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EXPERIMENTAL ARTICLES

Effect of the Nitrogen Source on the Biosynthesis, Composition, and Structure of the Exopolysaccharides of *Aureobasidium pullulans* (de Bary) Arnaud

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Abstract—The effect of the nitrogen source and the C/N ratio of the growth medium on the biosynthesis, composition, and structure of the exopolysaccharides (EPSs) of *Aureobasidium pullulans* (de Bary) Arnaud var. *aubasidani* Yurlova var. nov. and *A. pullulans* var. *pullulans* was studied. *A. pullulans* var. *pullulans* and *A. pullulans* var. *aubasidani* strains synthesized the maximum amounts of EPSs in the presence of, respectively, a reduced nitrogen source ($(NH_4)_2SO_4$) and an oxidized nitrogen source ($NaNO_3$) in the medium. The data presented confirm the validity of using the chemical composition and structure of the major cetavlon-precipitated fraction of *A. pullulans* EPSs for the characterization of intraspecies taxa.

Key words: Aureobasidium pullulans var. *aubasidani, A. pullulans* var. *pullulans*, nitrogen source, exopolysaccharides.

The recently described variety of *A. pullulans* (de Bary) Arnaud, *A. pullulans* var. *aubasidani* Yurlova var. nov. [1], differed from the *A. pullulans* var. *aubasidani* variety in the nitrogen source that is optimal for the biosynthesis of exopolysaccharides (EPSs) and in the chemical structure of EPSs synthesized under optimal conditions. It was found that a reduced nitrogen source (ammonium sulfate) is optimal for the synthesis of EPSs in *A. pullulans* var. *pullulans* strains, the EPSs being dominated by pullulans. At the same time, *A. pullulans* var. *aubasidani* strains synthesize maximum amounts of EPSs in media with an oxidized nitrogen source (sodium nitrate), the EPSs being dominated by aubasidans [1–4]. The type of nitrogen source also affects the activity of some pathways of carbon metabolism, such as glycolysis, the tricarboxylic acid cycle, the pentose phosphate cycle, and gluconeogenesis, in *A. pullulans* strains [5]. The maximum activities of carbon metabolism enzymes in *A. pullulans* var. *pullulans* SPCPA 11 were revealed when this strain was grown in media with ammonium sulfate as the carbon source. On the other hand, the maximum activities of such enzymes in the type strain *A. pullulans* var. *aubasidani* F-448 were found when this strain was grown in the presence of sodium nitrate.

These interesting data encouraged us to study in more detail the effects of the reduced and oxidized forms of the nitrogen source and the carbon-to-nitrogen (C/N) ratio of the growth media on the yield, composition, and structure of EPSs produced by different intraspecies taxa of *A. pullulans, A. pullulans* var. *aubasidani,* and *A. pullulans* var. *pullulans*.

MATERIALS AND METHODS

In this study, the *Aureobasidium pullulans* var. *pullulans* variety was represented by two strains, VKPM F-371 and the type strain VKM F-179 (also known as the type strain *Pullularia pullulans* var. *fusca* (Browne) Berkhout and *A. pullulans* (de Bary) Arnaud var. *melanogenum* Hermanides–Njihof). The *A. pullulans* var. *aubasidani* Yurlova variety was also represented by two strains, VKM F-2204 and the type strain VKPM F-448.

The synthesis of EPSs by these strains was studied in Czapek–Dox and Ueda media (with or without yeast extract) [6] containing inorganic nitrogen sources ($NaNO_3$ or $(NH_4)_2SO_4$) in amounts corresponding to the C/N ratios typical of Ueda medium (C/N = 157) or Czapek–Dox medium (C/N = 40) (see Table 1).

Cultivations and the preparation of native polysaccharide (polysaccharide + biomass), polysaccharide alone, and biomass alone were carried out as described earlier [7].

The chemical composition and structure of EPSs were studied using the following approaches: complete acid hydrolysis with the subsequent identification of monosugars by ascending paper chromatography, partial acid hydrolysis [2], periodate oxidation [8], methylation by the Hakomori method [9] with the subsequent identification of methylglycosides by gas–liquid chromatography [10], IR spectroscopy on a UR-20 spectrophotometer, and measurement of the optical rotation of 0.1% aqueous solutions of polysaccharides using a Perkin-Elmer model 241 automatic polarimeter.

