CHARACTERISTICS OF THREE YEAST STRAINS FOR WASTEWATER TREATMENT

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Abstract:
The selection of desirable strains with certain activities that have quick adaptivity and effectively remove contaminants from water is of great importance for the technologies for biological wastewater treatment.Three yeast strains are investigated. One of them was isolated from soil, and others are provided by a yeast strains' collection and denoted as C1 and C2. The identification of the yeast strains is done using the test kit API 20 C Aux (Biomerieux, France) for rapid identification of yeasts. Determination of the enzyme profile is obtained by applying the test kit API ZYM (Biomerieux, France). The three yeast strains accumulate high concentrations of viable cells. They are similar in their ability to oxidize S – containing compounds (elemental S, $Na_2S_2O_3$) under alkaline conditions lowering the pH of the medium and to exhibit catalase activity. Yeast strain Y exceeds the rest in the expression of lipolytic activity. That is confirmed in the subsequent analysis of its enzyme profile through identification system API ZYM. Through it are found and alpha –, beta – glucosidase and aminopeptidase activities.

Keywords: *morphological, physiological and biochemical methods, API ZYM, API 20 C Aux, Candida famata*

1. Introduction

One of the most important compounds of the planet is water [1]. The continuous decrease of potable water determines the need for different methods for its purification. Technologies for biological wastewater treatment with the participation of microorganisms, including yeasts, are becoming more and more current.
Among the microorfanisms, participating in the wastewater treatment process are representatives of the genera *Candida*, *Pichia*, *Torulaspora*, *Yarrowia*, *Geotrichum*, *Saccharomyces*, *Cryptococcus*, *Rhodotorula*, *Trichosporon* [2, 3, 4].

The selection of desirable strains with certain activities that have quick adaptivity and effectively remove contaminants from water is of great importance.
The aim of this work is the analysis and identification of yeast strains suitable for specific applications in the field of biological wastewater treatment.

2. Materials and methods

2.1. Microorganisms

Three yeast strains, marked as Y, C1, C2 are used in this study. Yeast strain Y is isolated from soil and strains C1 and C2 are provided by the microorganism

collection of the Department of Organic Chemistry and Microbiology at the University of Food Technologies, Plovdiv, Bulgaria.

2.2.Nutrient media

2.2.1. Malt extract medium. Composition: malt extract (Kamenitza, Bulgaria) in 1:1 ratio with tap water (vol/vol). pH=6,5 – 7,0. The medium is sterilized for 25 minutes at 121ºC [5].

2.2.2. Malt extract medium. Composition: malt extract (Kamenitza, Bulgaria) in 1:1 ratio with tap water (vol/vol) + 2 % agar – agar (w/vol). pH=6,5 – 7,0. The medium is sterilized for 25 minutes at 121ºC [5].

2.2.3. Luria – Bertani glucose medium (LBG). Composition (g/dm^3): triptone (Difco) – 10 g, yeast extract – 5 g, NaCl – 10 g, glucose (Scharlau) – 10 g. pH=7,5. The medium is sterilized for 25 minutes at 121ºC.

2.2.4.Soy – caseine broth medium. Composition (g/dm^3): triptone (Difco) – 17g, soy peptone (Scharlau) – 3 g, NaCl – 5 g, K_2HPO_4 – 2,5 g, glucose (Scharlau) – 2,5 g. pH=7,3 ± 0,2. The medium is sterilized for 25 minutes at 121ºC.

2.2.5.Citrate utilization medium (Simons medium). Composition (g/dm^3): $Na(NH_4)_2PO_4$ – 1,5 g; KH_2PO_4 – 1 g; $MgSO_4.7H_2O$ – 0,2 g; Na – citrate – 3 g; alcohol solution of bromthymol blue – 1%; agar – agar – 2%. The medium is sterilized for 25 minutes at 121ºC [6].

