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DETECTION OF RESPIRATION DEFICIENCY MUTANTS OF *SACCHAROMYCES CEREVISIAE* BY MBRT TEST

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ABSTRACT
Respiration deficiency mutants have been investigated extensively. Several methods have been reported. In our studies we applied methylene blue reduction test for detection of respiration deficiency mutants of *Saccharomyces cerevisiae* generated by exposure to ethidium bromide and ultra violet radiations. Petite colonies grown on glucose deprived glycerol media were subjected to MBRT test. All the petite colonies were showing MBRT test negative and wild type was MBRT positive. This method gives results within 4 to 5 hours thus provide a quick and simple technique for diagnosis of respiratory deficiency.

KEYWORDS: MBRT test, Respiration deficiency, *S. cerevisiae*,

INTRODUCTION

The respiration deficient (RD) mutant drew attention first by virtue of its small colony size of yeast when grown in limited sugar concentration, French designated it as 'petite colonie' (Alvarez *et al.*, 1957; Des Clark-Walker, 2007). The colony size was often considered indicative of respiratory deficiency. But small colony is not necessarily RD mutants so is unreliable (Nagai *et al.*, 1961). Several workers modified this colony size method by modifying the nutrient media, the normally used carbon source; sugar is omitted (Raut, 1954), or replaced by acetate, lactate (Ogur, 1956; Yanagishima, 1956), glycerol and succinate (Yanagishima, 1956; Sophie Brun *et al.*, 2005). The basis of these modifications is based on the fact that the carbon sources mentioned can be utilized only by oxidative degradation thus doesn't support the growth of RD mutants. The RD mutants can be identified by replica plating of suspected colonies on media with carbon sources mentioned, the yeast that fail to grow are RD mutants. But this multistep procedure is time consuming and require prolong incubation.

More precise diagnosis methods are based on differences in enzymatic characters. Spectroscopic examination shows the absence of the absorption bands of cytochromes *a* and *b* in the RD mutant. Cytochrome oxidase activity is assessed by Nadi test, the mutants with no cytochrome oxidase activity makes the Nadi test negative. It is extremely time consuming to apply these methods to each colony (Nagai *et al* 1961). The still most commonly used method employs 2,3,5-triphenyltetrazolium chloride (TTC) as the color indicator (Ongur 1957; Heslot *et al* 1970; Zong-wen Pang *et al* ., 2010 and Ilona Pfeiffer *et al.*, 2010). In this method melted soft agar containing TTC is poured onto the colonies grown on a normal nutrient agar, after incubation (1 to 3 hr.) normal colonies become red and RD remain white. But sometimes the RD colonies also appeared red tinted hence color differentiation between the two types is vague. Another problem is TTC itself produces the RD mutant (Laskowski, 1954; Yanagishima, 1956).

Another color differentiation employs methylene blue incorporation in nutrient media (Gause *et al.*, 1957). Referring this line, in this paper we have explained use of methylene blue reduction test (MBRT) to confirm respiration deficiency of *S. cerevisiae* mutants generated by exposure to UV light and ethidium bromide. MBRT is routinely done in dairy industry to assess quality of milk. During metabolic activities the respiring microorganisms consume the dissolved oxygen (DO) and lower O-R potential to level where methylene blue is reduced to luco compound (white colored). Microbes that lack components of RETC do not consume DO and methylene blue remain oxidized (Patel and Patel, 2000). This method gives results within few hours. To reduce this time further we added 1% glucose to milk as it is used rapidly compared to lactose in milk. Pasteurization done to resident microflora and aseptically the suspected colonies inoculated in milk + methylene blue. Mutants that do not reduce methylene blue are not respiring and those reduced indicate RETC is still active.

MATERIALS AND METHODS

Induction of petite (RD) mutation in *Saccharomyces cerevisiae*:

Present study, the commercially available active dried baker's yeast (*S. cerevisiae*) used as as test organism. The dried yeast was reconstituted in *S. cerevisiae* broth (Glucose 2%, Peptone 2%, Yeast extract 1%, Ammonium sulphate 1%, pH 5.5). Total viable count was determined by serial dilution method using *S. cerevisiae* agar (*S. cerevisiae* broth + 2% agar agar). The dilution giving good isolated colonies was selected and 0.1mL of it was spread on glucose deprived glycerol media plates (Glycerol 2%, Glucose 0.01%, Peptone 2%, Yeast extract 1%, Ammonium sulphate 1%, pH 5.5).