The homogeneity of EPS preparations was estimated by fractionating them with a 2.5% aqueous solution of cetavlon (hexadecyltrimethylammonium bromide) and by gel filtration on a Sepharose 4B column [4].

Pullulan used in this study was purchased from BDH Chemicals Ltd. (United Kingdom), and aubasidan was obtained from the Department of Microbiology, St. Petersburg State Chemical Pharmaceutical Academy (SPCPA).

Data were statistically processed by conventional methods.

Table 1. Characterization of the nutrient media used in this study

Medium	Nitrogen source	Nitrogen content, g/l	C/N ratio
Czapek–Dox	$NaNO_3$	3.0	40
	$NaNO_3$	0.77	157
	$(NH_4)_2SO_4$	2.33	40
	$(NH_4)_2SO_4$	0.6	157
Ueda without yeast extract	$NaNO_3$	3.0	40
	$NaNO_3$	0.77	157
	$(NH_4)_2SO_4$	2.33	40
	$(NH_4)_2SO_4$	0.6	157
Ueda with yeast extract	$NaNO_3$	3.0	40
	$NaNO_3$	0.77	157
	$(NH_4)_2SO_4$	2.33	40
	$(NH_4)_2SO_4$	0.6	157

RESULTS

As can be seen from Tables 2 and 3, *A. pullulans* var. *pullulans* F-179 and F-371 strains typically produced higher amounts of native polysaccharides in media with the reduced nitrogen source (ammonium sulfate) than in media with the oxidized nitrogen source (sodium nitrate), although in Ueda medium with yeast extract, which is an additional source of nitrogen, strain F-371 produced greater amounts of native polysaccharides in the presence of sodium nitrate than in the presence of ammonium sulfate (Table 3). Therefore, provided that the growth medium contains only an inorganic nitrogen source, *A. pullulans* var. *pullulans* strains produce the maximum amounts of polysaccharides in the presence of ammonium sulfate.

At high concentrations of both inorganic nitrogen sources, strains F-179 and F-371 accumulated more biomass, as a result of which the productivity of EPS synthesis decreased (this effect was most pronounced in media with ammonium sulfate). For instance, when the concentration of $(NH_4)_2SO_4$ in media was raised from 0.6 g/l (C/N = 157) to 2.33 g/l (C/N = 40), the productivity of EPS synthesis by strain F-179 in Ueda medium without yeast extract decreased by 2.12 times (Table 2), and the productivity of EPS synthesis by strain F-371 in this medium and in Czapek–Dox medium decreased by 2.7 and 3.1 times, respectively (Table 3). This implies that high concentrations of ammonium sulfate are inhibitory to EPS synthesis.

Conversely, *A. pullulans* var. *aubasidani* F-448 and F-2204 strains produced greater amounts of native polysaccharide and exopolysaccharide in media with sodium nitrate than in media with ammonium sulfate as the nitrogen source (Tables 4 and 5). The maximum yield of polysaccharides was observed in Ueda medium with yeast extract and sodium nitrate (Table 1).

Unlike *A. pullulans* var. *pullulans* strains, which produced greater amounts of polysaccharides in media with lower concentrations of nitrogen (i.e., at C/N = 157), *A. pullulans* var. *aubasidani* strains produced more polysaccharides in media with C/N = 40. Thus, the productivity of EPS synthesis by strain F-448 in media with sodium nitrate at C/N = 40 was 1.3 times (Czapek–Dox medium), 1.7 times (Ueda medium with yeast extract), and 2.4 times (Ueda medium without yeast extract) higher than in the same media with sodium nitrate at C/N = 157 (Table 4). In Czapek–Dox medium with 0.6 g/l ammonium sulfate (C/N = 157), strain F-448 virtually did not produce EPSs. In Ueda media with and without yeast extract containing 0.6 g/l $(NH_4)_2SO_4$ (C/N = 157), the productivity of EPS synthesis by strain F-448 was, respectively, 1.7 and 4.9 times lower than in the same media containing 2.33 g/l $(NH_4)_2SO_4$ (C/N = 40) (Table 4).

