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EXPERIMENTAL ARTICLES

Isolation of Tremelloid Yeasts on Glucuronate Medium

V. I. Golubev

All-Russia Collection of Microorganisms, Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, Pushchino, Moscow oblast, 142292 Russia

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Abstract—D-Glucuronate-containing agar is suggested for evaluating the population density and diversity of tremelloid yeasts in natural cenoses. This medium is superior to the commonly used wort agar on which many representatives of tremelloid yeasts cannot be revealed.

Key words: tremelloid yeasts, isolation, D-glucuronate.

Yeasts are a group of unicellular fungi that includes representatives of various classes of eumycetes [1] which differ in their requirements for growth conditions. However, to determine the density and diversity of natural yeast populations, wort agar (WA) is most commonly used [2], which was first employed in the fermentation industry to satisfy, primarily, the trophic requirements of saccharomycetes.

In the past decades, the yeasts were found to be extremely heterogeneous over the whole scope of their biology [3], and, therefore, the evaluation of the density and composition of natural yeast populations is impossible with a single medium [4]. It is evident that various media and growth conditions are necessary to meet the particular growth requirements of individual yeast groups.

Basidiomycetous yeasts of the tremelloid affinity utilize cyclite, *i*-inositol, and D-glucuronate, which is the first intermediate of inositol catabolism [5]. Ascomycetous and sporidiobolaceous yeasts, with a few exceptions, do not utilize these compounds as the sole carbon sources in the medium. Based on these facts, we developed a new medium to reveal and isolate tremelloid yeasts and to determine their population density and taxonomic composition. In this work, we present the results of testing the glucuronate-containing medium, performed during a study of the yeast mycobiota of the Prioksko-Terrasnyi reserve (Moscow oblast).

MATERIALS AND METHODS

Samples were taken in sterile parchment packets from a plot with fescue-herbaceous vegetation (section no. 34a) and from a plot with an oak forest and herbage (at the boundary of sections nos. 41 and 41a) in the middle of September 1997. The samples were minced, placed into flasks with sterile water, and shaken for 10 min. The 10^{-2} and 10^{-3} dilutions were plated in triplicate on WA and on media containing 1% *i*-inositol or D-glucuronate and the following compounds: $(NH_4)_2SO_4$, 5 g/l; KH_2PO_4, 0.85 g/l; yeast extract, 0.5 g/l; and agar, 20 g/l. The media were acidified with lactic acid after sterilization in an autoclave. A solution of glucuronic acid was sterilized separately and added to the melted media immediately before they were poured into Petri dishes (pH 4.5). The plated samples were incubated at 20°C.

After the grown colonies were counted, several isolates of each morphotype were plated on WA and examined for their purity under a microscope and by replating. The species were identified according to the manuals [3, 6], taking into consideration new taxa described recently, or by direct comparison with type strains. To confirm their taxonomic affinity, the isolates obtained were tested for their sensitivity to the mycocins of *Cryptococcus laurentii, Cr. podzolicus, Cystofilobasidium bisporidii, Filobasidium capsuligenum, Rhodotorula glutinis, Rh. pallida*, and *Sporidiobolus pararoseus* [7].

RESULTS AND DISCUSSION

Since tremelloid yeasts represent only a portion of the natural mycobiota, the total population density evaluated by plating onto inositol- and glucuronate-containing media was expected to be considerably lower than that evaluated on WA. Indeed, the population density determined on WA was higher in the samples of dead leaves, wood residues (small branches), and in the sample of a decayed wood trunk. However, in the rest of the samples, the overall yeast population density evaluated on inositol- and glucuronate-containing agar was several times higher than that determined on WA (Table 1); the results obtained on inositol- and glucuronate-containing media were similar.

