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EXPERIMENTAL ARTICLES

Trisporoids and Carotenoids in *Blakeslea trispora* Strains Differing in Capacity for Zygote Formation

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Abstract—The study of sexual interaction in seven (+) and four (–) *Blakeslea trispora* strains from the All-Russian Collection of Microorganisms showed that all the strains were capable of forming zygotes, albeit to a varying degree. Thus, pairs of strains with active zygote formation and not forming zygospores were identified. It was established that, irrespective of their capacity for zygote formation, all pairs of the (+) and (–) strains synthesized trisporoids in submerged culture. However, zygote-forming pairs accumulated significantly more trisporoids and carotenoids than the strain pairs incapable of forming zygospores. As a result, positive correlation between the capacity for zygote formation in surface culture and trisporoid and carotenoid synthesis in submerged culture was revealed in wild strains.

Keywords: *Blakeslea trispora*, zygote formation, carotenogenesis, trisporoids, trisporic acids

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Sexual interaction substantially stimulates carotenoid synthesis, as the number of mRNA genes *carB* (phytoene dehydrogenases) and *carRA* (phytoene synthetases and lycopene cyclases) formed in the joint culture of the (+) and (–) mating types of *Blakeslea trispora* strains increases 128- and 148-fold, respectively, after 48 h, whereas upon separate growth, it increases only 10-fold [1]. Moreover, in the course of sexual interaction, induction of expression of the *tsp3* gene occurs, which encodes β-carotene oxygenase, the enzyme responsible for the cleavage of β-carotene molecules, i.e., the first step in the synthesis of the sex hormone, trisporic acids (TSA) [2]. This hormone is a trigger of the process of sexual reproduction; it controls sexual interaction between heterothallic strains, the formation of sex structures, zygospores, stimulates carotenogenesis, and regulates its own feedback-type synthesis. When heterothallic strains are grown together, extracellular synthesis occurs of the family of trisporoids (C_{18} or C_{19}-isoprenoid compounds with the C_{14} main chain), including the TSA isoforms and their precursors.

An obvious close relationship between these processes allows for a suggestion that the capacity for zygote formation and TSA synthesis may be the criteria for the carotenogenic activity of these fungi. Although a "correlation relationship" between zygote formation and carotenogenesis was previously noted [3], the work was performed on mutant strains, and it was not clear how this was associated with TSA synthesis. The difficulty lies in the fact that zygote formation is only observed in surface culture, while submerged cultivation is used for studying carotenoid and trisporoid synthesis. The study of carotenogenesis in surface culture showed that zygote-forming pairs contained much less carotenoids than the pairs not forming zygospores [4]. This is due to the fact that carotenoids are absolutely necessary for the process of zygote formation, because they are used to form sporopollenine, a structural component of the zygospore cell wall, as well as trisporic acids, which are a sex hormone. In addition, carotenoids (about 0.5% of the dry mass) are accumulated in fungal zygospores [5]. The inhibition of carotenogenesis in surface culture therefore blocks the process of zygote formation [6].

In submerged culture, sexual interaction results in the formation of a multitude of expansions of different shapes filled with lipid–carotene vacuoles, which is more characteristic of the (–) mating type strains playing the main role in carotenogenesis. Earlier, it was found that the optimum conditions for carotenogenesis differed from those for TSA formation [4]. In order to reveal these conditions, a two-stage process is used. Initially, the inoculum of the (+) and (–) strains is grown separately in liquid culture. The inoculum obtained is then used in different ratios for the main fermentation. An equal (+) to (–) strain ratio in the inoculum is beneficial for TSA synthesis, whereas the prevalence of the (–) strain (7 : 1) is required for

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intense carotenogenesis. Moreover, it was shown on a small sample of two pairs of strains in submerged culture that the zygote-forming 812(+) × 921(–) pair synthesized more trisporic acids but less carotenoids than the 812(+) × 826(–) pair incapable of forming zygotes [4]. These contradictions cannot be explained from the position of the present-day notions of the role of TSA in carotenogenesis. Therefore, in order to understand the relationship between zygote formation and trisporoid and carotenoid synthesis, it is expedient, firstly, to expand the strain spectrum and, secondly, to investigate in greater detail the trisporoid (TS) composition, rather than the amount of trisporic acids alone.

The goal of the present study was to investigate a relationship between the fungus capacity for zygote formation and trisporoid and carotenoid synthesis using a representative sample of *B. trispora* (+) and (–) mating types of strains.