A. pullulans var. *aubasidani* F-2204 also produced less EPSs in media with lower concentrations of the nitrogen source. This effect was most pronounced in media with ammonium sulfate, which is not an optimal nitrogen source for strains of this variety. The productivity of EPS synthesis by strain F-2204 in Czapek–Dox medium with the $(NH_4)_2SO_4$ concentration corresponding to C/N = 157 was 0.12 g EPS/g biomass and only 0.02 g EPS/g biomass in Ueda medium with the same concentration of ammonium sulfate (Table 5). The addition of yeast extract to the cultivation media of strain F-2204 promoted the accumulation of biomass, with the respective decrease in the productivity of EPS synthesis. For instance, in Ueda medium with yeast extract, the productivity of EPS synthesis by strain F-2204 was 2.3 times ($NaNO_3$, C/N = 40), 2.1 times ($NaNO_3$, C/N = 157), 2 times ($(NH_4)_2SO_4$, C/N = 40), and 36 times ($(NH_4)_2SO_4$, C/N = 157) lower than in Ueda medium without yeast extract (Table 5).

Table 2. Effect of cultivation conditions on the synthesis of polysaccharides by *A. pullulans* var. *pullulans* F-179

Medium	Nitrogen source	C/N ratio	Native polysaccharide yield, g/l	Relative yield of native polysaccharide, %	Pure polysaccharide yield, g/l	Biomass content in the native polysaccharide preparation, %	Pure polysaccharide content in the native polysaccharide preparation, %	Productivity of EPS synthesis, g EPS/g biomass
Czapek–Dox	$NaNO_3$	40	7.63 + 0.32	39	5.18 + 0.21	32	68	2.12
	$NaNO_3$	157	3.28 + 0.21	17	2.10 + 0.10	36	64	1.77
	$(NH_4)_2SO_4$	40	11.05 + 0.78	57	5.53 + 0.12	50	50	1.00
	$(NH_4)_2SO_4$	157	10.76 + 0.72	55	5.60 + 0.17	48	52	1.08
Ueda without yeast extract	$NaNO_3$	40	5.83 + 0.30	30	4.08 + 0.19	30	70	2.33
	$NaNO_3$	157	10.67 + 0.63	55	7.79 + 0.20	27	73	2.70
	$(NH_4)_2SO_4$	40	12.07 + 0.81	62	7.72 + 0.42	36	64	1.77
	$(NH_4)_2SO_4$	157	11.41 + 0.81	59	9.01 + 0.84	21	79	3.76
Ueda with yeast extract	$NaNO_3$	40	15.46 + 0.73	79	8.97 + 0.75	42	58	1.35
	$NaNO_3$	157	13.18 + 0.89	68	8.43 + 0.64	36	64	1.86
	$(NH_4)_2SO_4$	40	19.25 + 1.02	99	11.16 + 0.86	42	58	1.38
	$(NH_4)_2SO_4$	157	19.47 + 0.93	100	12.65 + 0.87	35	65	1.86

Table 3. Effect of cultivation conditions on the synthesis of polysaccharides by *A. pullulans* var. *pullulans* F-371

Medium	Nitrogen source	C/N ratio	Native polysaccharide yield, g/l	Relative yield of native polysaccharide, %	Pure polysaccharide yield, g/l	Biomass content in the native polysaccharide preparation, %	Pure polysaccharide content in the native polysaccharide preparation, %	Productivity of EPS synthesis, g EPS/g biomass
Czapek–Dox	$NaNO_3$	40	2.37 + 0.34	14	0.95 + 0.10	60	40	0.67
	$NaNO_3$	157	3.55 + 0.46	21	1.50 + 0.88	58	42	0.72
	$(NH_4)_2SO_4$	40	5.06 + 0.72	30	1.16 + 0.72	77	23	0.30
	$(NH_4)_2SO_4$	157	7.15 + 0.67	42	3.43 + 0.17	52	48	0.92
Ueda without yeast extract	$NaNO_3$	40	5.62 + 0.89	33	1.41 + 0.11	75	25	0.33
	$NaNO_3$	157	10.48 + 2.34	62	5.34 + 0.66	49	51	1.04
	$(NH_4)_2SO_4$	40	18.81 + 1.04	110	5.08 + 0.78	73	27	0.37
	$(NH_4)_2SO_4$	157	17.43 + 1.55	102	8.72 + 1.32	50	50	1.00
Ueda with yeast extract	$NaNO_3$	40	26.99 + 2.77	158	3.78 + 0.89	86	14	0.16
	$NaNO_3$	157	34.03 + 1.56	200	11.91 + 2.25	65	35	0.54
	$(NH_4)_2SO_4$	40	19.80 + 2.12	116	9.50 + 1.45	52	48	0.92
	$(NH_4)_2SO_4$	157	17.03 + 3.13	100	6.13 + 0.98	64	36	0.56

Thus, high concentrations of either type of inorganic nitrogen source promote EPS synthesis by *A. pullulans* var. *aubasidani* strains.

