Differential Reduction of Tellurite by Growing Colonies of Normal Yeast and Respiration-Deficient Mutants

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ABSTRACT

NAGAI, SUSUMU (National Women's University, Nara, Japan). Differential reduction of tellurite by growing colonies of normal yeast and respiration-deficient mutants. *J. Bacteriol.* **90**:220-222, 1965.—A differential reduction of sodium tellurite was observed between normal and respiration-deficient mutant colonies of several species of *Saccharomyces*. Normal colonies turned black in contrast to mutant colonies which remained nearly white when grown on an agar medium containing 30 to 40 mg per liter of tellurite. Schopfer's medium enriched with yeast extract and a mixture of vitamins was most suitable to develop such black-and-white contrast. The difference was far less obvious when the asparagine of this medium was replaced by other nitrogen sources such as glutamate, peptone, or Casamino Acids. Addition of ammonium sulfate to the medium weakened and sometimes completely reversed the contrast. The usefulness of tellurite medium for diagnostic color differentiation of respiration deficiency was considered.

Tellurite is often reduced by living yeast to metallic tellurium which produces a dark color in the cells and colonies. The extent of the reduction is sometimes dependent on the cultural conditions and the physiological properties of the yeast. Normal (wild-type) yeasts and their respiration-deficient (RD) mutants often show characteristic differences in their reactions with various exogenous substances such as dyestuffs and indicators, as observed by Gause (1958), Ogur, St. John, and Nagai (1957), and Nagai and Nagai (1958). The reduction of tellurite by normal and RD mutant yeasts was tested to determine whether any differences could be observed. This report describes the reduction of tellurite by growing colonies and the darkening of such colonies on several media. The differences in the extent of darkening by normal and RD mutant colonies depended on the nutrient composition of the tellurite medium. Selenite has also been known to be similarly reduced by yeast, and was tested in a similar manner for comparison.

MATERIALS AND METHODS

The basal medium principally used was composed by modifying Schopfer's medium, which Schwartz (1959) used for studying the action of acridine orange on baker's yeast. Another composite medium, SP3, similar to that used for diagnosing RD mutants (Nagai, 1963), was also used for comparison. Table 1 shows the composition of

these media. A stock solution of sodium tellurite, Na_2TeO_3 (20 g per liter), and basal media were sterilized separately by steaming at 100 C for 50 and 75 min, respectively; these were mixed together after cooling to about 55 C. The tellurite media were dispensed into 9-cm petri dishes to make plates about 5 mm thick (1 liter for 30 plates).

Normal cultures of *Saccharomyces cerevisiae* (IFO 0044) and Fleischmann's yeast, *S. carlsbergensis* (IFO 0565), *S. chevalieri* (IFO 0210), and their RD mutants, were used to compare the darkening of colonies on these tellurite plates. The yeasts in diluted suspensions containing about equal numbers of normal and RD mutant cells were inoculated onto the surface of plates to form about 100 to 150 colonies per plate, and were allowed to grow at 30 C.

RESULTS

Tellurite is appreciably toxic to the yeast, and the colony growth was completely inhibited at 40 mg per liter with the original Schopfer's medium, at 80 to 90 mg per liter with its enriched modifications, and at 120 mg per liter with medium SP3. Hence, moderate concentrations, i.e., 30 to 40 mg per liter with modified Schopfer's medium and 50 to 60 mg per liter with medium SP3, were used to examine the reduction by growing colonies. Differential darkening of the colonies, if any, without growth inhibition was observed at these concentrations.

TABLE 1. *Composition of basal nutrient agar media**

Component	Schopfer's medium					SP3
	Original	Enriched modification				
		A	B	C	D	
Glucose	50.0	30.0	50.0	30.0	30.0	30.0
KH ₂ PO ₄	1.5	1.5	1.5	1.5	1.5	1.5
MgSO ₄ ·7H ₂ O	0.5	0.5	0.5	0.5	0.5	1.0
Asparagine	1.0	1.0	1.0	1.0	1.0	
Dehydrated yeast extract		1.5	1.5	1.5	1.5	1.5
(NH ₄) ₂ SO ₄			2.0		2.0	1.5
Peptone						1.5
Agar	15.0	15.0	15.0	15.0	15.0	15.0
Vitamin mixture†				2.0	2.0	

* Ingredients are expressed in grams per liter, except for vitamin mixture which is in milliliters per liter.

† "Panvitan liquid" (Takeda Yakuhin Kogyo Co., Osaka, Japan) containing (per ml): vitamin A (palmitate), 5,000 international units (IU); thiamin, 2 mg; riboflavin, 3 mg; pyridoxine, 2 mg; D-panthenol, 5 mg; nicotinic acid amide, 20 mg; ascorbic acid, 75 mg; vitamin D₂, 500 IU.