MATERIALS AND METHODS

The study was conducted with the collection (VKM) strains of the representative of the zygomycete fungi *Blakeslea trispora* Thaxter: the (–) strains VKM F-811, 921, 987, 826 and the (+) strains VKM F-666, 701, 812, 902, 904, 989, 1201, as well as two strains from the collection of cultures of the Institute of Microbiology, Russian Academy of Sciences: T(+) and T(–). Strain T(–) was obtained by means of mutagenesis and is not considered to be wild.

The fungi were grown in submerged culture. Spore suspension (2–5 × 10^5 spores/mL medium) was used for inoculation. The inocula of the (+) and (–) mating types of the strains were grown separately in 100 mL of hydrolytic flour medium [7] in 750-mL flasks for 48 h on a rotary shaker (220 rpm) at 27–28°C. In order to study carotenogenesis, 50 mL of the flour medium [7] was inoculated with the inocula of the (+) and (–) mating types of the strains at a ratio of 1 : 7 and an amount of 20% (vol/vol); 5% (vol/vol) of sunflower oil was added, and the mixture was grown for four days. For investigation of trisporoid formation, the flour medium was inoculated with the (+) and (–) strains (10% vol/vol) at a 1 : 1 ratio, supplemented with 3% (vol/vol) of sunflower oil, and incubated for 4 days.

In order to study the capacity for zygote formation, the slabs of the surface-grown mycelium of the (+) and (–) mating types of *B. trispora* strains (7–8 mm in diameter) were placed on the opposite sides of a petri dish with wort agar 7°B and cultivated for 10 days at 25°C in the dark. The zygote formation rate was assessed by the width of the contact band. The absence of zygospores was controlled by light microscopy at 300× magnification (Jenaval, Germany).

Trisporoids were extracted from the culture fluid with a chloroform–isopropanol (20 : 1) mixture at a 2 : 1 ratio [8] twice for 20 min at room temperature, initially at pH 8.0 followed by pH 2.0. All the operations related to the isolation of trisporoids were performed under scattered light. The antioxidant butylhydroxytoluene was added to the extracts, which were then washed off with water three times and dried with Na_2SO_4. The solvents were removed in a rotary evaporator, and the residue was dissolved in 96% ethanol to the concentration of 50 μg/μL. The trisporoid composition was investigated by ascending TLC using the system of solvents: chloroform–ethyl acetate–butylmethyl ether–acetic acid (50 : 30 : 15 : 5) [8]. Trisporoids (50–100 μg) were applied on a 20 × 20 cm plate with silica gel 60 F_{254} (Merck). Retinol (Sigma) was used as the standard. Detection was carried out in the ultraviolet light on a Sorbfil-M densitometer (Sorbpolimer, Russia). The absorption spectra in the 220–400-nm region [9] and results of the biological test were used for trisporoid identification. Quantitative analysis of TSs was carried out using the Dens software package (Lenkhrom, Russia) in the mode of direct approximation from the calibration curves for the standard retinol solutions.

The isolation of trisporoids for the biological test was carried out by TLC using the 20 × 20 cm silica gel plates to which 10–15 mg of trisporoids was applied. Chromatography was performed in the systems described above, but during detection in the UV light, the part of the plate from which the trisporoids were subsequently eluted was covered with foil to avoid their oxidation. The elution was performed with ethanol three times for 20 min at room temperature. After evaporating the solvents, the residue was dissolved in 0.2 mL of 96% ethanol and stored at –21°C.

To determine the biological activity of the trisporoids, we used the test strains *Mucor mucedo* VKM F-1355(–) and 1356(+) and the modified technique [8]. The inocula of the (+) and (–) mating types of strains were grown on petri dishes with a 3-mm layer of wort agar at 23°C for seven days. A 8 × 8 mm square slab from the colony-growing zone was placed on the edge of a microscope slide on which 2.5 mL of the following medium (g/L) was preliminarily applied: maltose, 20; KNO_3, 10; KH_2PO_4, 5; $MgSO_4 \cdot 7H_2O$, 2.5; yeast extract, 1; agar, 12 [10]. The slide was placed in a petri dish on the filter paper wetted with 0.2 mL of water to create moist conditions. The cultures were grown at 23°C (optimum growth temperature) for two days until the mycelium reached the middle of the slide. TS solutions in ethanol (30–300 μg) were applied onto small discs of filter paper (5 mm in diameter), which, after ethanol evaporation, were placed at a distance of 3 mm from the growing mycelium front. After that, cultivation of the fungi continued at 21°C (the optimum temperature for zygote formation) for 28 h. The biological activity of trisporoids was assessed by their ability to induce zygophore formation in the (+) and (–) mating types of *Mucor mucedo* strains. The zygophores were enumerated in the 6 mm^2 area between the disc and the band of the initial mycelial

