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Evaluation of *Saccharomyces cerevisiae* (yeast) growth response to arsenic stress

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Abstract

The research was performed at Botany Department, Nusrat Jahan College Rabwah, Pakistan to determine the growth responses of yeast on exposure to arsenic stress. Yeast strain was obtained from National Agricultural Research Centre (NARC), Pakistan. Few drops of different concentrations of sodium arsenite (1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 mg/L) were applied to yeast grown on Cysteine Lactose Electrolyte Deficient (CLED) agar media to determine the zone of inhibition through disk, well and spread method. Stress applied yeast petri plates were incubated for 24 hours at 37°C. After incubation, zones of inhibition were measured. Our study has shown that the yeast is not resistant to arsenic stress, with increasing values of arsenic, yeast growth has declined. This reduction of yeast growth due to arsenic stress can pose severe problems for the soil and overall plant growth. Hence strong competent measures are required to overcome the arsenic issue.

Key words: Yeast, arsenic, CLED, well method, spread method, disk method

Full length article **Corresponding Author*, e-mail: maleeha.umber@njc.edu.pk

1. Introduction

Many noxious contaminants are present in our environment especially in soil, where plants grow on which humans and animals are primarily dependent. These contaminates are adversely affecting our food chain and causing various diseases and disorders in living organisms. In a study, researchers came to know that rice grains contained the high amount of arsenic and it constitute the most customary way of arsenic contamination via food chain. On the other hand, it has been seen in various studies that some microorganisms present in soil become resistant to these contaminants by developing mechanisms to cope up the harmful effects of these contaminants [1]. One of the major soil contaminants includes arsenic. Arsenic is significantly pernicious heavy metal at old wood infusing plants because it has relatively higher solubility than other metals. It is poisonous to soil microorganisms in higher amounts because it is capable of interfacing with radicals [2].

It was investigated that arsenic contamination diminishes the growth, physiological activities and especially metabolic miscellany in many crops. Soil microorganisms under certain conditions of arsenic concentrations and compounds, can combat the poisonous effects of these contaminants. Therefore, it is crucial to study the soil microorganisms' resistance activities in order to eliminate the factor of noxious effect of these contaminants [3]. The sources of arsenic in environment includes arsenic mines and smelters, industries using arsenic, arsenic litter from geothermal electric power stations and fungicides containing arsenic [4].

Considering the current situation of the day by day increasing arsenic contamination in soils due to multiple types of anthropogenic activities, this study was designed to analyze the growth activity of *Saccharomyces cerevisiae* (Yeast) under sodium arsenite stress of varying concentrations to determine the yeast resistant to sodium arsenite. As soil is the most important growth media for the plants, it is worth noting that the soil microbes which promote plant growth symbiotically are resistant to arsenic poisoning (otherwise, it will ultimately cause detrimental effects on plant growth). *Saccharomyces cerevisiae* is a unicellular fungus that perpetuates by budding. It occurs in colonies and morphological conditions of different strains depend on the reactions by environmental stimuli [5]. The cells of yeast vary in diameter i.e., 3-4 µm. Yeast shows ultimate growth at temperature varying from 20-28°C and at pH varying from 3.5-4.

Different medium are used for isolating yeast includes potato dextrose agar and yeast mould agar etc. Yeast cells have potential to ferment carbohydrates into carbon dioxide and ethanol. It has significantly economic importance as it is used in brewing and baking industries. It is also proved to be beneficial by providing vitamins and proteins. It has also vital role in bioremediation of most of the industrial wastes [6]. The current planned study is therefore aimed to check the growth response of yeast under arsenic stress.

2. Materials and methods

The current study was done at Botany Department, Nusrat Jahan College Rabwah, Pakistan to determine the growth responses of yeast on exposure to arsenic stress. Carefully sterilized apparatus has been used throughout the present study. Three different procedures were used to study the effects of arsenic including spread, well and disc methods. Yeast strain was obtained from NARC, Pakistan. Few drops of different concentrations of sodium arsenite (1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 mg/L) were applied to yeast grown on CLED media.

2.1 CLED media preparation

CLED medium was prepared by weighing the 36 g of solid CLED agar and mixing it with 1000 ml of distilled water. The contents of solution were dissolved by heating with continuous shaking with a sterilized glass stirrer and boiled for 1 minute (for proper cessation). After cooling to 50°C, the contents were poured into sterilized disposable labeled petri plates. Arsenic stress was applied after solidification except in spread method in which arsenic stress is given before media solidification. The colour of media was green.

