THE STUDY OF VEGETATIVE INCOMPATIBILITY STRAINS OF BASIDIOMYCETES

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Abstract. The genetic relationship of dikaryotic cultures (strains, isolates) of the studied strains of certain types of basidiomycetes and influence of this factor on their biosynthetic properties by examining the vegetative incompatibility of introduced crops based on antagonism are determined. The analysis of the results of di-di splice revealed that the strains were dominated a moderate vegetative incompatibility response from 100% in *Agaricus bisporus*, *Stropharia rugosoannulata*, *Fistulina hepatica*, *Laetiporus sulphureus*, *Schizophyllum commune* and *Sparassis crispa* to 72.5% in *Flammulina velutipes* and 47.3% in *Pleurotus ostreatus*, a strong antagonistic reaction was very rare: from 13.2 in *P. ostreatus* to 6.6% in *F. velutipes*. The exception is *Lentinula edodes*, where 2 out of 3 cases have strong reactions (66,7%). In the presented samples of the strains, no case of clonal origin was detected, as different manifestations of the vegetative incompatibility reaction were observed in all splices. This indicates dominance of sexual reproduction in the studied populations and the genetic diversity of the strains of one species and their cultural and morphological and biosynthetic properties.

1. Introduction

Biotechnology is one of the priorities for the development of mycology, the development of technologies using of vegetative crops of basidiomycetes for biologically active substances, including those that have therapeutic properties. This is confirmed by the results of recent decades, which proved that biologically (including pharmacologically) active substances of

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fungi, in comparison with the products of chemical synthesis, are less toxic and more effective in medical practice.

As a result, numerous growing technologies and patented formulas for biologically active supplements based on mycelium of macromycetes have been developed [4, p. 4–6; 8, p. 1324].

Scientific bases of modern technologies of artificial cultivation of edible fungi and production of sowing mycelium were developed by a team of scientists the M.G. Kholodny of Institute of Botany of the National Academy of Sciences of Ukraine. Numerous collection of Cap fungi cultures has been created, which is the basis for current and future research of macromycetes species, in particular as objects of biotechnology. Multi-year experimental studies have been carried out on the development and cultural and morphological features of edible mushrooms at different stages of the life cycle and on the breeding of new indigenous high-yield strains of these fungi, selection of substrates, establishment of optimal technological parameters for fruit body cultivation and seed mycelium, study the interaction between edible mushrooms and micro-organisms that dominate the substrates during their fermentation. Biologically active substances have been found not only in fungi's fruit bodies but also in vegetative fungi mycelium obtained by liquid-phase or solid-phase cultivation [4, p. 5–8; 6, p. 5; 8, p. 1325].

The practical use of fungi in a variety of industries requires the constant search for new strains of different uses, based on the selection, establishment of cultural and biosynthetic characteristics of dicaryons and monocaryons as units of selection [3, p. 66–67, 212–217].

Due to the complexity of the organization, higher basidiomycetes, as objects of biotechnology, occupy an intermediate position between microorganisms, plants and animals. Their life cycle consists of morphologically and functionally distinct stages of development of monocaryon and dicaryons [2, p. 32–35; 3, p. 66–67, 212–217]. The dicaryon or secondary mycelium is a specialized type of heterocaryon in which two genetically distinct haploid nuclei are associated in pairs in each cell.

A number of works have shown that mycelium fungi have a common characteristic: in the physical contact of the mycelium, their cells are fused (anastomoses hyphae, plasmogamy). Because the fungal hyphae are covered with multilayered membranes, the merger is preceded by local dissolution of the cell walls. This property may have originated initially as a result of the need to fusion vegetative structures in the gametes left without gametes. It is also believed that anastomosis was based on mycoprazitism, in which it is described as haustorial and non-haustorial types of fusion.

Hyphae anastomoses have been found to play an important role in the adaptation of fungi to environment. If the fused cells contain genetically different nuclei, then in anastomoses they fall into the common cytoplasm (the phenomenon of heterocaryosis). Mitoses provide for the proliferation of different allelic states of individual genes in the heterocaryon. In fungi with haploid nuclei, heterocariosis replaces diploidy, as in the phenotype of heterocaryotic cells, exhibits the same allelic ratio (dominant, recessive, codominant) as in heterozygous.