An investigation of the composition and structure of EPSs showed that *A. pullulans* var. *pullulans* F-371 grown in Czapek–Dox and Ueda media with ammonium sulfate (the optimal nitrogen source for this strain) synthesized exoglucans close in structure to pullulan (Table 6). The exopolysaccharides produced by this strain in Ueda medium with and without yeast extract in the presence of sodium nitrate were also sim-

Table 4. Effect of cultivation conditions on the synthesis of polysaccharides by *A. pullulans* var. *aubasidani* F-448

Medium	Nitrogen source	C/N ratio	Native polysaccharide yield, g/l	Relative yield of native polysaccharide, %	Pure polysaccharide yield, g/l	Biomass content in the native polysaccharide preparation, %	Pure polysaccharide content in the native polysaccharide preparation, %	Productivity of EPS synthesis, g EPS/g biomass
Czapek–Dox	$NaNO_3$	40	5.85 + 0.88	100	3.45 + 0.22	41	59	1.44
	$NaNO_3$	157	6.56 + 1.12	112	3.48 + 0.17	47	53	1.13
	$(NH_4)_2SO_4$	40	3.46 + 0.12	59	0.62 + 0.11	82	18	0.22
	$(NH_4)_2SO_4$	157	2.38 + 0.14	41	0.02 + 0.01	99	1	0.01
Ueda without yeast extract	$NaNO_3$	40	4.67 + 0.20	80	2.94 + 0.81	37	63	1.70
	$NaNO_3$	157	6.81 + 0.27	116	2.86 + 0.13	58	42	0.72
	$(NH_4)_2SO_4$	40	2.61 + 0.17	45	1.15 + 0.10	56	44	0.79
	$(NH_4)_2SO_4$	157	3.83 + 0.15	66	0.54 + 0.21	86	14	0.16
Ueda with yeast extract	$NaNO_3$	40	12.91 + 1.04	221	9.68 + 0.78	25	75	3.00
	$NaNO_3$	157	13.23 + 1.15	226	8.47 + 0.60	36	64	1.77
	$(NH_4)_2SO_4$	40	10.02 + 0.92	171	4.51 + 0.41	55	45	0.82
	$(NH_4)_2SO_4$	157	8.92 + 0.90	152	2.94 + 0.34	67	33	0.49

Table 5. Effect of cultivation conditions on the synthesis of polysaccharides by *A. pullulans* var. *aubasidani* F-2204

Medium	Nitrogen source	C/N ratio	Native polysaccharide yield, g/l	Relative yield of native polysaccharide, %	Pure polysaccharide yield, g/l	Biomass content in the native polysaccharide preparation, %	Pure polysaccharide content in the native polysaccharide preparation, %	Productivity of EPS synthesis, g EPS/g biomass
Czapek–Dox	$NaNO_3$	40	9.10 + 1.17	100	6.64 + 1.78	27	73	2.70
	$NaNO_3$	157	9.19 + 2.45	101	5.61 + 0.85	39	61	1.56
	$(NH_4)_2SO_4$	40	7.42 + 0.89	82	4.38 + 0.75	41	59	1.44
	$(NH_4)_2SO_4$	157	6.16 + 0.99	68	0.68 + 0.67	89	11	0.12
Ueda without yeast extract	$NaNO_3$	40	12.27 + 1.78	135	8.59 + 1.25	30	70	2.33
	$NaNO_3$	157	13.56 + 2.98	149	8.00 + 1.08	41	59	1.44
	$(NH_4)_2SO_4$	40	9.69 + 1.56	106	6.20 + 0.93	36	73	1.78
	$(NH_4)_2SO_4$	157	8.54 + 1.67	94	3.59 + 0.55	58	42	0.72
Ueda with yeast extract	$NaNO_3$	40	31.71 + 2.18	348	15.85 + 2.86	50	50	1.00
	$NaNO_3$	157	21.86 + 3.10	240	8.96 + 1.90	59	41	0.69
	$(NH_4)_2SO_4$	40	17.33 + 1.98	190	8.14 + 1.88	53	47	0.89
	$(NH_4)_2SO_4$	157	9.03 + 0.77	99	0.18 + 0.41	98	2	0.02

