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YEAST β-MANNANASE ACTIVITY

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The aim of the research was to determine the mannan-degrading activity of yeasts cultures isolated from various sources and select strains with high β-mannanase activity. As a result of screening of 245 yeast strains, which are the representatives of 7 genera and 14 species, the active producers of extracellular β-mannanase were identified. To increase β-mannanase activity, the cultures were grown under submerged conditions using guar gum galactomannan as a carbon source and an inducer. β-Mannanase activity was determined by dinitrosalicylic method. The most active biosynthetic species were *Cryptococcus albidus, C. gastricus, C. magnus, C. terreus, C. laurentii, Saccharomyces cerevisiae, Williopsis californica, Metschnikowia pulcherrima, Pichia anomala* and *P. guilliermondii*. The activity in culture supernatant was ranged from 0.2 to 75 U/ml. α-Galactosidase activity was found in two strains (*Debaryomyces polymorphus* UCM Y-152 and *Debaryomyces hansenii var. fabryi* UCM Y-2400). None of the tested cultures demonstrated both β-mannanase and α-galactosidase activity, that is, they are unable to attack both the main and side chains of galactomannan.

Key words: yeast, β-mannanase, α-galactosidase, galactomannan.

High demand for enzymes that hydrolyze lignohemicelluloses determines the need for the research for screening and isolation of new high active mannanases producers (1,4-β-D-mannan mannohydrolases or β-mannanases, EC 3.2.1.78). These enzymes catalyze the hydrolysis of β-mannoside bond in the main chain of hemicellulose, as well as in gluco- and galactomannans with the formation of mannooligosaccharides, mannose, glucose and galactose. Cellulose and hemicellulose, due to their chemical properties, are the substrates of great biotechnological value. On the one hand, waste from the wood, paper industries and agriculture can be environment pollution factors, and on the other hand, they have a great technological potential as a source of poly- and oligosaccharides. Because of the ability to hydrolyze hemicellulose, β-mannanase has found an application in various industries: pharmaceutical, pulp and paper, gas; in biofuel and cheap energy, prebiotic mannooligosaccharides, as well as in food and feed production [1].

Mannanases are isolated from plants, invertebrates, bacteria and fungi. The basic requirements for enzyme producers are the simplicity of isolation of enzymes resistant to high temperature, salt concentrations, and their effectiveness over a wide pH range, i.e., biocatalysts must have the physico-chemical properties necessary for technological processes. Preferred sources of enzymes are microorganisms due to rapid growth, high productivity and the specificity of the action. β-Mannanases have been found in many species of microorganisms [2]. Currently the mannan-degrading enzymes of bacteria of *Acinetobacter* sp. [3], *Bacillus amyloliquefaciens* [4], *Bacillus* sp. [5, 6], *Cellulosimicrobium* sp. [7], *Klebsiella oxytoca* and *Klebsiella edwardsii* [3, 8], *Clostridium tertium* [9], *Scopulariopsis candida* [10], *Streptomyces* sp. [11], of micromycetes of the genera *Aspergillus, Penicillium, Trichoderma, Trichosporonoides* and many others are described [12–16].

The least studied group of β-mannanase producers are yeasts. For these microorganisms α-mannanase activity as a component of their lytic complex is more characteristic. However, even among them, the cultures with high β-mannanase activity are described, although not as numerous as among bacteria and micromycetes [1, 2]. The undoubted advantage of yeasts as enzyme producers over other microorganisms is their resistance to infections and ease of separation from the culture medium due to large cell sizes.

It is known that mannans constitute an extremely diverse group of glycopolymers, including homomannans and galacto-, gluco-, galactoglucomannans. The degradation efficiency of these polysaccharides depends on the complex of enzymes of different specificity, which is due to the nature of the raw materials used in this or that biotechnological process. Therefore, the search for producers of the enzymes of the mannan-degrading complex of definite specificity remains an actual problem.

This work is devoted to the study of the mannan-degrading activity of yeast cultures isolated from various sources, in particular from plant material, soil, water, gastrointestinal tract (GIT) of fish and warm-blooded animals, in order to select high-activity β-mannanase producers among them.

Materials and Methods

245 strains of yeast, representatives of 7 genera and 14 species from the Ukrainian Collections of Microorganaisms maintained at the Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine were studed. Strains were isolated from various sources (Table 1).

Cultivation of yeasts was carried out in wort broth containing 1% guar gum under submerged conditions in tubes containing 10 ml of nutrient medium at 25 °C and shaker rotation speed of 220 rpm for 4–5 days.