2.2.6.Gelatinase activity medium. Composition (g/dm^3)**:** triptone (Difco) – 10 g; yeast extract (Scharlau) – 5 g; NaCl – 10 g; glucose (Scharlau) – 10 g; gelatine (DDR) – 250 g. pH 7,5. Medium is dispensed into tubes and is sterilized for 25 min at 121ºC [6].

2.2.7. Proteolytic activity medium. Composition: malt agar medium with 10 % (vol/vol) solution additive (10 cm^3 milk/100 cm^3 water) of skimmed milk powder (Scharlau) [6].

2.2.8.Lipolytic activity medium (Tween – 80 compounds hydrolysis). Composition (g/dm^3): peptone (Scharlau) – 10 g; NaCl – 5 g; $CaCl_2$ – 0,1 g; Tween – 80 (Merck) – 10 cm^3; agar – agar – 20 g. pH 7 – 7,4. The medium is sterilized for 25 minutes at 121ºC [5].

2.2.9. Sulphur–containing compound oxidation mediua.

2.2.9.1. Starckey broth medium. Composition (g/dm^3): elemental S – 10 g; KH_2PO_4 – 3 g; $MgSO_4.7H_2O$ – 0,2 g; $CaCl_2.2H_2O$ – 0,2 g; $(NH_4)_2SO_4$ – 0,5 g; $FeSO_4$ – traces; indicator – bromocresol purple. pH 8,0. The medium is prepared in two versions: with glucose (5 g/ dm^3) and without glucose. It is sterilized at Koch apparatus for 30 min on three consecutive days [7].

2.2.9.2. NCL – broth medium. Composition (g/dm^3): elemental S – 10 g; $(NH_4)_2SO_4$ – 0,2 g; $MgSO_4.7H_2O$ – 0,5 g; $CaCl_2.2H_2O$ – 0,25 g; $FeSO_4$ – traces; indicator – bromocresol purple. The medium is prepared in two versions: with glucose (5 g/ dm^3) and without glucose. It is sterilized at Koch apparatus for 30 min on three consecutive days [7].

2.2.9.3. Thiosulphate agar medium. Composition (g/dm^3): $Na_2S_2O_3$ – 5g; K_2HPO_4 – 0,1 g; $NaHCO_3$ – 0,2 g; NH_4Cl – 0,1 g; agar – agar – 20 g. pH 8,0. The medium is prepared in two versions: with glucose (5 g/ dm^3) and without glucose. It is sterilized at Koch apparatus for 30 min on three consecutive days [7].

2.2.10. NO_3^- – reductase activity medium. Composition (g/dm^3): peptone (Scharlau) – 5 g; meat extract (Scharlau) – 3 g; KNO_3 –

1 g. pH 7.0. The medium is sterilized for 25 minutes at 121ºC [8].

2.2.11. NH_4^+ – citrate medium (for nitrifying activity). Composition (mol/dm^3): Na – citrate.$2H_2O$ – $9{,}5.10^{-3}$; NH_4Cl – $9{,}35.10^{-3}$; KH_2PO_4 – $1{,}47.10^{-3}$; $MgSO_4.7H_2O$ – $1{,}62.10^{-4}$; $CaCl_2$ – $1{,}36.10^{-7}$; $FeSO_4.7H_2O$ as EDTA – complex – $3{,}6.10^{-5}$. The medium is sterilized for 25 minutes at 121ºC [9].

2.2.12. Blood agar (NCIPD, Bulgaria). Composition (g/dm^3): casein hydrolysate – 14 g; NaCl – 5 g; peptone – 4,5 g; yeast extract – 4,5 g; defibrinated sheep blood – 70 cm^3; agar – agar – 12,5 g. pH 7,3 ± 0,2. All medium components are sterilized for 15 min at 121ºC. After cooling to 45ºC – 50ºC 70 cm^3 defibrinated sheep blood is added aseptically and is poured in sterile Petri dishes [8].