Perceptions about the role of hyphal interactions formed on the basis of studies performed with genetically marked laboratory strains of fungi [1; 2; 3] are described. As soon as a large number of naturally isolated strains were studied, most isolates in pairs did not form heterocaryons because of the vegetative incompatibility. Since vegetative incompatibility prevents the formation of heterocaryons with their adaptive properties, the existence of a strong selection in favour of incompatibility has drawn attention to both the phenomenon of incompatibility and its evolutionary consequences.

Vegetative incompatibility is manifested by the presence of two different strains of different genes responsible for the incompatibility. Thus, it is opposite to sexual compatibility, for the manifestation of which requires heteroallelic for compatibility genes. Therefore, the vegetative incompatibility was called heterogeneous and sexual homogeneous by the German geneticist K. Esser [3, p. 66–67, 212–217].

Vegetative incompatibility is a widespread phenomenon among fungi, oomycetes and myxomycetes, in which anastomoses between hyphae of strains heteroalellic at specific loci (*het*-loci), accompanied by death of the fused cells, and those adjacent to them, inhibits the interchange of nuclei and cytoplasm elements. Vegetative incompatibility of fungi has all the features that allow it to be considered an immune response. It is a form of recognition and rejection of the "stranger" because the greater the contacting strains of heteroallelic *het*-loci, and therefore the greater the difference in their genomes, the stronger the rejection reaction. Populations of many fungi break up into a large number of vegetative compatible (v – c) groups, leading to the mutual isolation of neighboring colonies. Probably, there are some factors that select genes of vegetative incompatibility and cause their accumulation in the population [1; 2; 3, p. 66–67, 212–217].

Among the hypotheses that explain the ecological role of vegetative incompatibility, the most valid hypothesis is about the protective function: protection against cytoplasmic elements, that are reproduces autonomously. In the cytoplasm of fungi there is found a large number of elements that are replicated autonomously (plasmids, viruses); some of them reduce the adaptability of their hosts, causing phenomena such as hypervirulence, aging, and others.

Not one of these elements, including viruses, is capable of infecting hyphae from the external environment (apoplastic). There are only two known ways of their distribution in fungal populations: vertical, by spores from parents to offspring, and horizontal transfer, via anastomoses between the hyphae of two growing strains.

Vertical transmission serves as protection of the sexual process, and protection against horizontal transmission is cytoplasmic incompatibility, since the efficiency of transmission of infection through the barrage zone is reduced, or it is not possible at all. Protection against vertical transmission is provided by the sexual process, and protection against horizontal transmission is cytoplasmic incompatibility, since the efficiency of transmission of infection through the barrage zone is reduced, or it is not possible at all.

Based on the above, the analysis of vegetative incompatibility reveals the distribution of different, i.e. biosynthetic, properties and phenotypic polymorphism of certain samples of basidiomycetes strains. The study of fundamental issues related to the phenomenon of compatibility in higher basidiomycetes is also of relevance as a scientific basis for the selection of high-yielding strains used in biotechnology, and for the regulation of the relationship between phytopathogenic wood-destroying fungi and the host plant to limit the spread of dangerous pathogens.

2. Materials and methods

Research objects are biosynthetically active strains of *Basidiomycota* fungi, selected in previous studies and related to *Agaricales* order and *Polyporales* order: *Agaricus bisporus, Lentinus edodes, Stropharia rugosoannulata, Fistulina hepatica, Laetiporus sulphureus, Flammulina* *velutipes, Pleurotus ostreatus, Schizophyllum commune, Sparassis crispa and* others. [5, p. 291; 6, p. 5; 7, p. 318]. The systematic position of the species of strains under study has been established according to current literature [4, p. 11]. The study of vegetative compatibility, growth rate of vegetative mycelium, cultural and morphological features of the experimental strains of basidiomycetes were performed according to well-known methods: natural beer-wort agar (BWA) and complex of potato-glucose agar (PGA) of nutrients. For calculations of the results of biological experiments and their statistical processing standard methods on a computer software package were used.

3. Determination of temperature optimum and radial growth of crops

The study of growth characteristics in pure culture is one of the most important stages of experimental studies of fungi, which allows to characterize the strains at the rate of growth and to optimize the conditions of their cultivation according to the set goals of the study.