ilar to pullulan. However, when grown in Czapek–Dox medium with sodium nitrate (i.e., under conditions nonoptimal for EPS synthesis), strain F-371 synthesized aubasidan-like exoglycans (Table 6). This inference is confirmed by the results of the partial acid hydrolysis of EPS, as well as by low values (+26, +46) of the optical rotation angle of EPS solutions, intense IR absorption at 890 cm^{-1} indicating the presence of β-glycosidic bonds, and a considerable amount (48 and 31%) of 1–3 bonds in the polymer molecule.

The cetavlon-aided fractionation of polysaccharides produced by strain F-371 in Ueda medium with $NaNO_3$

Table 6. Effect of cultivation conditions on the structure of exopolysaccharides synthesized by *A. pullulans* var. *pullulans* F-371

Medium	Nitrogen source	C/N ratio	Monosaccharide composition	Polysaccharide type	Optica rotation angle, $[\alpha]_D^{20}$*	Relative number of glycosidic bonds (periodate oxidation), %		
						1–6**	1–4***	1–3
Czapek–Dox	$NaNO_3$	40	Glucose	A	+46	20	49	31
Czapek–Dox		157	Glucose	A	+26	38	14	48
Ueda⁻		157	Glucose	P	+137	36	52	12
Ueda		157	Glucose	P	+150	15	72	13
Czapek–Dox	$(NH_4)_2SO_4$	40	Glucose	P	+95	51	44	5
Czapek–Dox		157	Glucose	P	+153	33	47	20
Ueda⁻		157	Glucose	P	+147	42	38	20
Ueda		157	Glucose	P	+118	17	60	23
Pullulan			Glucose	P	+190	29	65	6
Aubasidan			Glucose	A	+16	31	24	45

Note: A, aubasidan; P, pullulan; Ueda⁻, Ueda medium without yeast extract.
* Optical rotation of 0.1% aqueous solutions.
** 1–6 bonds and/or terminal nonreducing groups.
*** 1–4 and/or 1–2 bonds.

Table 7. Effect of the nitrogen source on the fractional composition of exopolysaccharides synthesized by *A. pullulans* var. *pullulans* F-371 and *A. pullulans* var. *aubasidani* F-448 in Ueda medium

Strain	Nitrogen source	Cetavlon-precipitated fraction	Yield, %	Polysaccharide type	Optical rotation angle, $[\alpha]_D^{20}$	Relative number of glycosidic bonds (periodate oxidation), %		
						1–6	1–4	1–3
F-371	$NaNO_3$	I	7	A	+44	10	12	78
		II	93	P	+254	24	62	14
	$(NH_4)_2SO_4$	I	19	A	+23	24	17	59
		II	81	P	+128	26	70	4
F-448	$NaNO_3$	I	91	A	+36	26	29	45
		II	9	P	+195	–*	–	–
	$(NH_4)_2SO_4$	I	15	A	+10	–	–	–
		II	85	P	+178	30	69	1

* "–" stands for "not detected." For other notes, see Table 6.

or $(NH_4)_2SO_4$ at C/N = 157 allowed two fractions to be obtained (Table 7). Fraction II was prevalent in media with either nitrogen source (the yield of this fraction was 81 and 93% in media with $(NH_4)_2SO_4$ and $NaNO_3$, respectively). The structure of the EPSs of this fraction was close to that of pullulan. The yield of fraction I was low (19 and 7% in media with $(NH_4)_2SO_4$ and $NaNO_3$, respectively). The EPSs of this fraction were similar to aubasidan but contained relatively low amounts of 1–6 bonds (10–24% in comparison to 31–33% typical of aubasidan) (Table 7).