The mannanase activity determination was performed by dinitrosalicylic method; guar gum galactomannan was used as a substrate [17]. A reaction mixture containing 0.5 ml of culture liquid (CL) and 0.5 ml of 1% galactomannan in 0.1 M phosphate-citrate buffer, pH 5.2, was incubated for 20 min at 45 °C, then 1 ml of dinitrosalicylic reagent (DSR) was added and the mixture was boiled for 10 min. The color intensity was evaluated spectrophotometrically at 540 nm. Mannose was used as the standard. One unit of enzyme activity was defined as the amount of the enzyme that releases 1 μmol of mannose per 1 min under experimental conditions.

α-Galactosidase activity was determined using p-nitrophenyl-α-D-galactopyranoside [18].

All experiments were performed in at least 3–5 repetitions. Statistical processing of

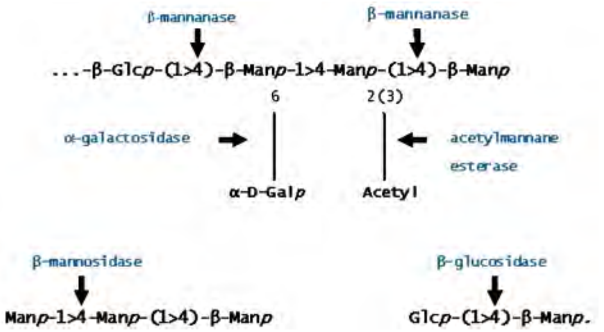
Table 1. **Sources of yeast strains isolation**

№№	Genus	Strains amount	The source of isolation
1	*Candida*	41	Sewage, water of the Dnieper, silage, GIT of longliver of the State *Institute* of Gerontology of AMS of Ukraine
2	*Cryptococcus*	97	Vegetables, alfalfa, trees, soil of Ukraine, Crimea, Yakutia, Israel
3	*Debaryomyces*	22	Israel Soil, beet, trees, sedge
4	*Metschnikowia*	23	Vegetables, fruits, lake water
5	*Pichia*	25	Vegetables, fruits, silage, gills, skin and fish GIT, oil contaminated soil
6	*Saccharomyces*	17	GIT of long-liver of the State *Institute* of Gerontology of NAMS of Ukraine, wine, soil, wort, kvass, pistachios
7	*Williopsis*	20	Vegetables, cereals, soil

experimental series results was carried out by standard methods using Student's t-test at 5% significance level.

Results and Discussion

Mannans are the main component of the hemicellulose of coniferous trees, and also widely distributed in the tissues of other plants: ivory nuts and coconuts, coffee beans, fenugreek, guar, caesalpinia, soy, carob, lichens. The main mannan-degrading enzymes are β-mannanase, β-mannosidase (EC 3.2.1.25) and β-glucosidase (EC 3.2.1.21). This group also includes α-galactosidase (EC 3.2.1.22) and acetylmannane esterase (EC 3.1.1.6), hydrolyzing the side chains of heteromannans with the galactose splitting off and acetyl ester bonds cleavage. The enzymatic hydrolysis of heteromannan is carried out as follows:



Various plant substrates, including agricultural waste, can contain lignin, cellulose, hemicellulose, as well as activators and inhibitors of unknown nature, which in turn makes them a rich substrate for the isolation of specific enzymes. Due to its chemical composition, such a substrate is easily colonized by microorganisms and can serve as a source of highly active enzymes involved in the degradation of biopolymers. Various vegetable substrates are used as sources of carbohydrates for the synthesis of mannanases: wheat, rice and corn bran, potato peel, cassava, pineapple, acacia seeds, palm, coconut and peanut oil cake [13, 15].

Therefore, the aim of our work was to investigate the extracellular mannan-degrading activity of yeasts, which are mostly isolated from plant substrates, such as the surface of vegetables, fruits, herbs, and trees. This choice is due to the fact that microorganisms adapt to the utilization of a substrate that is abundant in their habitats, and therefore form the enzymes that react with this substrate. In addition, the activity of cultures isolated from other sources, in particular from soil, silage, freshwater reservoirs, GIT of fish and mammals was studied.

Guar gum — galactomannan, whose main chain consists of 1,4-linked mannose residues, to which in the side chain single α-D-galactosyl residues are appended (in the ratio of 2:1), is added as a substrate for activating the synthesis of enzymes and for determining the mannan-degrading activity. Earlier it was shown [9] that guar gum serves as optimal carbon source for the synthesis of *Clostridium tertium* mannanases. The presence of α-linked galactose in the side chains of guar galactomannan gives reason to assume also the induction of α-galactosidase synthesis in the presence of this substrate. Therefore, to evaluate the effectiveness of hydrolysis of this galactomannan, both β-mannanase and α-galactosidase activity of yeast were studied in parallel.