Table 1. Zygote formation in different pairs of *B. trispora* strains

Strains	Width of the zygote formation band, mm				
	811(–)	921(–)	826(–)	987(–)	T(–)
1201(+)	–	3	1	4	–
666(+)	s	4	1	2	–
904(+)	2	4	2	6	–
812(+)	3	3	–	7	–
989(+)	5	8	2	7	–
701(+)	6	3	s	6	s
902(+)	2	2	s	7	–
T(+)	1	4	–	7	–

Note: "s" stands for single immature zygospores with unstained membrane; "–" denotes the absence of zygospores.

front using a Jenaval light microscope (Germany) at 300× magnification and a 50× dissecting microscope.

The carotenoid composition was determined by the method developed by us [11]. The experiments were performed in triplicate; the results show the data of the typical experiment reflecting general regularity: the scatter of the results did not exceed 10%.

RESULTS

Determination of the capacity of the wild strains of *B. trispora* for zygote formation made it possible to conclude that they were all capable of forming zygotes, although to a varied degree (Table 1). Two of the four (–) strains, 921 and 987, formed zygospores with all (+) strains, whereas 811(–) did not form zygospores with 1201(+) and 666(+), nor did 826(–) with a number of strains, e.g., with 812(+), 902(+), 701(+), and T(+). Importantly, 811(–) actively formed zygospores with the other (+) strains, while weak zygote formation only with several (+) strains was inherent in 826(–). Among the (+) strains, it was also possible to identify weak and strong zygote formers. Intense zygote formation was inherent in 989(+) and 904(+) strains, which formed zygospores with all wild (–) strains. Our findings enabled us to select zygote- and nonzygote-forming pairs of strains for our further research into carotenogenesis, namely, 1201(+) and T(+), which in pairs with all (–) strains could produce various combinations based on their capacity for zygote formation.

Since it was shown earlier that the optimum conditions for carotenoid and trisporoid synthesis occur under different strain ratios in the inoculum [4], each process was studied under the optimum conditions revealing the maximal fungus activity. Moreover, a pair of strains of carotenoid overproducers, T(–) and T(+), were at our disposal. Investigation of these strains could be helpful for the understanding of the relationship in question. It must be emphasized that this pair does not belong to wild-type strains, since the (–) T strain was obtained by mutagenesis.

The study of carotenogenesis in submerged culture under the optimal conditions in pairs of strains with a different capacity for zygote formation made it possible to conclude that zygote-forming pairs had the highest carotenogenic activity among wild strains. For example, the following carotenogenic activity series can be constructed for pairs with 1201(+): 921(–) > 826(–) > 987(–) (all zygote-forming) > 811(–) (does not form zygospores) (Table 2). The same general pattern was observed in pairs with T(+): 921(–) > 987(–) = 811(–) (all zygote-forming) > 826(–) (does not form zygospores). Note that no proportional dependence was found between the rates of zygote formation and carotenogenesis.

Table 2. Carotenogenesis in the joint cultures of heterothallic *B. trispora* strains differing in their capacity for zygote formation

Strains	Width of the zygote formation band, mm	Biomass, g/L	Carotenoids, % of dry mass		
			β-carotene	lycopene	sum
T(+) × 987(–)	7	48.2	0.15	0.02	0.17
T(+) × 921(–)	4	44.1	0.25	0.01	0.25
T(+) × 826(–)	–	48.8	0.07	0.005	0.07
T(+) × 811(–)	1	40.7	0.16	0.01	0.17
T(+) × T(–)	–	42.3	1.28	0.02	1.30
1201(+) × 987(–)	4	50.0	0.21	0.03	0.24
1201(+) × 921(–)	3	43.4	0.73	0.03	0.75
1201(+) × 826(–)	1	38.9	0.47	0.09	0.55
1201(+) × 811(–)	–	28.2	0.02	0.005	0.02
1201(+) × T(–)	–	43.6	0.84	0.15	0.99

Note: "–" denotes the absence of zygospores.