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Table 1. Total population density of yeasts (thousand CFU/g) revealed by plating on wort agar, *i*-inositol agar, and D-glucuronate agar

Substrates	Wort agar	Inositol agar	Glucuronate agar
Fescue-herbaceous steppe			
Herbaceous vegetation	153.6	642.4	680.0
Turf	8.5	24.8	32.1
Soddy-podzolic soil, A1	0.0	13.0	2.5
Oak forest with herbage			
Herbaceous vegetation	2.8	99.2	123.3
Dead leaves	28.0	7.4	7.4
Wood residues	74.7	38.4	26.1
Decayed wood pulp	26.0	4.0	4.0
Forest litter	0.0	11.9	9.6
Soddy-podzolic soil, A1	3.0	6.5	8.7

Identification of the 180 yeast strains isolated revealed principal differences in the taxonomic composition determined by plating onto WA and onto inositol or glucuronate agar. The proportion of ascomycetous yeast grown on WA was 26%, whereas on the latter two media, it was about 6%. The relative content of basidiomycetous yeasts on WA was 74% (59% tremelloid yeasts). On inositol and glucuronate agar, basidiomycetous yeast comprised 94–95% (81 to 91% tremelloid yeasts).

Still more profound distinctions were revealed in the species composition. In samples with a population density on WA higher than that determined on inositol or glucuronate agar (Table 1), ascomycetous (*Candida* sp.) and inositol- and glucuronate-negative basidiomycetous yeasts (*Rh. fujisanensis*) predominated on WA (Table 2). Tremelloid yeasts isolated from both steppe and forest plots correlated well in all parameters not only with the standard species descriptions but also with the type strains. On each plot, no more than seven species were revealed using WA, which is two to four times less than the number of species revealed on inositol and glucuronate media. The glucuronate medium was more efficient, because some yeasts do not utilize inositol but assimilate glucuronate [8, 9].

Thus, the yeasts detected on the media suggested are more diverse; evidently, many organisms of the natural populations do not form colonies on WA. Several organisms, growing only on inositol and glucuronate media, seem to be unknown species. Formally, most of these isolates can be identified as *Cr. aerius, Cr. albidus*, and *Cr. laurentii*, whose descriptions contain many characteristic variables [3, 6]. Direct comparison of these isolates with type species strains revealed morphological, physiological, biochemical, and cultural differences, as well as differences in the sensitivity to mycocins [7]. We were able to distinguish several types, whose number is indicated in Table 2 in parentheses.

Platings from the diluted (1000–3000 cells/ml) pure cultures of tremelloid yeasts detected only on inositol and glucuronate media showed that either all or the majority of their cells did not form colonies on WA. One of these cultures, PTZ-4, identified as *Cr. laurentii*, was shown to form on the glucuronate medium colonies whose number corresponded to the cell concentration in the suspension (as calculated in a Goryaev chamber), whereas only 4% of the same cells formed colonies on WA. In strain PTZ-18A, identified as *Cr. albidus*, 20% of cells (or 25%, if nonviable, methylene blue-stained cells were discounted) formed colonies on glucuronate agar, whereas no growth occurred after repeated platings on WA. These data suggest that WA contains some factors that inhibit the growth of tremelloid yeasts. It should be noted that the inhibition was observed when the cell content in the substrate analyzed was low; no inhibition occurred with abundant inoculum. The colonies grown on glucuronate agar can be transferred to WA and then maintained by reinoculating WA slants.

Thus, only a portion of the yeast mycobiota of natural substrates, mainly, already known and widespread forms, can be detected when only WA is used. I suggest the use of glucuronate agar for the determination of the population density and diversity of tremelloid yeasts.