2.2.13. Gorodkova medium (for yeast sporulation). Composition (g/dm^3): peptone – 10 g; NaCl – 5 g; месен meat extract – 10 g; glucose – 2,5 g; agar – agar – 20 g. pH 6,5 – 7,0. The medium is sterilized for 25 minutes at 121ºC [5].

2.2.14. Acetate agar medium (for yeast sporulation). Composition (g/dm^3): $NaOOCCH_3.3H_2O$ – 5 g; agar – agar – 20 g. pH 6,5 – 7,0. The medium is sterilized for 25 minutes at 121ºC [8].

2.2.15. Minimal agar medium (for yeast sporulation). Composition (g/dm^3): agar – agar – 20 g. pH 6,5 – 7,0. The medium is sterilized for 25 minutes at 121ºC [8].

2.2.16. Rice agar medium (Fluka Analitycal) (for pseudomycellium identification). Composition (g/dm^3): rice extract powder – 0,7; agar – agar – 20 g. The medium is prepared in two versions – with and without Tween – 80 (Merck) (1 cm^3/dm^3). pH 5,8 ± 0,2. The medium is sterilized for 25 minutes at 121ºC.

2.2.17. Motility medium (NCIPD, Bulgaria). Composition (g/dm^3)**:** peptone – 10 g, meat extract – 3 g, NaCl – 5 g, agar – agar – 4 g. pH 7,4 ± 0,2. The medium is sterilized for 25 minutes at 121ºC.

2.3.Cultivation and storage of the analyzed microorganisms.

Yeasts grow in malt extract medium at 30ºC in thermostat for 48 hours and are stored in a refrigerator at 4ºC for 2 months.

2.4. Analytical methods.

2.4.1. Morphological and cultural methods

2.4.1.1. Cellular and colonial morphology. Description of cellular and colonial morphology of the studied yeast strains is performed by microscopic observation the developed on malt agar single colonies of the studied strains.

2.4.1.2. Identification of pseudomycellium formation ability. A part of the cultural medium of the studied strain, that has been cultivated for 48 hours in a thermostat is taken with flamed and cooled bacteriological loop and spread in touch on agar plates with rice-agar. The inoculated plates are incubated under aerobic conditions for 2-4 days at 23ºC - 28ºC.

A microscopic preparation is prepared from the developed colonies of the tested strain, which is examined for the presence of specific pseudomycellium or other specific formations in yeasts. The presence of vegetative cells only determines the result as negative.

2.4.1.3.Identification of spore formation ability.

The ability of the yeast to form spores under unfavorable environmental conditions - lack of nutrients - is examined.

Using a flamed and cooled bacteriological loop a part of the pre-developed on malt agar 48 - hour colonies of the studied strain is taken and is stroked on a Petri dish with minimal agar medium Gorodkova agar or acetate agar. Incubation conditions - 20ºC - 25ºC for 7 days. A microscopic preparation is prepared from the biomass and it is stained by the method of Moller. Spores are rubine red and vegetative bodies – blue.

2.4.1.4. Determining the number of viable cells in the development of the cultures in liquid medium. Sterile malt extract is inoculated under aseptic conditions with yeast suspension, 5 cm^3 medium is inoculated with 1 cm^3 of the inoculum of the studied cultures. After incubation in a thermostat at 30ºC for 48 h, tenfold dilution method is done and malt agar plates are inoculated. Petri dishes are thermostatted at 30ºC for 48 h so that the strain would form single colonies, which are then counted.

2.4.2. Physiological and biochemical methods

2.4.2.1. Citrate utilization. Developed in containers with 30 cm^3 1:1 malt extract at 30ºC for 48h yeast cultures are centrifuged at 3000 min^{-1} for 10 min. The supernatant is discarded and the biomass is resuspended in 5 cm^3 sterile physiological solution. The yeast inoculum is taken using a sterile bacteriological loop and it is streak-spread on petri dishes with Simons medium. The inoculated plates are incubated at 30ºC for 48h. Positive results are recorded in a case of a colour change of the medium from green to blue.

2.4.2.2. Determination of the gelatinase activity of the tested strains.

The preparation method of the yeast inoculum is analogous to that in 5.1.