To assess the growth of higher basidiomycetes crops a method of analysis of the dynamics of increasing the radius of colonies from the time of cultivation was used. Radial growth rate – *Vr* of vegetative mycelium was quantified in the phase of linear growth of colony radius from the time of cultivation.

Obtained experimental data from the temperature optimum of the radial growth rate of strains of some types of basidiomycetes allowed determining the optimal conditions for their cultivation in order to study the biosynthetic possibilities and obtain a seed inoculum on agar media. Used in the work nutrient media agar wort (WA, natural) and potato-glucose agar (PGA, complex) are suitable for growing the species of fungi. On the PGA higher values of *Vr* were recorded at the same optimum temperature, individual for each strain. It is established that in the analysis of data on the growth characteristics of basidiomycetes, special attention should be paid to the quantitative composition of nutrient media, since the concentration of substances can both accelerate and inhibit the growth processes of organisms.

The results of this stage of the research became the basis for choosing the optimal conditions for cultivation of strains to study their vegetative compatibility.

4. Study of vegetative incompatibility of Pleurotus ostreatus strains

In order to establish the genetic affinity of dikaryotic cultures (strains, isolates) of the examined species of basidiomycetes and the influence of this factor on their biosynthetic properties, they studied the vegetative incompatibility of introduced cultures on the basis of antagonism.

Analysis of the results of 105 di-di splice showed (Table 1.) that all the studied cultures of oyster mushroom (*Pleurotus ostreatus*) during splicing on agar nutrient medium formed in the contact area mycelial roller, which was a phenotypic manifestation of the vegetative incompatibility reaction.

However, the manifestations of antagonism at contact of different strains were different (Table 1; Fig. 1). Three types of mycelia morphology were identified in the contact zone of natural dicarions of oyster mushroom: the first type was characterized by that the contact area was poorly expressed, the reversum was not pigmented along the contact line, the mycelium hyphae crawled on one another, forming a roller, but did not splice; in the second type of mycelium morphology in the contact area a clear pigmented barrage 3-4 mm formed wide; in the third type of mycelium morphology, the formation of dense, corrugated or pigmented barge more than 4-5 mm wide was observed in the contact area. Depending on the amount of pigment highlighted, its color varied from pale yellow or red (second type) to dark brown (third type). In the first type of exudate and pigmentation in the contact area were absent.

According to Vilgales' classification, the first type of mycelium morphology in the contact zone was characterized as W (*weak*) – weak reaction of vegetative incompatibility; second – N (*normal*) – moderate reaction, light yellow pigmentation (N1) or red (N2) pigmentation, third – S (*strong*) – strong reaction, dark brown pigmentation. In the control variants of the splice, there was a fusion of hyphae without noticeable antagonistic reactions, which corresponds to the reaction of complete vegetative compatibility – C (*compatibility*) [3, p. 66–67, 212–217].

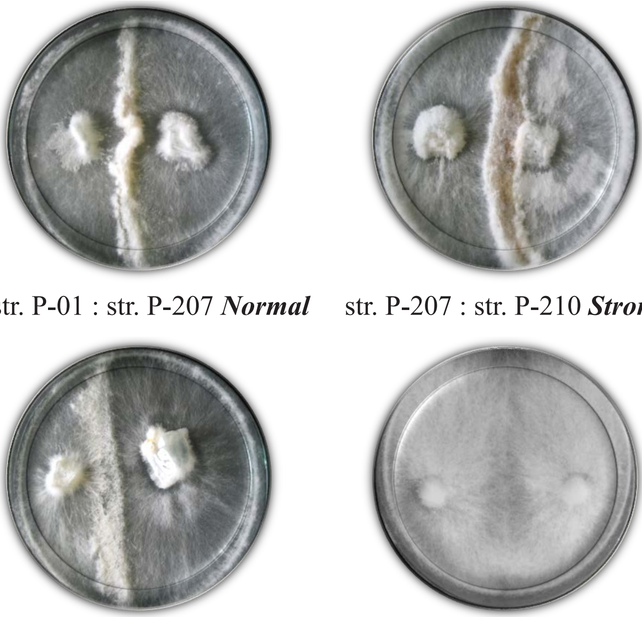
The degree of antagonism did not depend on the substrate – the tree species on which the wild fruiting bodies – the sources of the strains, and the time and place of their collection, grew. At the same time, in pairs splicing with several genetically different dicaryons, the same strain (or isolate) could show different degrees of antagonism, even all three types of vegetative incompatibility reactions (Table 1). At the same time, with multiple