The type strain F-448 of *A. pullulans* var. *aubasidani* grown in Czapek–Dox and Ueda media with sodium nitrate at either concentration studied (C/N = 40 and C/N = 157) and in Czapek–Dox medium with ammonium sulfate (C/N = 40 and C/N = 157) synthesized mainly aubasidan-like exoglucans (Table 8). At the same time, in Ueda medium without yeast extract and with the growth-limiting concentration of ammonium sulfate (C/N = 157), strain F-448 produced a polysaccharide composed of glucose and trace amounts of mannose (less than 1%). This polysaccharide differed from other polysaccharides synthesized by strain F-448 in that it had a high value of optical rotation angle ($[\alpha]_D^{20}$ = +83 as compared to +16...+45 typical of aubasidan-like exopolysaccharides), almost equal intensities of IR absorption at 840 cm^{-1} (α-glycosidic bonds) and 890 cm^{-1}, and a low content (about 25%) of 1–3 bonds (Table 8).

Table 8. Effect of cultivation conditions on the structure of exopolysaccharides synthesized by *A. pullulans* var. *aubasidani* F-448

Nitrogen source	Medium	C/N ratio	Monosaccharide composition	Polysaccharide type	Optical rotation angle, $[\alpha]_D^{20}$	Relative number of glycosidic bonds (periodate oxidation), %		
						1–6	1–4	1–3
$NaNO_3$	Czapek–Dox	40	Glucose	A	+16	31	24	45
	Czapek–Dox	157	Glucose	A	+40	59	4	37
	Ueda⁻	157	Glucose	A	+28	42	22	36
	Ueda	157	Glucose	A	+45	32	31	37
$(NH_4)_2SO_4$	Czapek–Dox	40	Glucose	A	+20	–*	–	–
	Czapek–Dox	157	Glucose	A	+23	32	12	56
	Ueda⁻	157	Glucose and mannose (<1%)	P	+83	42	33	25

* "–" stands for "not detected." For other notes, see Table 6.

EPSs synthesized by strain F-448 in Ueda medium with low concentrations of $NaNO_3$ and $(NH_4)_2SO_4$ (C/N = 157) could also be fractionated with cetavlon into two fractions (Table 7). The proportion of these fractions depended on the cultivation conditions, the type of nitrogen source in particular. The EPSs produced in Ueda medium with $NaNO_3$ were mainly represented by fraction I (91%) containing aubasidan-like polysaccharides. The EPSs synthesized by strain F-448 under nonoptimal conditions (Ueda medium with $(NH_4)_2SO_4$ at C/N = 157) were mainly represented by fraction II (85%) containing pullulan-like polysaccharides, whereas the content of aubasidan-like polysaccharides was low (15%). For comparison, the commercial preparation of aubasidan produced by strain *A. pullulans* var. *aubasidani* F-448 in Czapek–Dox medium with sodium nitrate at C/N = 40 must contain no less than 60% of cetavlon-precipitated fraction I, no less than 45% of β(1–3) glycosidic bonds, no more than 22–24% of α(1–4) glycosidic bonds, and no more than 31–33% of β(1–6) glycosidic bonds; the optical rotation angle of 0.1% aqueous solutions must be from +12° to +16° [11].

DISCUSSION

Ammonium sulfate is considered to be an optimal nitrogen source for the production of pullulan by *A. pullulans* [12–14], whereas sodium nitrate is an optimal nitrogen source for the production of aubasidan by the type strain F-448 [2, 11, 15]. Theoretically, ammonium sulfate, as a reduced nitrogen compound, must be more preferable to sodium nitrate in all cases.

As follows from the results presented in this paper, ammonium sulfate is an optimal nitrogen source for the biosynthesis of EPSs by *A. pullulans* var. *pullulans* F-179 and F-371 strains. Low concentrations of both nitrogen sources (C/N = 157) favored the production of EPSs, whereas their high concentrations (C/N = 40) favored the production of biomass. The inhibitory action of high concentrations of nitrogen sources on EPS synthesis was also shown for other *A. pullulans* var. *pullulans* strains producing pullulan [12, 13]. It is most likely that the induction of active growth of micromycetes by high nitrogen concentrations leads to a rapid exhaustion of nutrients from the medium, as a result of which the biosynthesis of polysaccharides is limited by a deficiency of the carbon source.