Table 3. Composition of the main trisporoids in the pairs of heterothallic *B. trispora* strains differing in the capacity for zygote formation

Variant	Biomass, g/L	β-Carotene, % of dry mass	Trisporoids									
			% of Σ								mg/g dry mass	g/L
			TN + TL	X_{290}	TSA-A	TSA-B	TSA-C	Σ TSA	X_{260}	MTSA		
T(+) × T(–)	23.9	0.26	31.7	–	1.7	17.8	32.0	51.5	–	–	94.4	2.26
T(+) × 826(–)	30.3	0.05	39.5	–	3.4	–	18.0	21.5	–	–	23.3	0.71
T(+) × 921(–)	32.0	0.06	36.8	–	3.8	8.8	31.8	44.4	4.1	–	71.4	2.28
1201 (+) × T(–)	26.2	0.10	26.6	18.5	–	7.7	26.2	33.9	–	4.3	23.3	0.61
1201 (+) × 811(–)	27.2	0.02	–	43.3	–	3.3	23.3	26.7	–	–	3.0	0.08
1201 (+) × 921(–)	24.6	0.08	35.7	–	–	10.2	15.8	26.0	–	–	37.3	0.92

Note: TN + TL, trisporins and trisporols; TSA-A, B, C, trisporic acids A, B, C; MTSA, methyltrisporates; X, unidentified compounds; "–", not determined.

Table 4. Biological activity of the trisporoids isolated from the joint culture of T(+) × 921(–)

Trisporoids, fraction	(–) *M. mucedo* strain		(+) *M. mucedo* strain	
	Trisporoids, μg	Number of zygophores	Trisporoids, μg	Number of zygophores
Trisporins/trisporols, NF	130–260	–	20–150 240	++ +++
X_{60}, NF	50	++	50–80 100–200	+ ++
X_{290}, NF	15–150	–	90–180	+
MTSA, NF	80	++++	80–160	++
TSA-A, AF	20 50	++ +++	20 40–80	+ ++
TSA-B, AF	20–60	+++	25–120 150	++ +++
TSA-C, AF	30 50	++++ ++++	30–50 60–100	++ +++

Note: NF, neutral fraction; AF, acid fraction; "–", not determined. X, unidentified compound. MTSA, methylsporates. The number of zygophores: + fewer than 10; ++ 10–50; +++ 50–100; ++++ more than 100.

To investigate trisporoid formation, we selected the pairs of strains contrasted by zygote formation. In submerged culture where zygote formation was not marked, all the cultures, regardless of their capacity for zygote formation, were under the same conditions. When the (+) and (–) strains interacted, carotenogenesis was intensified and formation of a number of trisporoids was observed (Table 3). It should be emphasized that the pairs of strains not forming zygospores also turned out to produce trisporoids in submerged culture, although at much lower concentrations than in active zygote formers. Trisporins and trisporols with maximum absorption at 300 nm (Figs. 1, 2) and the ability to induce zygophores only in the (+) *M. mucedo* strain (Table 4) were the main components of the neutral trisporoid fraction. It should be noted that trisporols/trisporins were present in both the neutral and acid fractions and represented by two spots on a thin-layer chromatogram (Fig. 1); however, the maxima of their absorption spectra and the biological activity data coincided; consequently, we considered them as one fraction and assessed their total amount. Methyltrisporates with maximum absorption at 323 nm and the biological activity in relation to both strains were a minor component in the neutral fraction. In the acid fraction, the main component was trisporic acids (Figs. 1, 3) with a positive reaction of the biological test to both the (+) and (–) *M. mucedo* strains (Table 4). The trisporic acids A (λ_{max} = 315 nm), B (λ_{max}= 322 nm), and C (λ_{max} = 327 nm) could be identified by their absorption spectra [8].

The results shown in Table 3 indicate that, among wild strains, far more trisporoids (three times as many) were formed in the strains capable of forming zygotes and synthesizing more carotenoids. However, the strain 1201(+), compared with T(+), formed consid-

erably less trisporoids (2–3-fold) with the (–) strains studied. Differences between the pairs forming and not forming zygotes were also observed in the trisporoid composition. For example, in the case of the zygote-forming pair T(+) × 921(–), three times as many trisporoids were formed than in the pair T(+) × 826(–) not forming zygotes. Moreover, TSA-B was absent in the latter case and the TSA share in the trisporoid composition was half as much. However, the relative content of the neutral precursors, trisporins/trisporols, slightly differed. The absence of TSA-A was a feature of the trisporoid composition in pairs with 1201(+). The differences between the amount of trisporoids, depending on the capacity for zygote formation in pairs with 1201(+), were stronger by a factor of 10–12. Furthermore, in the pair 1201(+) × 811(–), which did not form zygotes, an additional spot with the maximum at 290 nm accounting for approximately the same share as in the zygospore-forming pair 1201(+) × 921(–) was present in the chromatogram instead of the typical trisporins/trisporols. In terms of biological activity, this compound was similar to trisporins, because it induced zygophore formation only in (+) *M. mucedo*, but its biological effect was by far weaker than in trisporins/trisporols (Table 4).