A clear-cut difference developed in 2 to 3 days on medium A; normal colonies appeared black in contrast to RD mutant colonies which remained nearly white (Fig. 1). The contrast was emphasized further (Fig. 2 and 3) by addition of a mixture of vitamins (medium C). A queer reversal of the contrast to white-and-black took place on medium B, which contains ammonium sulfate. The difference was scarce at first, because both normal and RD colonies appeared almost equally dark. After 1 week or more, however, normal colonies turned much lighter or nearly white, whereas RD colonies remained dark or turned nearly black (Fig. 4). This fact was further confirmed by replica-plating transfers from a tellurite-free master plate to the tellurite media A, B, and C. The reversing effect of ammonium sulfate was also observed on medium D, which consisted of medium C plus ammonium sulfate. The contrast was greatly weakened as compared with that on medium C.

Since medium C was found to lead to clear-cut differences between normal and RD colonies, its asparagine was substituted by some other nitrogen sources to determine whether asparagine is specifically affecting the differential reduction of

FIG. 1-6. Normal and RD mutant colonies grown on tellurite agar plates. (1) *Saccharomyces carlsbergensis* on medium A with 30 mg per liter of tellurite; (2) *S. chevalieri* on medium C with 30 mg per liter of tellurite; (3) *S. chevalieri* on medium C with 40 mg per liter of tellurite; (4) *Fleischmann's* yeast on medium B with 40 mg per liter of tellurite; (5) *S. chevalieri* on glutamate-substituted medium A with 40 mg per liter of tellurite; and (6) *S. cerevisiae* on SP3 medium with 50 mg per liter of tellurite. Normal colonies are dark in contrast to RD colonies which appear light, except in Fig. 4 where the contrast is reversed, and in Fig. 5 where the colonies are dark altogether.

tellurite. The difference was about equally as clear-cut with monosodium aspartate and alanine, but was vague with monosodium glutamate, peptone, and Casamino Acids. Similar substitutions (such as glutamate, Fig. 5) in medium A resulted in an over-all darkening of both normal and RD colonies.

Sodium formate (10^{-2} and 5×10^{-3} M) and methionine (5×10^{-3} M) were added to medium C in view of the finding of Falcone and Nickerson (1963) that these additions diminished the reduction of selenite by yeast. These additions rendered normal colonies lighter and RD colonies darker than those on the ordinary medium C. The contrast was consequently obscured.

SP3 medium, which is similar to that used in the author's diagnostic color plate technique (Nagai, 1963), also allowed appreciable color differences in *S. cerevisiae* (Fig. 6), but the differences were variable and rather poor in the other yeasts. Sabouraud and Henneberg's media supplemented with 30 to 60 mg per liter of tellurite made both normal and RD colonies black, and, therefore, the use of these media was discontinued. Sodium selenite (Na_2SeO_3 , 50 to 100 mg per liter) was also tried in place of tellurite in media A and C. The yeasts turned reddish-orange, and scarcely any difference was observed between normal and RD colonies.

DISCUSSION

The darkening of colonies on tellurite media is apparently brought about by the tellurite-reducing activity of the yeast, as pointed out by Nickerson (1954), who compared the normal (yeast form) and filamentous mutant cultures of *Candida albicans* on tellurite and selenite media. In the species of *Saccharomyces* investigated here, the differential reduction of tellurite occurring between normal and RD colonies is strikingly clear on certain types of the enriched Schopfer's media. The selenite-reducing system was later analyzed at the enzymatic level by Falcone and Nickerson (1963) and Nickerson and Falcone (1963). Selenite appeared to be bound to protein through vicinal thiol groups, and to be released therefrom as metallic selenium after accepting 4 electrons. Presuming that the tellurite-reducing system is similar to the selenite-reducing system, it is interesting to note that the activity of the tellurite-reducing system in RD mutant cells depends on the cultural conditions. Various metabolites in the nutrient media might affect the ease of electron flow to produce metallic tellurium. The mechanism by which the presence of ammonium sulfate reverses the black-and-white contrast is not yet clear. This may be due to the lowering of pH when ammonium sulfate is used

up. Such a lowering in pH was observed by the author (Nagai, 1964) in a medium which contained *p*-dimethylaminoazobenzene (butter yellow) as an effective indicator. A reversal, such as the one seen with medium D, is prevented somewhat by the presence of vitamins.

The differential reduction of tellurite occurring between normal and RD mutant colonies appears to provide another convenient procedure of diagnostic color differentiation for respiration deficiency in addition to various methods hitherto recommended. Comparing the tellurite plate with the mixed-dye plates recommended earlier (Nagai, 1963, 1965), the advantage of the former is that the preparation of diagnostic medium is simpler, and the development of contrast is faster. A drawback, however, is that an overall darkening appears with tellurite unless the nutrient composition of the medium is carefully adjusted.

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