Enzymatic activity was studied in 245 strains, belonging to 7 genera and 14 species. The most numerous group consisted of representatives of the genus *Cryptococcus* (97 strains) (Fig. 1). Extracellular β-mannanase activity in the CL supernatant of producers ranged from 0.2 to 75 U/ml. It should be noted that a fairly high percentage of active strains were found among the yeasts of the genus *Cryptococcus*. Thus, 28 % of the strains of *C. albidus*, 100% of *C. gastricus*, 11% of *C. humicolus*, 27% of *C. magnus*, 66% of *C. terreus* and 29% of *C. laurentii* showed β-mannanase activity. Although β-mannanase activity was present in CL of many strains of this group, the rate of hydrolysis of galactomannan was low (0.2–15 U/ml). It should also be noted that there is no α-galactosidase activity in all cases.

Representatives of the species *Saccharomyces cerevisiae* also actively hydrolyzed guar gums galactomannan. Among this group of microorganisms, 41 % of cultures showed activity. Among representatives of the species *Williopsis californica* and *Metschnikowia pulcherrima* 40% and 30% of studied strains demonstrated mannanase activity. The lowest number of active strains was found among representatives of the species *Candida krusei* — 14% and the genus *Debaryomyces* — 13.6%, and the most active group were yeasts of the *Pichia anomala* species — 76% of active strains (Fig. 2).

Thus, we established a higher frequency of β-mannanase activity in the yeast cultures

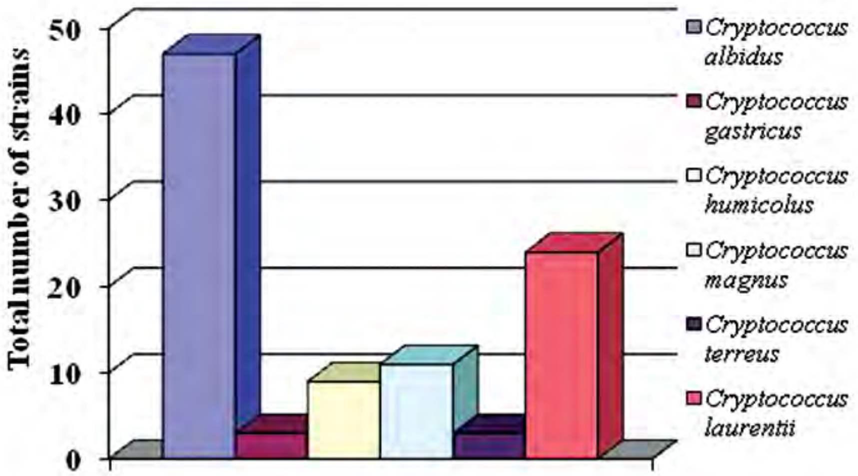


Fig. 1. **Species diversity of *Cryptococcus* cultures used in the screening process**