Thus, it is the first time we have shown in this study on four pairs of wild-type strains with a different capacity for zygote formation that there exists a positive correlation between the capacity for zygote formation in surface culture and trisporoid and carotenoid synthesis in submerged culture.

M. mucedo test strains are used for investigation of the biological activity of trisporoids because they form zygophores, which can easily be enumerated on aerial hyphae. Unlike this fungus, the zygophores in *B. trispora* and *Phycomyces blakesleeanus* are formed on substrate hyphae, and these strains are normally used only for studying the carotenogenic effect of trisporoids [12]. In *M. mucedo*, the zygophores appear as characteristic short, curved aerial hyphae with a thickened membrane, the (+) strain having longer zygophores.

The results of the test showed that trisporoids from the zygote-forming pair T(+) × 921(–) differed in their sexual activity (Table 4). Thus, trisporins, trisporols, and the unidentified substance with maximum absorption at 209 nm caused an effect only in the (+) strain; TSA-A, TSA-B, and TSA-C were efficient for both strains. A much stronger reaction to trisporoids in the (–) *M. mucedo* strain was a general pattern consistent with the literature data [7]. On the contrary, the (+) strain not only reacted to higher trisporoid concentrations, but also formed substantially fewer zygophores than the (–) strain. TSA-B had the strongest effect on the (–) strain among the trisporic acids, which agrees with the literature data [13], while no such feature was noted for the (+) strain. The unidentified compound with the maximum absorption at

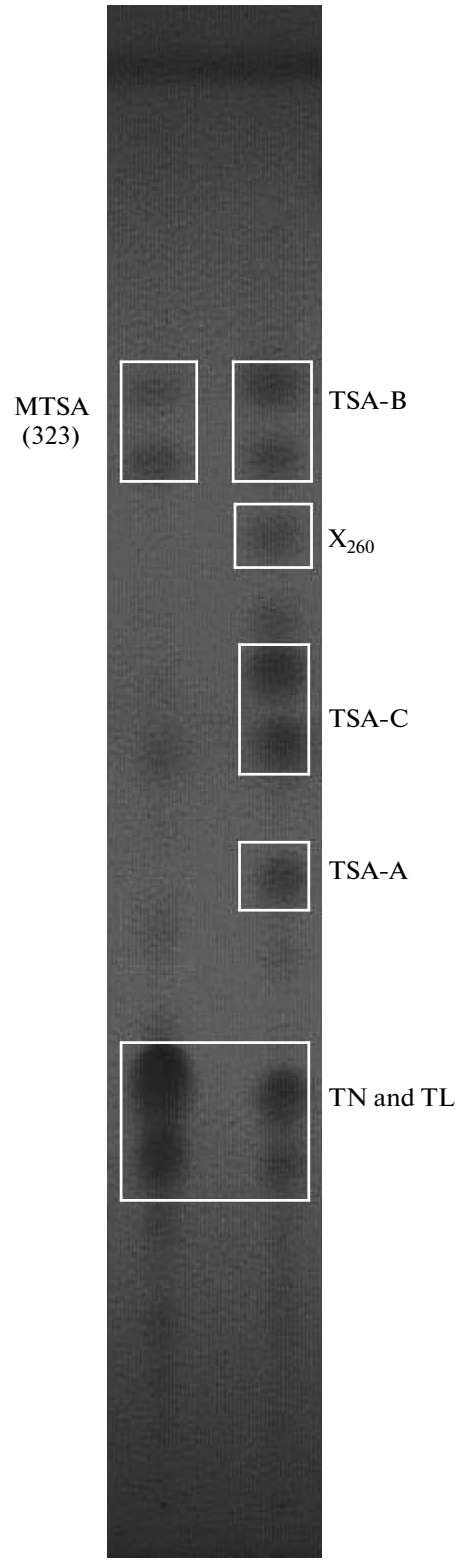

Fig. 1. Chromatogram of trisporoids of the neutral (left) and acid (right) fractions from the joint culture of *B. trispora* T(+) × 921(–) strains. TSA-A, -B, and -C are the trisporic acids A, B, C; X is an unidentified compound; TN and TL are trisporins and trisporols; M-TSA stands for methyltrisporates.