The suspension of the tested strain is taken with a sterile bacteriological loop and is put in point onto a pre-sterilized medium with gelatin. The tubes are incubated for 7 days at room temperature and the presence of crater melting is monitored [6].

2.4.2.3. Proteolytic activity determination using fusion agar method. The ability of the yeast strains to hydrolyze milk proteins in agar medium with milk additive is investigated. Wells with d = 6 mm are made on Petri dishes with medium for proteolytic activity. After inoculation, the plates are incubated at 30ºC for 48 hours. Results are reported as positive in the case of a formation of a brighter halo around the wells of the plates. A lack of halo is a sign of an inability to utilize milk proteins.

2.4.2.4. Determination of the lipolytic activity using fusion agar method. Lipolytic activity includes the ability of the analyzed strains to hydrolyze the compounds of Tween - 80. Wells with d = 6 mm are made on the Petri dishes with agar medium. After inoculation the Petri dishes are cultivated at 30ºC for 1 - 7 days. Formation of a turbid zone around the wells, due to the precipitation of Ca - salts of the formed free fatty acids, indicates the presence of lipolytic activity.

2.4.2.5. Analysis on the ability of the studied strains to oxidize S-containing compounds. The analysis of the oxidation of S-containing compounds includes development of the yeast strains on selective media. Tubes with sterile liquid Starkey medium and NCL medium "with" and "without" glucose are inoculated with 1 cm^3 yeast suspension, prepared as in 5.1. With a bacteriological loop the same suspension is used to streak plates with thiosulphate media "with" and "without" glucose. The plates and tubes are cultivated in a thermostat at 30ºC for 15 days. Changes in the color of the indicator bromocresol purple (from purple to yellow on Starkey and thiosulphate media; and

from yellow to dark yellow or purple on NCL – broth) are reported as positive results.

2.4.2.6.Determination of nitrifying activity of the studied strains. Nitrifying activity includes the development of the analyzed yeasts on liquid medium with ammonium salts being the sole nitrogen source. A suspension of the tested yeast strains is prepared, as described in 5.1.
1 cm^3 of it is used to inoculate tubes with 5 cm^3 of ammonia - acetate medium and the tubes are incubated at 30ºC for 24 hours. If the yeast strain has nitrifying activity, NO_3^- iones would be formed in the medium. Their presence is determined by test - strips (110,020 Nitrate Test Merckoquant, Merck).
Some microorganisms have the ability to reduce NO_3^- to NO_2^-. Therefore, the content of NO_2^- in the medium is determined, using a test - strip (110,022 Nitrate Test Merckoquant, Merck). Results are recorded as positive in the presence of NO_3^- and NO_2^- or negative - when NO_3^- and NO_2^- are absent in the medium.

2.4.2.7. Determination of the nitrate - reductase activity of the studied strains.
Prepared as in 5.1., the suspensions of the studied cultures are used to inoculate medium with KNO_3 - 1 cm^3 suspension is used to inoculate 5 cm^3 of sterile medium for determination of nitrate - reductase activity. Incubation is carried out for 7 hours at 30ºC. The presence of NO_2^- in the medium is considered as a positive result. Their presence is determined by test - strip (110,022 Nitrate Test Merckoquant, Merck). Result are recorded as positive - in the presence of NO_2^- or negative - in the absence of NO_2^- in the medium.

2.4.2.8.Determination of the hemolytic activity of the investigated cultures. Some microorganisms have the ability to utilize blood, breaking down red blood cells. Depending on the mechanism of their hydrolysis there are three types of hemolytic activity. In α - hemolysis iron from hemoglobin is oxidized and the colonies become dark - green. In β - hemolysis the erythrocytes and the hemoglobin in them are degraded, and a bright halo is formed around some of the colonies. γ – hemolysis is observed in the case of no hemoglobin hydrolysis.
Using a bacteriological loop inoculum is taken from the suspension of the tested yeast strain, prepared as in 5.1. and it is stroked on blood agar. The inoculated plates are cultured for 48 hours at 30ºC. The presence of hemolytic activity is recorded: in the cases of α - or β – hemolysis, the result is positive, while for γ – hemolysis, it is negative.