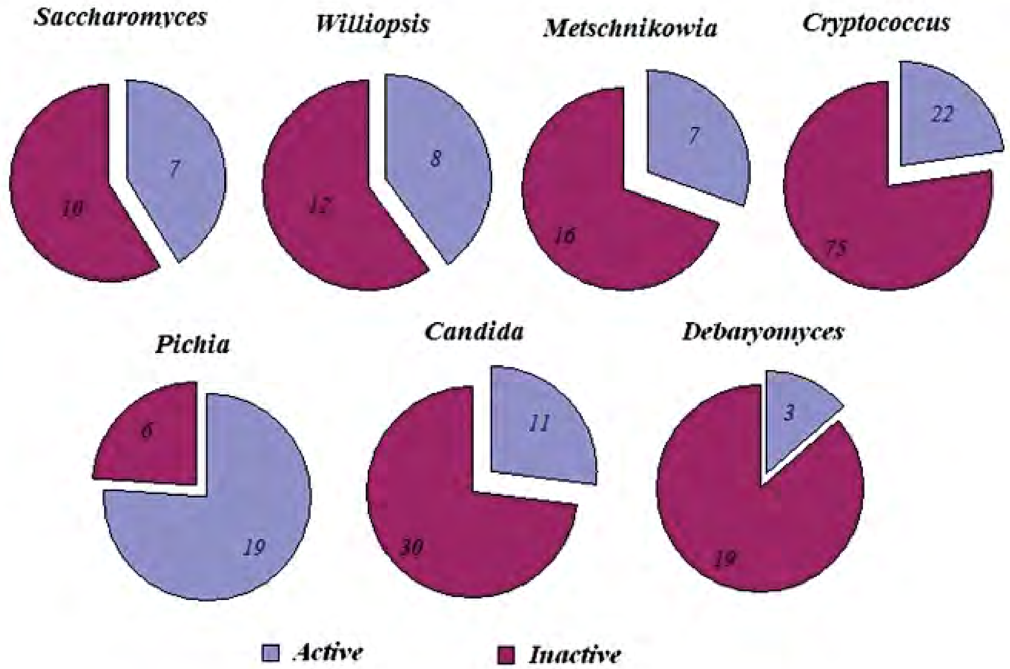


Fig. 2. **The ratio of active (showing β-mannanase or α-galactosidase activity) and inactive strains among representatives of different yeast genera**

studied than in other studies, where it was shown that only 7% of strains of the genus *Cryptococcus* and less than 3% of strains of the genus *Pichia* exhibited extracellular β-mannanase activity [19, 20]. This may be due to the sources of producer strains used by us, as extracellular enzymatic activity often depends on inducers and substrates from the external environment.

In contrast to micromycetes and actinobacteria, which, as established earlier [21], exhibited both α-galactosidase and β-mannosidase activity, no such producers were found among the yeast cultures. In the CL supernatant of only two yeast cultures — *Debaryomyces polymorphus* UCM Y-152 and *D. hansenii* var. *fabryi* UCM Y-2400 — α-galactosidase activity was detected in the absence of β-mannanase. This indicates that all studied yeast strains are capable of attacking either the main galactomannan chain or its side chains. Probably, there is a phenomenon of antisinergism of the action of enzymes competing for the same substrate [22].

β-Mannanase activity in the CL supernatant of different yeast strains varied in a wide range and was depend upon both the strain and the species of the microorganism (Fig. 3). Thus, representatives of *S. cerevisiae* and various species of *Cryptococcus* showed low β-mannanase activity. The most active species were *W. californica* UCM Y-25 (tomatoes), UCM Y-258 (soil) and UCM Y-250 (oats), *M. pulcherrima* UCM Y-357 (birch), UCM Y-355 (hornbeam), UCM Y-445 (soil contaminated with crude oil) and *P. anomala* UCM Y-244 (silage), UCM Y-237 and UCM Y-231 (GIT of trout and carp, respectively; Table 1).

An analysis of the results shows that the ability to synthesize secondary metabolites is primarily a strain-specific and not a species-specific trait, and the activity of different strains within the genus and species can differ by several orders of magnitude. The ability of a microorganism to hydrolyze a mannan-containing substrate and to show β-mannanase activity depends largely on the source of culture isolation. Although there is no clear correlation between the level of hydrolytic activity and the source of strain isolation, a high occurrence of mannanase-producing strains was found among yeasts isolated from soil and plants (Table 2).