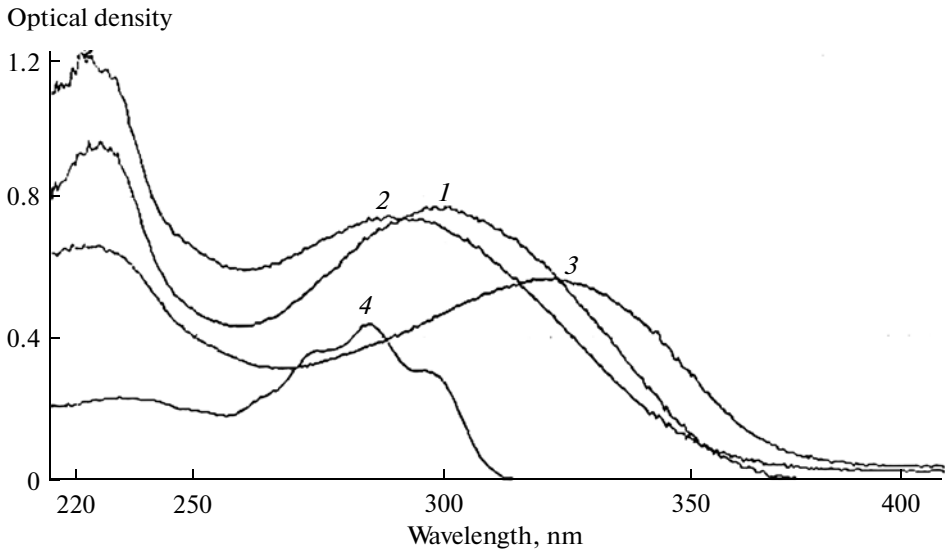

Fig. 2. Absorption spectra of trisporoids from the neutral fraction of the joint culture of *B. trispora* T(+) × 921(–) strains. Trisporins/trisporols (*1*); X_{290} (*2*); X_{323} (*3*); and 4-dihydromethyltrisporate (*4*).

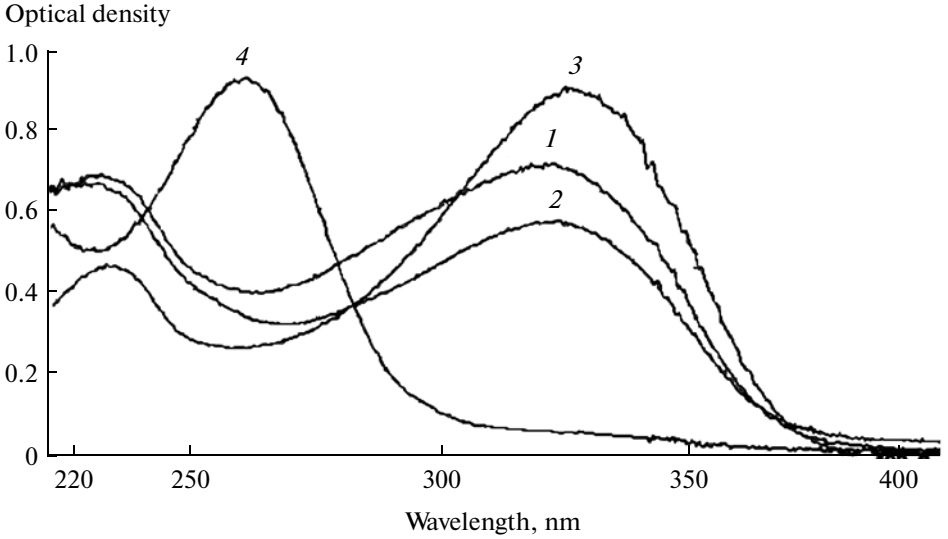

Fig. 3. Absorption spectra of trisporoids from the acid fraction of the joint culture of T(+) × 921(–). TSA-A_{315} (*1*); TSA-B_{323} (*2*); TSA-C_{327} (*3*); and X_{260} (*4*).

260 nm, which was a minor component, exhibited biological activity in relation to both strains.

Of special interest was the study of the interrelationship between zygote formation and trisporoid and carotenoid synthesis in the pair of the overproducer strains T(+) and T(–). The T(+) strain was shown to be a sufficiently active zygote former (Table 1), whereas the (–) strain differed fundamentally from the wild (–) strains, because it did not form zygospores either with any of seven wild (+) strains or with the T(+) strains. Note that in terms of the carotenogenic activity, the T(–) strain ranked among the leaders, because in combinations with 1201(+) and T(+), it synthesized more carotenoids than all the wild (–) strains, irrespective of their capacity for zygote formation (Table 2). It was found that the pair T(+) × T(–) formed large amounts of trisporoids comparable with that in the zygote-forming pair T(+) × 921(–) (Table 3). The high share of trisporic acids, in particular, TSA-B, the most active component according to the biological test data, was a feature of the trisporoid composition. Thus, the pair T(+) × T(–) formed large amounts of carotenoids and trisporoids, in particular, trisporic acids, but did not form zygospores, which was indicative of the inhibition of zygote formation at the later stages, but not at the stage of TSA synthesis. It is