Table 1

Vegetative incompatibility between strains of Pleurotus ostreatus

Strain	P-107	P-208	P-203	P-6v	P-207	P-191	P-210	P-kl	P-01	P-12	P-211	P-205	P-212	P-104
P-107	C	S	W	N1	N1	N2	W	W	N2	W	N2	W	N1	N2
P-208		C	N2	W	N2	N1	W	S	W	W	W	N1	N1	S
P-203			C	W	S	W	W	W	N2	W	N1	N2	W	S
P-6v				C	W	W	W	W	W	W	W	N1	N1	N2
P-207					C	S	S	W	N2	N1	W	S	W	N2
P-191						C	N1	S	N2	S	N2	N2	W	N2
P-210							C	W	W	W	W	N1	W	S
P-kl								C	N2	N2	N1	N2	N1	W
P-01									C	N1	N1	N2	N2	N2
P-12										C	N1	N1	W	N2
P-211											C	N1	N1	S
P-205												C	W	N2
P-212													C	W
P-104														C

Note: S – (Strong) – strong reaction; N1, N2 – (Normal) – moderate reaction; W – (Weak) – weak reaction; C – (Compatibility) – full crops compatibility



str. P-01 : str. P-207 ***Normal*** str. P-207 : str. P-210 ***Strong***

str. P-203 : str. P-107 ***Weak*** str. P-6v : str. P-6v ***Compatibility***

Figure 1. Types of reactions of vegetative incompatibility of some strains of *P. ostreatus*

splicing of the same pair of genetically different mycelium of dicarions, the same degree of antagonism was observed, indicating a strict genetic control of the processes of vegetative incompatibility in basidiomycetes.

The natural population of *P. ostreatus* was characterized by the frequency of detected types of reactions of vegetative incompatibility. In the study group of isolated cultures, a moderate vegetative incompatibility response (47.3%) was dominant, while a strong antagonistic reaction was observed only rarely (13.2%).

The biological diversity of *P. ostreatus* strains (natural isolates) by vegetative incompatibility was also estimated by the Zenon diversity index, which can range from 0.5 to 3.5 bits. In the studied sample of *P. ostreatus* strains, the Zeno index (H) was 1.01. The degree of vegetative incompatibility diversity observed during the splice was calculated using the Piel index (e). Its value is in the range from 0 to 1 vegetative incompatibility, and 1 of which corresponds to a situation of equal distribution of all reactions. In our case, the Piel index was e = 0.75, indicating a low heterogeneity of reactions of vegetative incompatibility in the study group of *P. ostreatus* strains.

To summarize this section of the work, it should be noted that the studied population of oyster mushroom is mainly composed of genetically different fungal individuals, which are reproduces mainly by spores of sexual reproduction. However, we do not deny the presence of vegetative reproduction in the natural population, because the results of several studies with other isolates that were not involved in further experiments indicate the presence of vegetative compatible clones that were isolated from fruit bodies growing on stumps of poplar tree and maple 5 meters from one another. In our opinion, single cases of vegetative reproduction do not influence the formation of the population structure of *P. ostreatus*.

5. Vegetative incompatibility between strains of several species of Basidiomy-cetes

The results of the study of vegetative incompatibility of strains of species of basidiomycetes *Agaricus bisporus, Lentinula edodes, Stropharia rugosoannulata, Fistulina hepatica, Laetiporus sulphureus, Schizophyllum commune and Sparassis crispa* are presented in table 2.

Analysis of the results of 36 di-di splices of strains these basidiomycetes within their species, showed that all the fungal cultures, when spliced on CA, were formed in the contact area mycelial roller – showed reactions of vegetative incompatibility. However, the antagonism expressed in the contact of the various strains was heterogeneous.

For strains of *A. bisporus, S. rugosoannulata, S. crispa* and *L. sulphureus*, a moderate reaction of vegetative incompatibility with light yellow pigmentation was recorded. The studied strains of *Schizophyllum commune* and *Fistulina hepatica* in a common culture had a more moderate response of vegetative incompatibility with red pigmentation of barrage.