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Table 2. Yeast species detected on various media

Wort agar	Inositol agar	Glucuronate agar
	Fescue-herbaceous steppe	
–*	–	*Bullera alba*
Bullera unica	*Bullera unica*	*B. unica*
Cryptococcus aerius	*Cryptococcus aerius*	*Cryptococcus serius* (4)
Cr. albidus	*Cr. albidus* (3)**	*Cr. albidus* (4)
Cr. ater	*Cr. ater*	*Cr. ater*
–	–	*Cr. hungaricus*
–	*Cr. laurentii* (3)	*Cr. laurentii* (3)
–	*Cr. magnus*	*Cr. magnus*
–	–	*Cr. podzolicus*
–	–	*Cr. terreus*
–	*Debaryomyces castellii*	*Debaryomyces castellii*
Rhodosporidium babjevae	*R. babjevae*	–
–	–	*Rhodosporidium* sp.
–	*Rhodotorula foliorum*	
–	*Rh. hordea*	*Rh. hordea*
–	*Tremella* sp. (7)	*Tremella* sp. (5)
–	*Trichosporon asahii* var. *faecalis*	–
–	*Tr. moniliiforme*	–
Udeniomyces piricola	–	–
	Oak forest with herbage	
–	–	*Bullera huiaensis*
Candida railenensis	*Candida railenensis*	*Candida railenensis*
C. santjacobensis	–	*C. santjacobensis*
Cryptococcus aerius	*Cryptococcus aerius* (4)	*Cryptococcus aerius* (4)
–	*Cr. albidus* (2)	*Cr. albidus* (3)
Cr. ater	–	–
Cr. podzolicus	*Cr. podzolicus*	*Cr. podzolicus*
Cr. laurentii	*Cr. laurentii* (2)	*Cr. laurentii* (4)
–	*Cr. luteolus*	*Cr. luteolus*
–	*Cystofilobasidium infirmominiatum*	*Cystofilobasidium infirmominiatum*
–	–	*Pichia inositovora*
–	–	*Rhodosporidium* sp.
–	*Rhodotorula auriculariae*	*Rhodotorula auriculariae*
Rhodotorula fujisanensis	*Rh. fujisanensis*	–
–	*Rh. graminis*	–
–	*Rh. hordea*	*Rh. hordea*
–	–	*Rh. pallida*
–	–	*Tremella* sp. (3)
–	*Tremella indecorata*	*T. indecorata*
–	–	*Udeniomyces piricola*

* The species was not detected.
** The number of types differing in morphological and physiological traits and sensitivity to mycocins.

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REFERENCES

1. Golubev, V.I., Evolution of the Concept of Yeast, *Usp. Sovrem. Biol.*, 1992, no. 5/6, pp. 715–724.
2. Bab'eva, I.P. and Golubev, V.I., *Metody vydeleniya i identifikatsii drozhzhei* (Methods for Isolation and Identification of Yeasts), Moscow: Pishchevaya prom-st', 1979.
3. *The Yeasts: A Taxonomic Study*, Kurtzman, C.P. and Fell, J.W., Eds., Amsterdam: Elsevier Sci., 1998.
4. Chernov, I.Yu., Microbial Diversity: New Possibilities of an Old Method, *Mikrobiologiya*, 1997, vol. 66, no. 1, pp. 107–113.
5. Golubev, V.I., i-Inositol Catabolism and the Taxonomic Significance of the Ability of Yeasts to Assimilate D-Glucuronate, *Mikrobiologiya*, 1989, vol. 58, no. 2, pp. 276–283.
6. Barnett, J.A., Payne, R.W., and Yarrow, D., *Yeasts: Characteristics and Identification*, Cambridge: Cambridge Univ. Press, 1990.
7. Golubev, W.I., Mycocins (Killer Toxins), *The Yeasts: A Taxonomic Study*, Kurtzman, C.P. and Fell, J.W., Eds., Amsterdam: Elsevier Sci., 1998, pp. 55–62.
8. Golubev, V.I., Okunev, O.N., and Vdovina, N.V., Assimilation of i-Inositol by Yeasts as a Diagnostic Feature, *Mikrobiologiya*, 1974, vol. 43, no. 6, pp. 1046–1050.
9. Golubev, V.I. and Blagodatskaya, V.M., On the Taxonomic Significance of the Ability of *Candida* Berkhout Yeasts to Assimilate D-glucuronic Acid, D-glucono-δ-Lactone, and 5-Keto-D-Gluconate, *Mikrobiologiya*, 1978, vol. 47, no. 5, pp. 841–848.