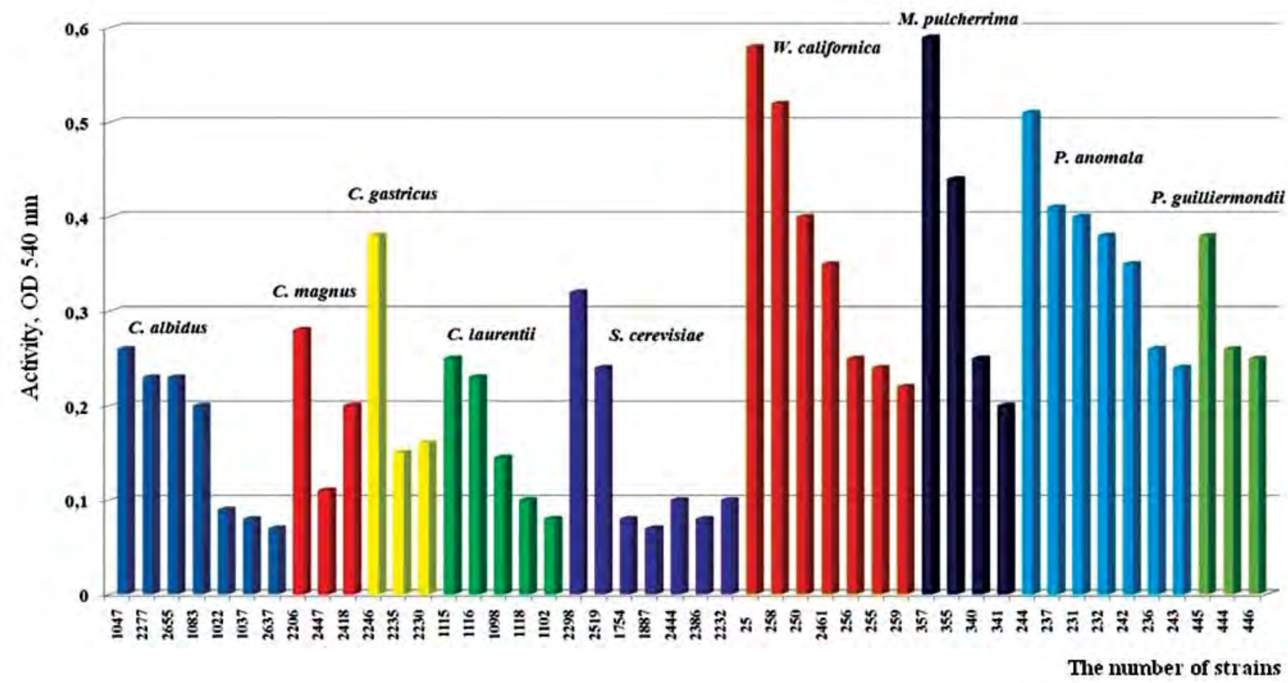


Fig. 3. **β-Mannanase activity in the culture liquid supernatant of the most active yeast strains**

Table 2. **Isolation sources of the most active yeast strains**

№№	№ of UCM-Y	Species	The strain origin (time, place of isolation or from where it was received)
1	2	3	4
1	1022	*Cryptococcus albidus*	Soil, 1969
2	1037	*Cryptococcus albidus*	Soil, Teremky, 1983
3	1047	*Cryptococcus albidus*	Soil, Teremky, 1983
4	1083	*Cryptococcus albidus*	Soil, Teremky, 1983
5	2277	*Cryptococcus albidus*	Israel, 2000
6	2637	*Cryptococcus albidus*	Soil under the juniper, Crimea, 2000
7	2655	*Cryptococcus albidus*	Soil, Yakutia, 2002
8	2230	*Cryptococcus gastricus*	Israel, 2000

end of table 2

№№	№ of UCM-Y	Species	The strain origin (time, place of isolation or from where it was received)
1	2	3	4
9	2235	*Cryptococcus gastricus*	Israel, 2001
10	2246	*Cryptococcus gastricus*	Israel, 2002
11	2206	*Cryptococcus magnus*	Israel, 2000
12	2418	*Cryptococcus magnus*	Soil, Evolutionary Canyon, Israel, 1999
13	2447	*Cryptococcus magnus*	Pistachio, Israel,1999
14	1098	*Cryptococcus laurentii*	Lupine, rhizosphere, 1967
15	1102	*Cryptococcus laurentii*	Tobacco, rhizosphere, 1969
16	1115	*Cryptococcus laurentii*	Cucumbers, leaves, 1969
17	1116	*Cryptococcus laurentii*	Cucumbers, rhizosphere, 1969
18	1118	*Cryptococcus laurentii*	Carrot, leaves, 1969
19	340	*Metschnikowia pulcherrima*	Cabbage, leaves, 1968
20	341	*Metschnikowia pulcherrima*	Apple tree, leaves, 1969
21	355	*Metschnikowia pulcherrima*	Grab, rhizosphere 1969
22	357Кат	*Metschnikowia pulcherrima*	Birch, rhizosphere, 1969
23	231	*Pichia anomala*	GIT of carp, 1977
24	232 Кат	*Pichia anomala*	Gills of carp, 1977
25	236	*Pichia anomala*	GIT of carp, 1977
26	237	*Pichia anomala*	GIT of trout, 1978
27	242	*Pichia anomala*	Gills of bream, the Gorky Reservoir, 1983
28	243	*Pichia anomala*	Leather of bream, Balkhash, 1983
28	244	*Pichia anomala*	Silage
30	444	*Pichia guilliermondii*	Soil contaminated with crude oil, West Ukraine
31	445	*Pichia guilliermondii*	Soil contaminated with crude oil, West Ukraine
32	446	*Pichia guilliermondii*	Soil contaminated with crude oil, West Ukraine
33	1754	*Saccharomyces cerevisiae*	Wine, received from Golovach
34	1887	*Saccharomyces cerevisiae*	Intestine of pheasant, Turkmenistan, 1983
35	2232	*Saccharomyces cerevisiae*	Israel, 2000
36	2298	*Saccharomyces cerevisiae*	Institute of Hematology
37	2386	*Saccharomyces cerevisiae*	Institute of Urology
38	2444	*Saccharomyces cerevisiae*	Pistachio, Israel, 2000
39	2519	*Saccharomyces cerevisiae*	Netherlands, Rotterdam, brewing (top fermenting yeast)
40	25	*Williopsis californica*	Tomatoes, rhizosphere, 1969
41	250	*Williopsis californica*	Oats, rhizosphere, 1967
42	255	*Williopsis californica*	Cucumbers, 1969
43	256	*Williopsis californica*	Tomatoes, 1969
44	258	*Williopsis californica*	Soil, Teremky, 1983
45	259	*Williopsis californica*	Soil, Teremky, 1983
46	2461	*Williopsis californica*	Soil, Znamenka, Kirovograd region, 1999