Lentinus edodes has a natural brown color of mycelium that develops with age. In our experiment, we observed a moderate reaction of vegetative incompatibility with red pigmentation of barrage for strains 340 and 511 and an increase in this reaction to strong in variants of compatible cultiva-

Table 2

Vegetative incompatibility between strains of several species of Basidiomycetes

Species	*Agaricus bisporus*			*Lentinula edodes*			*Stropharia rugoso-annulata*		*Fistulina he-patica*	
Strain	A-115	B-92	273	340	511	523	258	754	Fh-08	Fh-18
Fh-18										C
Fh-08									C	N2
754								C		
258							C	N1		
523						C				
511					C	S				
340				C	N2	S				
273			C							
B-92		C	N1							
A-115	C	N1	N1							

Species	*Laetiporus sulphureus*			*Schizophyllum commune*			*Sparassis crispa*		
Strain	C-096	Ls-201	Ls-08	H-184	H-185	Sc-01	B-003	B-004	B-014
C-096									C
Ls-201								C	N1
Ls-08							C	N1	N1
H-184						C			
H-185					C	N2			
Sc-01				C	N2	N2			
B-003			C						
B-004		C	N1						
B-014	C	N1	N1						

Note: S – (Strong) – strong reaction; N1, N2 – (Normal) – moderate reaction; W – (Weak) – weak reaction; C – (Compatibility) – full crops compatibility

tion with strain 523. Probably, the appearance of red and brown color of mycelium in the contact city is associated with the induction of pigment synthesis of fungus – carotenoids and melanin [5, p. 291; 7, p. 318].

6. Study of vegetative incompatibility of strains Flammulina velutipes

Analysis of the results of 105 di-di splice of *F. velutipes* strains (Table 3, Fig. 2) showed that all the investigated cultures of winter honeysuckle were formed in the contact area with a mycelial roller in the contact zone with CA – revealed a reaction of vegetative incompatibility.

However, the expressed antagonism between the different strains of flammulin was heterogeneous. We have recorded all types of mycelium morphology in the contact area of the dicarion colonies of *F. velutipes*.

The degree of antagonism did not depend on the substrate: tree species on which wild fruit bodies grew – the source of the strains, as well as the time and place where they were collected. At the same time, in paired splice with other dicarions, the same natural strain (or isolate) reveals different degrees of vegetative incompatibility reaction, as confirmed by multiple splicing.

The natural population of *F. velutipes* was characterized by the frequency of detection of types of vegetative incompatibility reactions. A moderate reaction of vegetative incompatibility (72.5%) prevails in the population studied, and a strong antagonistic reaction is very sporadic (6.6%).

The degree of antagonism did not depend on the substrate – the tree species on which the wild fruit bodies grew – the sources of the strains, and the time and place where they were collected. At the same time, in paired splice with other dicaryons, the same natural strain (or isolate) reveals different degrees of reaction of vegetative incompatibility, as confirmed by multiple splicing same pair of strains.

The natural population of *F. velutipes* was characterized by the frequency with which types of reactions of vegetative incompatibility were detected. A moderate reaction of vegetative incompatibility (72.5%) prevails in the population studied, and a strong antagonistic reaction is rare (6.6%).

Biodiversity of *F. velutipes* natural strains by vegetative incompatibility: Zeno index (H) was equal to 1.25; Piel's index was e = 0.85, which indicated a low heterogeneity of vegetative incompatibility reactions in the study population.

Therefore, the population of the enoki mushroom being studied is made up of genetically different fungal individuals, which are reproducing by sexual reproduction spores. We do not deny the presence in the Donetsk population of vegetative reproduction, because the results of several splices

