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ORIGINAL ARTICLE

EFFECT OF BARIUM ON THE GROWTH OF *SACCHAROMYCES CEREVISIAE* L. (BAKER'S YEAST)

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Abstract: *The effect of Barium was observed on Saccharomyces cerevisiae L. (brewer's/ bakers' yeast) in laboratory condition. In culture media, the growth levels of Saccharomyces cerevisiae, which were supplemented with Barium chloride salt in different concentrations (1-10,000 ppm) were recorded for 36 hours at the intervals of one and half hours. A gradual enhancement of growth was recorded in the in vitro culture in nutrient medium. Hence the overall response was dose-dependent. The growth of Saccharomyces cerevisiae was totally inhibited at 50,000 ppm concentration of Barium chloride. Saccharomyces cerevisiae is a member of soil microflora, are part of food chain in overall ecosystem and comes to atmosphere too. As Barium is very common in agriculture and technology, its accumulation can affect the natural growth of yeast in soil ecosystem adversely*.

Key words: *Barium, Saccharomyces cerevisiae, effect on growth.*

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1. INTRODUCTION

Barium, a metal though not an essential element, is found in nature in ample amounts as $BaSO_4$ or barite and $BaCO_3$ or witherite [1- 2]. This element is also present in feldspars and micas. In soil, Barium concentration ranges from 19 - 2300 mg/Kg [3]. Barium is used in petroleum industry, while its isotopes and alloys are used in drilling and in fireworks, rubber, paint, ceramics, optical glass, adhesives, batteries, etc. It is also used in manufacturing of oil paints and as rodenticides [4 -6]. Unlike other heavy metals, there are not many reports on the effects of Barium on plants and microorganisms. Anaerobic sulphur bacteria can derive sulphur from Barites for its respiration [7].

The exposure of barium mainly occurs when one inhales or ingests [8] which possess a serious problem to human health [9]. It leads to breathing problems, irritation of stomach, numbness of face, rise in blood pressure, arrhythmia, vomiting, liver, kidney spleen and heart problems which left untreated may be fatal [10-12].

The main objective of the present study was to study the effect of $BaCl_2$ on Yeast, *Saccharomyces cerevisiae*, which is predominantly found in soil and air. Yeast is widely used in brewery and in baking industries.

2. MATERIALS AND METHODS

Preparation of culture medium as the source material

The stock culture of *Saccharomyces cerevisiae* L. (yeast) was received from the Department of Botany, University of Calcutta, which was maintained by regular subculturing at periodic interval.

Media preparation with the test chemical and culturing method

The composition of the culture medium for the growth of *Saccharomyces cerevisiae* was as following:

1. Bacto-peptone (Difco Laboratories, Michigan) -1 g
2. Yeast extract (E. Merck, India) - 1 g
3. Glucose - 2 g
4. Double distilled water (solvent)- 100 ml

All the above components of growth medium were dissolved in distilled water and the solution was heated and constantly stirred to dissolve all the ingredients, and pH was maintained at 5.8.

Barium belongs to Group IIA in the Periodic Table. The test chemical used in the experiment was Barium Chloride [$BaCl_2$, molecular weight. 208.3 (Glaxo Laboratories, India)]. It is a water soluble, white-coloured crystal. In the present study, effect of Barium chloride was observed on the cell division of yeast in culture medium,

Barium chloride (in mg) was dissolved in the sterilized growth medium of *Saccharomyces cerevisiae* in appropriate amount. The solution was prepared in nephro-culture flasks to get the desired concentrations, prior to inoculation. The different concentrations were 10,000 ppm,1000 ppm, 100 ppm, 10 ppm and 1 ppm of Barium chloride salt (Table 1).

Table 1: Culture media preparation for the growth of yeast cells (*Saccharomyces cerevisiae*) with Barium chloride salt at different concentrations.

Test System	Concentration of Barium Chloride ($BaCl_2$) Salt		Amount of salt ($BaCl_2$) (mg)	Final volume with Growth Medium of *Saccharomyces* after dilution (ml)
	Percentage (%)	Level in ppm		
Saccharomyces cerevisiae in culture medium	1.0	1×10^4	200	20
	0.1	1×10^3	20	20
	0.01	1×10^2	2.0	20
	0.001	1×10	0.2	20
	0.0001	1.0	0.02	20

Duration of treatment period = 36 hours.

The set up of the cultures were made in the nephro-culture flasks. In each of the nephro-culture flask,20 ml of media was poured. All the flasks were plugged with non-absorbent cotton and wrapped with aluminum foil, with brown paper over it. All the flasks containing culture media were autoclaved at 121.6□ under the pressure of 20 Ibs/ Square Inch for 20 minutes for sterilization.

