99,4%.

The values of information entropy (H) of the microorganisms cells were 0,002, the storage capacity (H_{max}) 0,5 in both areas of plant tissue contamination.

Previously we've obtained informative characteristics for yeast and bacteria cells – producers of biotechnological foodstuff [19]. The information redundancy coefficient R was calculated for each type of microorganism.

For different yeast strains the values of redundancy coefficient R (%) were:

Saccharomyces cerevisiae – 82,02; *Mycoderma vini* – 68,84; *Rhodotorula gracilis* – 81,12. For different bacterial species the values of redundancy coefficient R (%) were: *Escherichia coli* – 88,46; *Bacillus subtilis* – 65,94; *Micrococcus sulfaticus* – 49,29; *Pseudomonas fluorescens* – 38,83.

Comparative analysis of information characteristics of the researched microorganisms (*Fusarium*, yeast, bacteria) showed that the values of the redundancy

coefficient R in the *Fusarium* cells causing potato dry rot were higher in 1.3 times than that of yeast cells. The values of the redundancy coefficient R in the *Fusarium* cells causing potato dry rot were higher in 1.6 times than that of bacterial cells.

These data should be explained by some facts. The yeast strains and bacterial species were grown in a nutrient culture media prepared to ISO 11133-1-2011. The microorganisms were in resting state in culture media. The *Fusarium* cells have demonstrated low values of information entropy H (0,002) compared to information capacity H_{max} (0,5). The redundancy coefficient R in *Fusarium* cells had high values (99,40% to 99,69%) thus demonstrated a high degree of orderliness and relative organization in microbial cells contaminating potato tubers.

It is known that phytopathogenic fungi of the genus *Fusarium* contaminating potato tubers are parasites. The fungi cells penetrate plant tissue and multiply in it by activating their metabolism [21]. Our research proves the maximum adaptation level of *Fusarium* cells in biocenosis with plant cells during microbial contamination of potato tubers.

It is important to study the *Fusarium* cell information characteristics in case of development and introduction new chemical and biological plant protecting preparations. It should be expected that in the case of the reaction of *Fusarium* cells on chemical or biological preparations the value of information entropy H should increase up to values of information capacity H_{\max} , and the value of the redundancy coefficient R will decrease. This may be accrued as a result of disruption structural and functional integrity of *Fusarium* cell system and will indicate the efficiency of new preparations .

Conclusions

The algorithm and the computer program based on morphometric analysis of *Fusarium* spp. macroconidium cells causing potato dry rot was created to study bioinformatic cell characteristics.

The values of bioinformatic cell characteristics such as the information entropy (H), the storage capacity (H_{\max}) and the information redundancy coefficient (R) were obtained to *Fusarium* spp. macroconidium cells causing potato dry rot.

The method of the bioinformatic modelling is demonstrated to help in study of adaptive reactions of *Fusarium* spp. causing potato dry rot and can be recommended as a testing system in development of new plant protecting preparations.

The method of the bioinformatic modelling can be recommended for the diagnostics of *Fusarium* infections in medicine.

The authors disclose that they have no conflict of interests.

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