Thus, as a result of the work, the data were obtained on the prevalence frequency of β-mannanases producers among yeast cultures. It is shown that the soil and sources of plant origin are the optimal medium for the isolation of active producers of the enzymes of mannan-degrading complex. For the first time the strains-producers of β-mannanase among representatives of *W. californica* and *M. pulcherrima* species have been identified. Yeast β-mannanases are promising for use in various fields of biotechnology, in particular in the processing of mannan-containing raw materials.

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β-МАНАНАЗНА АКТИВНІСТЬ ДРІЖДЖІВ

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Метою роботи було визначення манандеградувальної активності дріжджових культур, ізольованих із різних джерел, для відбору серед них високоактивних продуцентів β-мананаз. У результаті скринінгу серед 245 штамів дріжджів, представників 7 родів, 14 видів, виявлено активні продуценти позаклітинної β-мананази. Для оцінювання активності культури вирощували в глибинних умовах, як джерело вуглецю та індуктор використовували галактоманан камеді гуару. β-Мананазну активність визначали динітросаліциловим методом. Найбільш активними біосинтетиками виявились представники видів *Cryptococcus albidus*, *C. gastricus*, *C. magnus*, *C. terreus*, *C. laurentii*, *Saccharomyces cerevisiae*, *Williopsis californica*, *Metschnikowia pulcherrima*, *Pichia anomala* та *P. guilliermondii*. Активність у супернатанті культуральної рідини становила від 0,2 до 75 од/мл. У двох штамів *Debaryomyces polymorphus* УКМ Y-152 і *Debaryomyces hansenii* var. *fabryi* УКМ Y-2400 виявлено α-галактозидазну активність. Жодна з досліджених культур не виявляла одночасно β-мананазної та α-галактозидазної активності, що свідчить про нездатність їх атакувати як основний, так і бічні ланцюги галактоманану.

Ключові слова: дріжджі, β-мананаза, α-галактозидаза, галактоманан.

β-МАННАНАЗНАЯ АКТИВНОСТЬ ДРОЖЖЕЙ

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Целью работы было определение маннандеградирующей активности дрожжевых культур, выделенных из различных источников, для отбора высокоактивных продуцентов β-маннаназ. В результате скрининга среди 245 штаммов дрожжей, представителей 7 родов, 14 видов, выявлены активные продуценты внеклеточной β-маннаназы. Для оценки активности культуры выращивали в глубинных условиях, в качестве источника углерода и индуктора использовали галактоманнан камеди гуара. β-Маннаназную активность определяли динитросалициловым методом. Наиболее активными биосинтетиками оказались представители видов *Cryptococcus albidus*, *C. gastricus*, *C. magnus*, *C. terreus*, *C. laurentii*, *Saccharomyces cerevisiae*, *Williopsis californica*, *Metschnikowia pulcherrima*, *Pichia anomala* и *P. guilliermondii*. Активность в супернатанте культуральной жидкости составила от 0,2 до 75 Е/мл. У двух штаммов *Debaryomyces polymorphus* УКМ Y-152 и *Debaryomyces hansenii* var. *fabryi* УКМ Y-2400 выявлена α-галактозидазная активность. Ни одна из изученных культур не проявляла одновременно β-маннаназную и α-галактозидазную активность, что свидетельствует о неспособности их атаковать как основную, так и боковые цепи галактоманнана.

Ключевые слова: дрожжи, β-маннаназа, α-галактозидаза, галактоманнан.