Individual inoculation was done using disposable sterilized plastic syringe for all flasks containing culture media, with equal volume of *Saccharomyces cerevisiae* stock culture. Aseptic condition was maintained for the whole procedure. After inoculation, the flasks were plugged with cotton immediately and wrapped

with aluminum foil. The incubation was carried out at 37□ on a shaker. A blank set was also maintained against all the concentrations of Barium chloride and a control set too (Table 1).

Study of the growth of *Saccharomyces cerevisiae*

The optical density values for all the level of concentrations of the treatment set, were recorded with spectrophotometer along with the control and blank as reference. The recording was done for 36 hours at an interval one and half hours, from the time of inoculation. Three-times repetition was made for the whole experiment to minimize the human error. The net optical density for all the levels of concentration (after subtraction from the reading of relevant blank set) were plotted in the Y-axis against time (in X-axis), to depict the effect of Barium on the growth of yeast cells (*Saccharomyces cerevisiae)*.

3. RESULTS AND DISCUSSION

When the *Saccharomyces cerevisiae* cultures were exposed to the abiotic stress of $BaCl_2$ at *in vitro* condition (at a concentration range of 1×10^4 ppm to 1 ppm), there was an increase of growth in all concentrations for the first 36 hours, when compared to that of the control set. The growth was found to be at maximum level for 100 ppm at 24 hours, followed by 1000 ppm and10 ppm respectively. At 1 ppm also growth was maximum after 24 hours but it was less, compared to the above three higher concentrations. However, growth was least at 10,000 ppm, whereas 50,000 ppm was found to be lethal for growth (Table 2, Figure 1). Medium supplemented with 100 ppm, Ba^{2+} appeared to be stimulatory producing optimum enhancement in growth. The order of growth promotion was 100 ppm>1000 ppm>10 ppm> 1 ppm> Control (Figure 2). At 24 hours interval 1000 ppm and 10 ppm Ba^{2+} stimulated the growth to the same level as evident from optical density (O. D.) values.

Table 2. The level of growth (optical density) for *Saccharomyces cerevisiae* growing culture media after Barium chloride treatment at different concentrations.

	Time in hours																							
Concentration	0	1.5	3	4.5	6	7.5	9	10.5	12	13.5	15	16.5	18	19.5	21	22.5	24	25.5	27	28.5	30	31.5	33	34.5
Control	0	5	10	13	15	21	22	23	25	26	27	28	30	32	34	36	36	36	24	12	12	12	8.5	8
10,000 ppm	0	8	20	22	23	31	32	40	45	50	52	60	85	105	110	143	144	144	120	106	105	104	104	104
1000 ppm	2	6	22	49	80	100	110	110	120	140	180	210	240	252	258	262	263	263	254	198	145	139	130	128
100 ppm	6	5	21	35	41	55	80	120	140	200	220	260	280	300	332	346	350	350	299	108	106	105	106	106
10 ppm	0	5	5	25	26	52	75	100	110	130	140	200	220	250	254	260	263	263	106	107	103	90	84	84
1 ppm	5	7	15	40	84	95	97	99	100	120	140	150	170	180	192	195	195	195	191	84	72	72	74	74