2.4.2.9. Determination of the catalase activity of the analysed cultures. Catalase activity is determined by the method described in [6].

2.4.2.10. Determination of the oxidase activity of the investigated cultures.
Oxidase activity test includes an analysis of the suspension of the studied strains for the presence of the enzyme cytochrome - oxidase. In the presence of molecular oxygen, cytochrome - oxidase can reduce the number of organic substances, including reagent NaDi (1 - naphthol + diamine dimetilparaphenylene) with the formation of indophenole blue. Test - strips for oxidase activity (Microbiology Bactident Oxidase 1.13300.0001, Merck) are places in the suspension of yeasts prepared as in 5.1.
After 20-60 s the result is compared to a color scale. Positive result are recorded in the case of a color change of the strip from white to blue to blue - violet.

2.4.2.11. Determination of the profile of enzyme activity of the studied cultures.

The determination of the profile of enzyme activity is performed, using the test kit API ZYM (BioMerieux) for semiquantitative determination of the enzyme profile of the studied strain. Fresh 24-hour culture of the tested strain is centrifuged for 15 minutes at 5000 g, the obtained biomass precipitate is washed twice and resuspended in API suspension medium. The API ZYM strips are placed in the incubation boxes and the microtubes are inoculated with the prepared cell suspension. The sample is incubated for 4 to 4,5 hours at 30° C. After the incubation one drop of reagent A and one drop of reagent B are pipetted into each microtubule. After 5 min staining result is reported according to the colour scheme described in the manufacturer's instructions. The enzyme activity is determined according to the colour scale from 0 (no enzyme activity) to 5 (maximum enzyme activity).

2.4.2.12. Determination of the biochemical profile of the investigated cultures.

The system API 20 C Aux (BioMerieux SA, France) for identification of yeast species based on the consumption of 19 carbon sources is used for the determination of the biochemical profile of the tested cultures. Fresh 24-hour culture of the tested strain, developed on malt agar, is resuspended according the instructions of the manufacturer in API C resuspension medium. The honeycomb wells on the bottom of the incubation boxes are filled with sterile physiological solution. The API 20 C strips are placed in the incubation boxes and the microtubules are inoculated with the prepared cell suspension. The sample is incubated for 48h to 72h at the optimum temperature for each of the studied strains. Results are recorded according the change in turbidity in comparison to the control (microtubule 0). The results are processed with apiweb® identification software.

3. Results and discussion

The ability of the yeast strains to grow in three cultural media is investigated. The results represented in Table 1 show that the best liquid medium for the strains' development is malt extract (2.1.).
The amount of viable cells is determined during cultivation of the investigated yeast strains on malt extract liquid medium.
Test results (Table 2) show that each of the three yeast strains accumulate high concentration viable cells (over 10^{12} cfu/cm^3) for 48 h cultivation at 30°C.

Table1
Media for yeast development

Strain	Medium		
	LBG (Luria – Bertani with glucose)	ME (Malt extract)	SCB (Soy – Casein broth)
Y	–	Abundand sludge, Uniform turbidity	–
C1	–	Abundand sludge, Uniform turbidity	–
C2	–	Abundand sludge, Uniform turbidity	–

Table 2
Concentration of viable yeast cells, cultivated in malt extract liquid medium

Yeast strains	Average (cfu/cm^3)
Y	$5,6.10^{13}$
C1	$1,0.10^{13}$
C2	$1,4.10^{12}$

The colonial characteristics of the researched strains are determined after inoculating malt agar medium (2.2) and cultivation for 48 h (Table 3). Cell morphology is done with coloured microscope preparations.

By inoculating minimal agar media - Gorodkova agar, acetate agar and rice agar – it is determined, that researched yeast strains don't form pseudomycellium and chlamidospores.