The plates were exposed to UV (254nm) radiations from 1 to 10 min with interval of 1min. For another set EtBr (1mg/mL) treatment was given to 1mL of selected dilution with 1min interval for 10 min. Treated samples were spread on glycerol media plates.

Identification of RD mutants by MBRT:

We used colony size method and leuco dye reduction ability to detect respiratory deficiency. After mutagen treatment the colonies showing small size (petite colony) compared to larger colony of wild type on glucose-glycerol media were subjected to methylene blue reduction test. For MBRT test milk was supplemented with 1% glucose and few drops of 1% aqueous methylene blue till milk becomes dark blue colored. The milk was distributed in test tubes in 5mL quantity cotton plugged and then pasteurized at 60^{o}C for 30 min. to reduce resident microflora that might give false positive test. When cooled to room temperature the loop-ful portion of petite colony was aseptically inoculated in methylene blue + milk and incubated at 30^{o}C for 5hours. In control tube wild type *S. cerevisiae* was inoculated. After incubation tubes were observed for color change. Additionally catalase test was done using 30% hydrogen peroxide.

RESULTS AND DISCUSSION

The results shown in the Table-1 and Figure-1. The dilution 10^{-5} was giving good isolated colonies (1250/mL), hence it was used for mutagenesis. Survivors of mutagen treatment showing characteristic petite (small) colony size were subjected for MBRT test. Even after 5 hrs of incubation the mutants did not show change in color whereas wild *S. cerevisiae* decolorized the methylene blue within 1 hour. All the mutants were also found to be catalase negative. All the petite colony forming mutants of *Saccharomyces cerevisiae* were found to be MBRT and catalase negative. This confirms the absence of respiration activity in the mutants and proves the utility of MBRT for quick detection of respiration deficiency. It requires less time, involves few steps and obviates the need of agar overlay as in TTC method and thus no chances of technical problems like mixing due to dislodging or chances of contamination. The ease of this method makes this technique more suitable for the said purpose.

Figure: 1

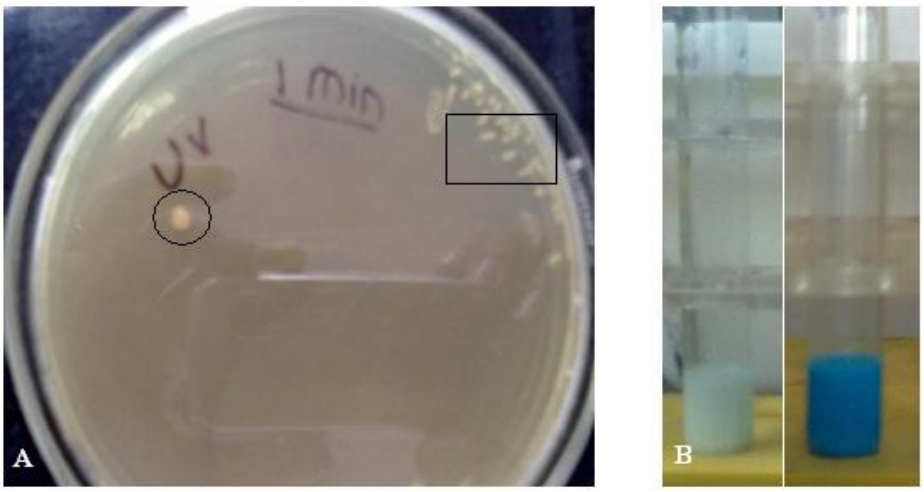

Figure-1. Petriplate showing colonies of wild type (in circle) and RD mutants (in square) of *S. cerevisiae*. (B) Results of MBRT: Wild type (left) reduced methylene blue and RD mutant (right) couldn't reduce methylene blue.

Table-1. Shows the strains of S. cerevisiae and the test results MBRTA, Catalase

Sr No	Strain of *S. cerevisiae*	MBRT test	Catalase test
1	Wild type	+Ve	+Ve
2	EtBr-8	-Ve	-Ve
3	EtBr-6	-Ve	-Ve
4	UV-1	-Ve	-Ve
5	UV-2	-Ve	-Ve
6	UV-3	-Ve	-Ve

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