Table 3

Vegetative incompatibility between Flammulina velutipes strains

Strain	F-03	F-04	F-1	F-102	F-104	F-203	F-3	F-4	F-7	F-8	F-9	Fv-1	Fv-7	F-vv
F-03	C	N1	N1	N1	N1	N2	N1	W	N2	W	N1	N1	N1	S
F-04		C	N1	N1	N2	N1	W	N1	W	N1	N1	N1	N1	N2
F-1			C	N2	S	N1	W	W	N2	W	N1	N2	W	N2
F-102				C	N1	N1	N1	N1	N1	N1	W	N1	N1	N2
F-104					C	S	N1	W	N2	N1	W	N1	W	N2
F-203						C	N1	S	N2	S	N1	N2	N2	S
F-3							C	W	W	W	W	N1	W	N2
F-4								C	N2	N2	N1	N2	N1	N2
F-7									C	N1	N1	N2	N2	N2
F-8										C	N1	N1	W	N2
F-9											C	N1	N1	N2
Fv-1												C	W	N2
Fv-7													C	N2
F-vv														C

Note: S – (Strong) – strong reaction; N1, N2 – (Normal) – moderate reaction; W – (Weak) – weak reaction; C – (Compatibility) – full crops compatibility

str. F-04 : str. F-vv ***Normal*** str. F-vv : str. F-03 ***Strong***

str. F-03 : str. F-4 ***Weak*** str. F-vv : str. F-vv ***Compatibility***

Figure 2. Types of reactions of vegetative incompatibility of some strains of *F. velutipes*

with other isolates, which were not used in further experiments, indicate the presence of vegetative compatible clones from fruiting bodies grown on stumps on maples located at a distance of up to 1 meter.

In our opinion, single cases of vegetative reproduction do not significantly influence the formation of the population structure of *F. velutipes*, as well as in the researched *P. ostreatus*.

The result of research is the study of the phenomenon of vegetative or heterokaryotic incompatibility in different taxonomic groups of fungi: the inability of fusion the mycelium of certain strains, prevents the formation of heterocaryons. It is shown that the incompatibility is manifested in the fact that anastomoses do not form or hyphae die after fusion, in the latter case, a strip of vacuolated dead cells is formed at the boundary of contact between the two colonies. According to the gene control mechanism, the vegetative incompatibility of opposite sexual compatibility, which requires heterogeneity on compatibility genes. Vegetative incompatibility, on the contrary, manifests itself if the strains have different genes responsible for compatibility, the German geneticist K. Esser called vegetative incompatibility as heterogeneous and sexual as homogeneous. It has been established from the results of experiments that natural strains (isolates) often differ in several het genes, resulting in a large number (tens) of mutually incompatible groups.

7. Ways of using in the modern biotechnology of vegetative incompatibility of Basidiomycota strains

As we have already noted, one of the priority areas for the development of mycology is biotechnology, namely the development of technologies using cells and vegetative cultures of basidium fungi to obtain biologically active substances, including those that have therapeutic properties. This has been confirmed by the results of researches in recent decades, which have shown that pharmacologically active fungi substances, as compared to chemical synthesis products, are less toxic and more effective in medical practice. As a result, numerous cultivation technologies and patented formulas for biologically active additives based on mycelium of higher fungi have been developed [4, p. 1–215; 6, p. 4–10; 8, p. 1323–1332].

The scientific foundations of modern technologies of artificial cultivation of edible mushrooms and production of sowing mycelium have been developed and continue to be developed in numerous specialized scientific institutions both in our country and in the world. Research in this scientific direction has intensified recently, due to a shortage of biologically/physiologically active substances used in various sectors and the functioning of society.

It should be noted that researchers' interest in natural compounds of any origin is linked to the search for new chemical structures. However, recently the properties of natural compounds, and, above all, the level and nature of their biological activity, are much more relevant and in demand. Recently the properties of natural compounds and the level and nature of their biological activity are much more relevant and in demand. High-effective screening (high-throughput screening – HTS) used by leading pharmaceutical firms has virtually exhausted the stocks of natural compounds and synthetic products in various scientific institutions, biological activity that has never been in research before. The requirements of the pharmaceutical industry have stimulated the development of combinatorial chemistry, which allows the synthesis of a large number of chemicals. However, combinatorial chemistry does not shorten the development of drugs, although it greatly expands their search.

In recent decades, natural product-based drug discovery (NPDD) activity in various countries of the world has been in strong competition with high-efficiency screening, combinatorial chemistry and genetic research. An analysis of NPDD activity in Japan showed that among the microorganisms studied, fungal organisms from such taxa and ecological groups as ascomycetes, mitospore, soil and fungi that live on forest floor were the most widely represented. At the same time, companies have encountered difficulties in obtaining biological material from other countries because of the Convention on Biological Diversity. However, companies have encountered difficulties in obtaining biological material from other countries due to the Convention on Biological Diversity. With biodiversity, opportunities for prospective strains are increasing. The study of fungal organisms with complex trophies and ecologies, which are rare, will allow expanding the search and will help to obtain new information on their biology and physiology, biochemistry and genetics.