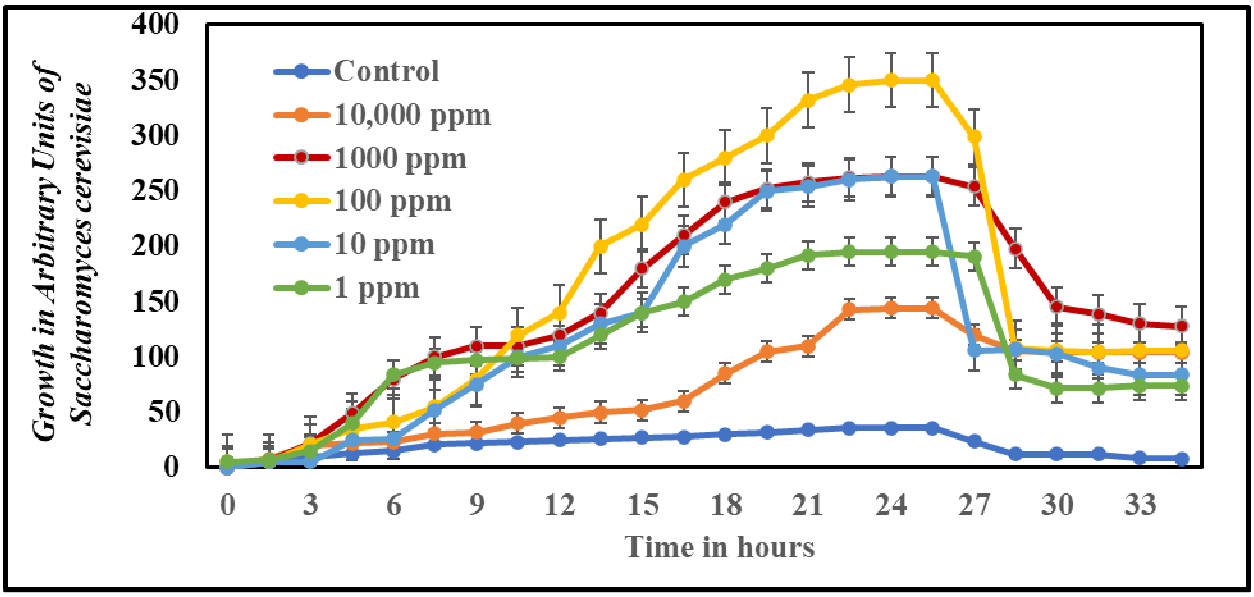

Figure 1. Effect of barium chloride on growth of *Saccharomyces cerevisiae* (bars indicate standard deviation)

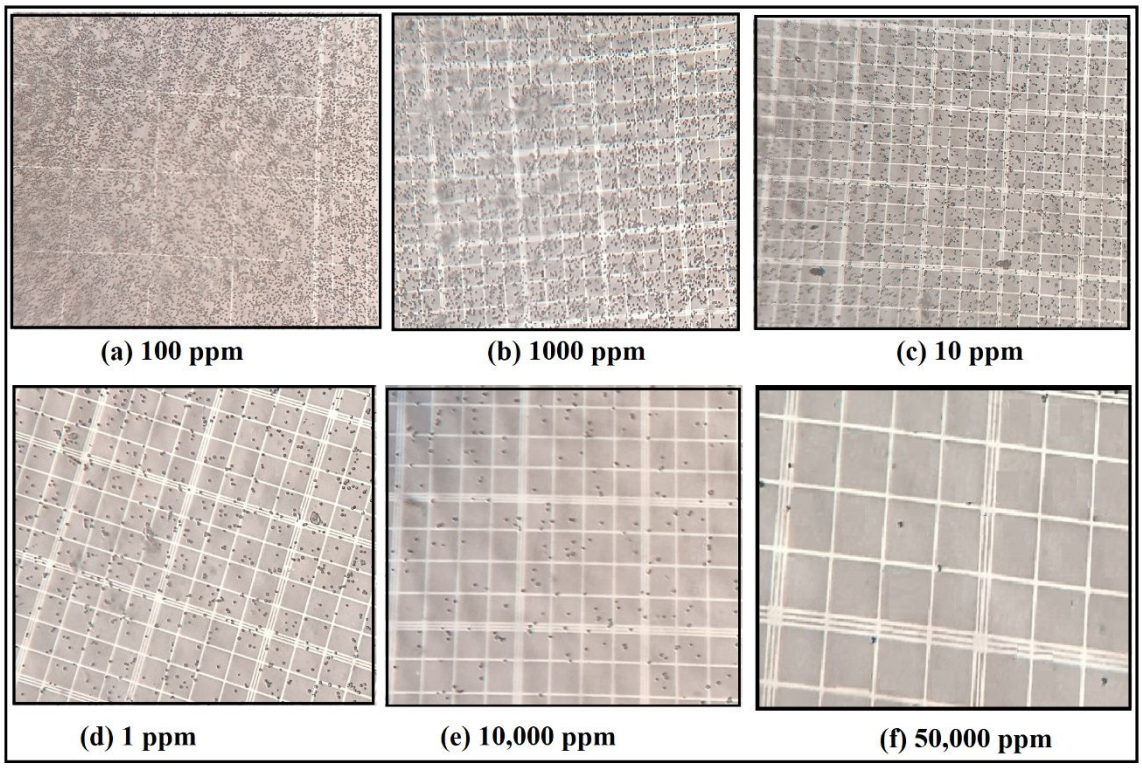

Figure 2 (a-f). Growth of yeast cells in nutrient media, treated with different concentrations (1-50,000 ppm) of Barium chloride, as observed with haemocytometer under compound light microscope.

A similar effect was noticed in case of seed germination of *Cucumis sativus* exhibiting 500 µM and 2000 µM of Ba stimulating germination [13]. In another report, 2 ppm Ba^{2+} increased wort fermentability and

ethanol production to 26.03% and 7.62% (v/v) respectively. Ethanol production and wort fermentability was reduced at 4ppm Ba^{2+} [14]. Addition of Ba^{2+} in wort medium initially increases the pH at all concentrations, followed by a decrease after 96 hours, which increases the acidity of the medium [15]. The effect of stress on Yeast depends on the genetic make-up of yeast strains, the physiological state of yeast, nature of fermenting medium and presence of other microbes in the medium which competes with yeast.

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