Experiment results, presented in Table 4, show considerable similarities between the investigated yeast strains. Three cultures don't hydrolyse gelatine, don't exhibit nitrifying and nitrate – reductase activities, are catalase – positive and oxidoreductase – negative. All of them are non – motile, without proteolysis activity. They can assimilate citrate, with the exception of yeast strain Y. They cannot hydrolyze hemoglobin (γ – hemolysis).

All analyzed strains don't assimilate sulphur compounds in non – glucose medium at pH < 5 and pH 8. C2 is an exception (NCL broth without glucose). Analogy between strains is also observed in S – containing compounds at pH 8 ($Na_2S_2O_3$ medium with glucose). As a difference from C1, Y and C2 are developed in NCL broth with glucose.

Yeast strain Y, isolated from soil, exceeds C1 and C2 in its values for the lipolytic activity.

Table 3
Colonial characteristics and cell morphology of the investigated yeast strains

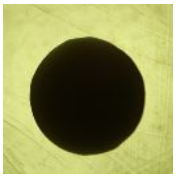
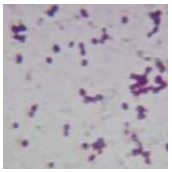
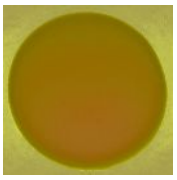
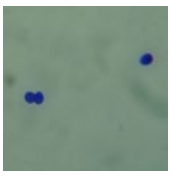
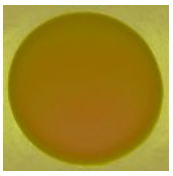
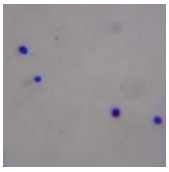
Strain	Colonial characteristics		Cell morphology	
	Colony description	Visualization	Cell description	Visualization
Y	Round colonies with wave – like ends, smooth surface, 4-5 mm in diameter, soft consistence, swelled, drop – like with plateau		Yeasts, ellipse shaped. With vegetative breeding, with budding, without pseudomycellium formation	
C1	Round colonies with wave – like ends, smooth surface, 4-6 mm in diameter, soft consistence, whitish in colour		Yeasts, ellipse shaped, with single arrangement and make clusters, with vegetative breeding, with budding, without pseudomycellium formation	
C2	Round colonies with wave – like ends, smooth surface, 4-6 mm in diameter, soft consistence, whitish in colour		Yeasts, ellipse shaped, with single arrangement and make clusters, with vegetative breeding, with budding, without pseudomycellium formation	

Table 4
Biochemical features of the analyzed yeast strains

Strain			Y	C1	C2
Lipolytic activity, mm			+ 13,7±1,4	–	–
Nitrifying activity			–	–	–
Gelatinase activity			– Facultative anaerobes	– Facultative anaerobes	– Facultative anaerobes
Oxidation of S – containing compounds	Mediums with glucose	$Na_2S_2O_3$	+ Growth	+ Growth	+ Growth
		Starkey	–	– Growth	– Growth
		NCL	+	–	+
	Mediums without glucose	$Na_2S_2O_3$	– Growth	– Growth	– Growth
		Starkey	–	– Growth	–
		NCL	–	–	+
Catalase activity			+	+	+
Oxidoreductase actividy			–	–	–
NO_3^- - reductase activity			–	–	–
Proteolytic activity	24 h		–	–	–
	48 h		–	–	–
Citrate utilization			–	+	+
Hemolytic activity			γ	γ	γ
Motility			–	–	–

Aiming at a more complete analysis of the possibilities for application of the yeast strain Y in the field of wastewater treatment its enzyme profile is investigated with API ZYM.

The performed investigations (Table 5) show the presence of alkaline phosphatase, esterase, esterase-lipase, lipase, leucine – aminopeptidase, valine – aminopeptidase, cysteine – aminopeptidase, acid phosphatase, phosphohydrolase, alpha – glucosidase and beta – glucosidase. The presence of different lipolytic enzymes confirms the results from the biochemical tests for the lipolytic activities, shown in Table 4.