Numerous collections of cultures of Basidiomycota, Ascomycota and other microorganisms have been created as promising objects of biotechnology. Many years of experimental studies were conducted to study their development and cultural and morphological features at different stages of the life cycle and substrates, selection of new strains, study of their interaction in compatible culture, layout of new substrates, establishment of new approaches and optimal technological parameters of cultivation. Many years of research have been conducted into their development and cultural and morphological features at different stages of the life cycle and substrates, the selection of new strains, their interaction in a shared culture, the composition of new substrates, the establishment of new approaches and optimal technological parameters of growing, made it possible to establish, that biologically active substances are found not only in fruit bodies but also in vegetative mycelia.

To summarize the results of this research, we note the following: the practical, biotechnological use of fungi in a variety of industries requires continuous search / selection of new strains of different uses, based on selection, life cycle features and natural isolate characteristics.

Due to the complexity of the organization, higher basidium fungi, as objects of biotechnology, occupy a special, intermediate position between microorganisms, plants and animals.

Mycelium fungi have a common characteristic: in the physical contact of the mycelium, their cells merge (anastomoses hyphae, plasmogamy). Because the fungal hyphae are covered with multilayered membranes, the merger is preceded by local dissolution of the cell walls. This property may have originated initially as a result of the need to merge vegetative structures in fungi without gametes. Probably that anastomosis was based on mycoprazitism, which is described as gaustorial and non-gaustorial types of merger.

Gif anastomoses have been found to play an important role in the adaptation of fungi to environmental conditions. If the fused cells contain genetically different nuclei, then in anastomoses they fall into the general cytoplasm (the phenomenon of heterocariosis). Mitoses provide for the proliferation of different allelic states of individual genes in the heterocarion. In fungi with haploid nuclei, heterocariosis replaces diploidy, as in the phenotype of heterocariotic cells, exhibits the same allelic relationships (dominant, recessive, codominant) as in heterozygous.

The ideas about the role of hyphal interactions, based on studies conducted with genetically labeled laboratory strains of fungi, are described. As soon as a large number of naturally isolated strains began to be studied, it was found that most isolates do not form heterocarions due to vegetative incompatibility when paired sowing. Since vegetative incompatibility prevents the formation of heterocarions with their adaptive properties, the presence of powerful selection in favor of incompatibility made it necessary to pay attention both to the incompatibility phenomenon itself and to its evolutionary consequences.

Vegetative incompatibility occurs when two different strains have different genes that are responsible for incompatibility. Thus, it is opposite to sexual compatibility, for the manifestation of which requires heteroallelic for compatibility genes. So the vegetative incompatibility was called heterogeneous, and the sexual was called homogeneous. Vegetative incompatibility is a phenomenon widespread among mushrooms, in which anastomoses between the hyphae of strains heteroallelic to certain loci (het-loci) are accompanied by the death of the fused cells and those adjacent to them, which impedes the mutual exchange of nuclei. The vegetative incompatibility of mushrooms has all the characteristics that make it possible to consider it as an immune response. It is a form of recognition and rejection of “alien” because the more the contacting strains of heteroallelic *het*-loci, and therefore the greater the difference in their genomes, the stronger the rejection reaction. Populations of many fungi break up into a large number of vegetative compatible (v – c) groups, leading to the mutual isolation of neighboring colonies. Probably some of the factors that breed the genes of the vegetative incompatibility and cause them to accumulate in the population.

Among the hypotheses that explain the ecological role of vegetative incompatibility, the most substantiated hypothesis of a protective function is protection against cytoplasmic, self-replicating elements. In cytoplasm in fungi a large number of elements, which have been found, autonomously replicate (plasmid, viruses) some of them reduce the adaptability of their hosts, causing phenomena such as hypervirulence, ageing and others.

Not one of these elements, including viruses coated with protein capsids, is capable of infecting hyphae from the external environment (apoplastic). There are only two known ways of their distribution in fungal populations: vertical way via spores from parents to offspring, and horizontal transmission via anastomosis between hyphae of two growing strains.