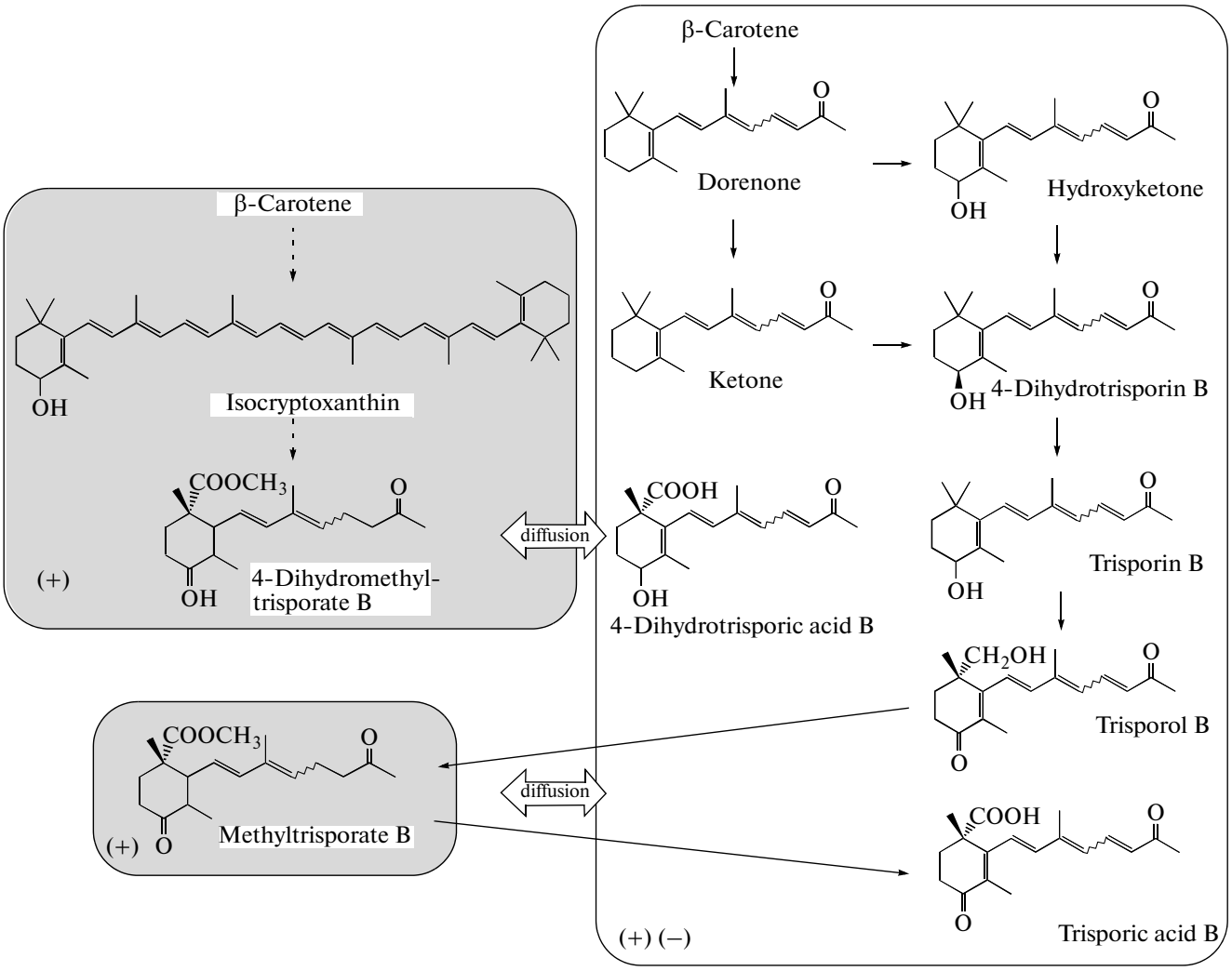

Fig. 4. Scheme of trisporic acid synthesis in mucoraceous fungi [15].

important to emphasize that positive correlation between TSA synthesis and carotenogenesis exists in these strains despite the defect in the process of zygote formation.