The ability of yeast strains to utilize 19 different carbon sources, included in the test kit API 20 C Aux for rapid identification of yeasts, is investigated. Pseudomycellium and chlamidospore formation is determined using rice agar.

The results from the analyses are generalized in Table 6.

After data processing with the software apiweb® yeast strain Y is identified as *Candida famata* (synonym *Torulopsis candida* [10] with reliability 99,9% (Table 7). C1 is identified as *Candida famata* - 53,8%, *Candida lusitaniae* – 22,7%, *Candida guillermondii* – 21,6%. C2 is identified as *Candida famata* - 54,7%, *Candida lusitaniae* – 23,1%, *Candida guillermondii* – 22%. Regardless of the lower confidential values for the identification of C1 and C2, they are undoubtedly representatives of the genus *Candida*. Other tests must be conducted in order to determine their species identification.

Table 5
Enzyme profile of the yeast strain Y

	Enzyme	Activity of yeast strain Y	
1	Control	-	
2	Alkaline phosphatase	3	
3	Lipase C_4	1,5	
4	Lipase C_8	0,5	
5	Lipase C_{14}	0,5	
6	Leucine-aminopeptidase	5	
7	Valine-aminopeptidase	3,5	
8	Cysteine-aminopeptidase	3	
9	Trypsin	-	
10	Chymotrypsin	-	
11	Acid phosphatase	4	
12	Naphthol – AS – BL – phosphohydrolase	1	
13	α-galactosidase	-	
14	β-galactosidase	-	
15	β-glucuronidase	-	
16	α-glucosidase	4	
17	β-glucosidase	5	
18	α-glucoseaminidase	-	
19	α-manosidase	-	
20	α-fucosidase	-	

Table 6
Ability of the investigated yeast strains to utilize the 19 carbon sources in API 20 C Aux

№	Substrate	Y	C1	C2
1	Control	–	–	–
2	D – glucose	+	+	+
3	Glycerol	+	+	+
4	Calcium 2 – keto – gluconate	+	+	+
5	L – arabinose	–	–	–
6	D – xylose	+	+	+
7	Adonitol	+	+	+
8	Xylitol	+	+	+
9	D – galactose	+	+	+
10	Inositol	–	+	–
11	D – sorbitol	+	+	+
12	Methyl – α – D – glucopyranoside	+	+	+
13	N – acetylglucoseamine	+	+	+
14	D – cellobiose	+	+	+
15	D – lactose (bovine)	+	–	–
16	D – maltose	+	+	+
17	D – saccharose	+	+	+
18	D – trehalose	+	+	+
19	D – melezitose	+	+	+
20	D - raffinose	–	–	–
21	Hyphae/Pseudohyphae	–	–	–

Table 7

Strains, identified according to the software apiweb®

Strain	Species	% of reliability
Y	*Candida famata*	99,9
C1	*Candida famata*	53,8
	Candida lusitaniae	22,7
	Candida guillermondii	21,6
C2	*Candida famata*	54,7
	Candida lusitaniae	23,1
	Candida guillermondii	22,0

4. Conclusion.

As a result of the conducted experimental analyses the following more important conclusions can be drawn:

1. A yeast strain is isolated and is identified using the methods of the conventional taxonomy *Candida famata* (synonym *Torulopsis candida* [10]);
2. The strain has the ability to utilize S – containing compounds in the presence of glucose, lowering the pH value;
3. It has clearly defined catalase and lipolytic activities. The last one is also confirmed by the presence of wide range of enzymes with such activity. Its beta – and alpha glucosidase activity, leucine –, valine – and cysteine – aminopeptidase activity, acid – and alkaline phosphatase activity, less significant – naphthol – AS – BL – phosphohydrolase activity and the least is C_4 –, C_8 – и C_{14} – lipase activity are also significant.

5. References.

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