Protection against vertical transmission is provided by the sexual process, and protection against horizontal transport is provided by cytoplasmic incompatibility, as the effectiveness of the transmission of the infection through the barrage zone is reduced or impossible.

As an example of the practical use in modern biotechnology of the vegetative incompatibility of Basidiomycota strains, the following may be mentioned. *Neurospora* fungi and *Blakeslea trispora* have long been classic with carotinogenesis research objects.

It has been established that the enzymes of the *N. crassa* fungus, which catalyze both the formation and the denaturization of phytine, are most likely photoinduced. For this fungus, the quantity and quality of the carotinoids also change due to the conditions of cultivation: the nature of the sources of food, which consists of carbon and nitrogen, their ratio in the nutrient medium, mineral salts, vitamins, aeration, pH and temperature.

An interesting example of chemical control of carotinogenesis is observed in *B. trispora*. In separate cultivation of (+) – and (-) – strains synthesize carotenoids in small quantities, whereas in mixed cultures intensive carotene synthesis occurs. This synthesis is induced by trisporic acid, a hormone produced by the combination of enzymes from two mixed culture strains. Trisporic acid is an intermediate product of carotine biosynthesis and its main function is to stimulate sporulation and reproduction. Carotinogenesis stimulation may be part of a mechanism to enhance trisporic acid synthesis. Recent work has shown that under the conditions of surface cultivation, the sexual interaction of heterotalic strains of *B. trispora* capable of intensive zygospore formation resulted in a decrease in the level of carotinogenesis compared to the strains that grew separately. Quite the opposite, the sexual intercourse of strains that are not capable of zygotic formation has led to the stimulation of carotinogenesis. In the deep culture of the pair of zygote-forming strains, synthesized more trisporic acids, but less carotinoids than strains do not form a zigospore. The inverse relationship between zygotic formation and carotinogenesis was revealed, which made it possible to propose as a criterion for selection of carotinogenic pairs of strains their inability to zygote formation.

Thus, an analysis of vegetative incompatibility reveals the distribution of different properties, i.e. biosynthetic properties and phenotypic polymorphism of certain samples of species strains of basidiomycotic strains. The study of fundamental issues related to the phenomenon of compatibility in higher basidium fungi is also of relevance as a scientific basis for the selection of high-yielding strains used in biotechnology, and for regulating the relationship between phytopathogenic wood-destroying fungi and the host plant to limit the spread of dangerous pathogens.

8. Conclusions

Thus, a total of 246 di-di splices of vegetative mycelium of basidial fungus strains of *Agaricus bisporus, Lentinus edodes, Stropharia rugosoannulata, Fistulina hepatica, Laetiporus sulphureus, Flammulina velutipes, Pleurotus ostreatus, Schizophyllum commune* and *Sparassis crispa* were carried out. As a result of experiments, all types of mycelium morphology were established in the contact zone of the colonies of these strains. These are: W (*weak*) – weak reaction, contact area was weakly expressed, not pigmented, hyphae of mycelium crawled on each other, but did not merge; N (*normal*) – moderate reaction, a clear, pigmented barrage 3-4 mm wide was formed in the contact zone; S (*strong*) – strong reaction, in the zone of contact formation of dense infrequently corrugated or pigmented barrage more than 4-5 mm wide, C (*compatability*) – full compatibility was observed.

Among the tested strains, a moderate vegetative incompatibility response from 100% in *A. bisporus, S. rugosoannulata, F. hepatica, L. sulphureus, S. commune* and *S. crispa* to 72,5% in *F. velutipes* and 47,3% in *P. ostreatus;* a strong antagonistic reaction was observed very rarely – from 13,2 in *P. ostreatus* to 6,6% – in *F. velutipes.* The exception is *Lentinus edodes*, where 2 out of 3 cases (66.7%) had strong reactions.

No cases of clonal origin were found in the samples of the strains presented, as different reactions of vegetative incompatibility were observed in all the splices. Based on a number of scientific publications [1; 2; 3], we approve the dominance of sexual reproduction in the populations studied and the genetic diversity of strains of the same species, and their cultural-morphological and biosynthetic properties.

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