DISCUSSION

It is of interest to consider our findings in the context of present-day views of the synthesis of trisporic acids in mucoraceous fungi. The scheme of TSA synthesis [14], which remained unchanged until 2008 when it was revised, was proposed more than 35 years ago. Based on the data obtained with tritium-labeled TSA precursors, a new scheme (Fig. 4) with several important distinctions was proposed [15]. The first β-carotene cleavage product is not retinol, but dorenone, from which β-C_{18}-ketone is subsequently formed. It was shown that trisporol rather than 4-dihydrotrisporin was the last common precursor for the (+) and (−) mating types of strains. It was proved that the (+) strain was capable of independently synthesizing small amounts of TSA. The authors suggested that there might be another pathway of TSA formation from the oxidized β-carotene derivative isocryptoxanthin, which was found earlier during oxidative degradation of β-carotene by enzyme preparations from *B. trispora* mycelium [16]. Last but not least, it was established that the methyltrisporates formed from trisporol only by the (+) strain were further hydrolyzed to TSA by both strains. Note that the hydrolysis rate in the (−) strain was considerably higher than in the (+) strain. The enzymes of the pathway of TSA synthesis have not been studied in detail to date. Three enzymes are presently known: β-carotene oxygenase [2], 4-dihydrotrisporate dehydrogenase [17], and 4-dihydrotrisporin dehydrogenase [18]. It was established that only one of them, β-carotene oxygenase, was induced in sexual interaction between the strains, while the remaining enzymes were constitutive.

Proceeding from the contemporary notions of TSA synthesis, it is clear that, when the (+) and (–) *B. trispora* strains were cultivated together, an excess of neutral precursors, trisporins/trisporols, which were formed by both strains and constituted up to 40% of the sum of trisporoids, was produced. Against this background, the methyltrisporates in the culture fluid, which are formed only by the (+) mating type of strain and are then converted to TSA by both strains, were the minor components. These data indicate that regulation of the TSA concentration may occur in the medium at the stage of methyltrisporate formation by the (+) strains. It is known from the literature that the strains of both mating types are capable of converting trisporins to trisporols at almost the same rate, but the hydrolysis rate of methyltrisporates in TSA in the (–) mating type of strains is considerably higher than in the (+) strains [15]. This suggests that in our case, the fact of accumulation of neutral TSA precursors in the culture fluid may be regarded as the formation of a depot for the maintenance of its required concentration. It remains to be understood why a different strain ratio in the inoculum, with a 7-fold predominance of the (–) strain, is required for carotenogenesis. It may be suggested that a certain concentration of trisporic acid, whish is attained precisely at such a strain ratio, may be required for the activation of carotenogenesis. This problem requires further study.

Attempts to investigate a relationship between zygote formation, TSA synthesis, and carotenogenesis have been made previously. In [3], Bobneva used the monosporic variants obtained from the industrial pair of strains (+) 5 and (–) 4, which were spontaneous mutants with a different zygote formation rate and did not form pairs incapable of producing zygotes. Although the author did establish a dependence between the zygote formation rate in surface culture and carotenogenesis in submerged cultivation, the closely related strains used in the work did not allow for a definitive conclusion about the relationship between these processes. Moreover, the author did not succeed in unraveling the relationship between carotenogenesis and TSA formation. In our previous study, we found a relationship between the capacity for zygote formation and carotenogenesis in the surface culture of *B. trispora* [4]. It was found that a considerably lower amount of carotenoids was observed in the mycelium of the zygote-forming pair than by the pair not forming zygotes. This may be explained by the fact that most of carotenoids are used for zygospore formation, so very little remains in the mycelium. In the same study, it was established that the zygote-forming pair 812(+) × 921(–) synthesized more TSA but somewhat less carotenoids than the pair 812(+) × 826(–), which is not a zygospore former. These contradictory findings and the impossibility to explain them from the positions of the role played by TSA in carotenogenesis determined the goal of the present study. Not only did we expand the strain spectrum, but we also studied the quantitative trisporoid composition. As a result, it was established that zygote-forming pairs of strains synthesized a significantly larger amount of both carotenoids and trisporoids, including TSA, than those not forming zygotes. However, no proportional dependence between the zygote formation rate and carotenogenesis was found. This might be connected with the fact that the zygote formation rate depends on a number of other factors. The capacity for zygote formation appears to be more significant for biotechnological purposes, because the strains not forming zygotes are considerably less productive in trisporoid and carotenoid formation. In this connection, the investigation of the pair of industrial producers is of special interest. Although this pair has a defect in zygospore formation, a positive correlation was revealed between carotenoid and trisporoid synthesis.

Thus, the finding of a positive correlation between the capacity of the (+) and (–) strains of *B. trispora* for zygote formation in surface culture and trisporoid and carotenoid synthesis in submerged culture was the main novel result of the present work. The composition of trisporoids in submerged culture has not been previously studied quantitatively. It was established that zygote-forming pairs form not only more trisporoids but also TSA, which results in the stimulation of carotenogenesis. These findings lead us to believe that the capacity for zygote formation and/or TSA synthesis may be the criterion for the selection of carotenogenic strains.

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