The data presented here and in our earlier publication [16] show that strains F-448 and F-2204 of the other variety, *A. pullulans* var. *aubasidani*, produce maximum amounts of EPSs in media with the oxidized nitrogen source, sodium nitrate. As opposed to the situation with *A. pullulans* var. *pullulans* strains, high concentrations of the nitrogen source promoted the synthesis of both biomass and EPSs. This fact can be explained biochemically. Indeed, as shown by Schuster *et al.* [14], the increase in the initial concentration of ammonium sulfate extends the period of its utilization and leads to a rapid exhaustion of the carbon source from the medium. As a result, the consumption rates of carbon and nitrogen sources appear to be more balanced than in the case of the lower concentrations of the nitrogen source. High rates of glucose consumption, which is an indication of the enhanced activity of metabolic processes in the cell, lead to a rapid exhaustion of glucose and to the limited growth of *A. pullulans*. Both of these factors can explain the rise in the biosynthesis rate of polysaccharides. Similar processes must occur during the growth of *A. pullulans* var. *aubasidani* strains in the presence of sodium nitrate as the nitrogen source, since its assimilation is associated with the assimilation of ammonium ions. These speculations cannot be applied to *A. pullulans* var. *pullulans* strains, since the *A. pullulans* var. *pullulans* and *A. pullulans* var. *aubasidani* varieties differ in carbon metabolism [5] and, probably, in the kinetics of biosynthetic processes.

As was shown earlier, the exopolysaccharides of *A. pullulans* var. *pullulans* and *A. pullulans* var. *aubasidani* synthesized under optimal conditions differed in their chemical composition and structure: *A. pullulans* var. *pullulans* strains grown in Ueda medium with $(NH_4)_2SO_4$ produced mostly pullulan-like polysaccharides, whereas *A. pullulans* var. *aubasidani* strains grown in Czapek–Dox medium with $NaNO_3$ produced mainly aubasidan-like polysaccharides [1, 4]. Different concentrations of the optimal nitrogen source corresponding to C/N = 40 and C/N = 157 did not affect the type of exopolysaccharide produced. However, under nonoptimal growth conditions, the situation changes. Indeed, under conditions unfavorable for EPS synthesis (Czapek–Dox medium with $NaNO_3$ at both C/N = 40 and C/N = 157), *A. pullulans* var. *pullulans* F-371 produced aubasidan-like EPSs (Table 6). Similarly, *A. pullulans* var. *aubasidani* F-448 in Ueda medium with $(NH_4)_2SO_4$ at C/N = 157 (in this case, three factors—growth medium, nitrogen source, and C/N value—were nonoptimal for EPS synthesis) synthesized the pullulan-like exoglycan composed of glucose and trace amounts (less than 1%) of mannose (Table 8).

An investigation of the fractional composition of the EPSs produced by *A. pullulans* var. *pullulans* and *A. pullulans* var. *aubasidani* strains showed that, under optimal conditions, the type of inorganic nitrogen source did not affect the composition of the major cetavlon-precipitated fraction of EPSs. Indeed, as can be seen from the data presented in Table 7 for *A. pullulans* var. *pullulans* F-371 grown in Ueda medium with $(NH_4)_2SO_4$ or $NaNO_3$ at C/N = 157, both preparations contained mainly pullulan-like polysaccharides. In nonoptimal medium, however, the type of nitrogen source affected the fractional composition of EPSs (Table 7). Thus, *A. pullulans* var. *aubasidani* F-448 grown in Ueda medium with ammonium sulfate at C/N = 157 (nonoptimal conditions for EPS synthesis) produced mainly (85%) pullulan-like polysaccharides. The same strain in the same medium but with sodium nitrate (the optimal nitrogen source for this strain) produced mainly (95%) aubasidan-like polysaccharides. These data confirm the validity of using the chemical composition and structure of the major cetavlon-precipitated fraction of EPSs produced under optimal conditions for the characterization of the intraspecies taxa *A. pullulans* var. *aubasidani* and *A. pullulans* var. *pullulans*, as was suggested by Yurlova [1, 2].

The effect of the type of nitrogen source on the composition and structure of EPSs produced by *A. pullulans* and other microorganisms has not yet been investigated in detail. Furthermore, little is known about the nitrogen and carbon metabolism of EPS producers. The results of our investigations presented in this paper and reported earlier [1, 5, 16] suggest that the regulation of carbon and nitrogen metabolism in *A. pullulans* is species specific. This explains the heterogeneity of the *A. pullulans* population with respect to the nitrogen source optimal for EPS synthesis and to the type of EPSs produced.

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