2.2 Spread method

In this method, the arsenic stress of varying concentrations was given before the inoculation of yeast on solidified media. For this purpose, the drops of varying concentrations of sodium arsenite in equal amounts are mixed well in liquid media with the help of sterilized toothpicks. Afterwards, the yeast was spread on solidified media with the help of cotton buds. Then these stress applied yeast petri plates were incubated for 24 hours at 37°C. Control group petri-plates were without arsenic stress.

2.3 Well method

In this method, the wells were made with the help of sterilized cork borer in solidified media. Yeast was spread in same manner as in spread method and then arsenic stress of varying concentrations of sodium arsenite was given in well with the help of droper. Then, these stress applied yeast petri plates were incubated for 24 hours at 37°C. In control group, no wells of arsenic were bored.

2.4 DISC method

In this method discs of filter paper (Whatman filter paper) of equal diameter were made with the help of page puncture. Then these discs were placed in solutions of sodium arsenite of varying concentrations for a specific time to let the concentration of sodium arsenite be infused into discs. Meanwhile the yeast was spread on solidified media and afterwards the discs of varying concentrations were placed centrally in the petri plates with the help of forcep in order to give arsenic stress while control group was without arsenic infused discs. Then, these stress applied yeast petri plates were incubated for 24 hours at 37°C.

2.5 Measurement of zones of inhibition

After 24 hours, zones of inhibition of different diameter or sizes were seen in well diffusion method and disc diffusion method. These inhibitory zones were measured using measuring scales.

3. Results and discussions

According to the results presented in figure 1, yeast growth is retarded along with an increase in sodium arsenite concentration. Results of well diffusion method depict that if sodium arsenite is present in liquid form in higher concentrations in soil, it will diffuse in surrounding regions of soil affecting the yeast growth.

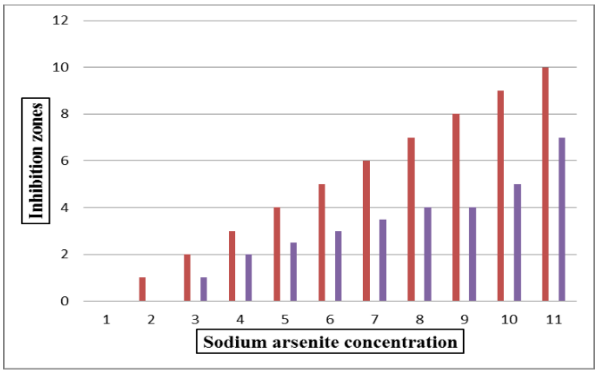


Figure 1: Representation of an increase in inhibition zones of yeast growth with increasing sodium arsenite concentrations through well diffusion method

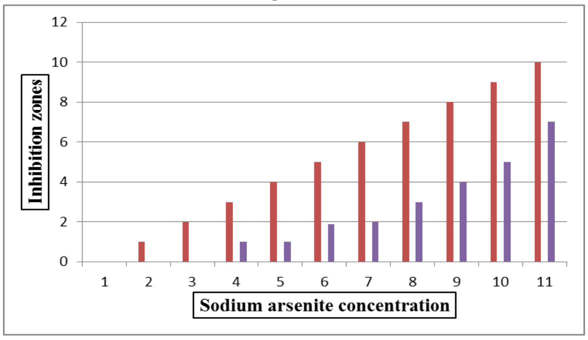
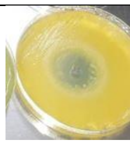
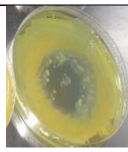
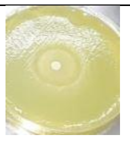
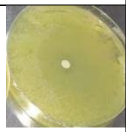
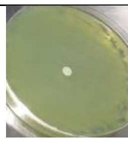


Figure 2: Representation of an increase in inhibition zones of yeast growth with increasing sodium arsenite concentrations through disk diffusion method

Yeast survives and resists the sodium arsenite stress upto 3 mg/L, after this level yeast growth was inhibited as sodium arsenite concentration increases (Figure 2). In accordance to the results presented in figure 2, it can be predicted that the sodium arsenite in solid form in soil could harm the microbes of its nearby region more pronouncedly.

In spread method, yeast growth retardation begun at very low concentration (1 mg/L) as all the levels of sodium arsenite stress were equally mixed in CLED media in respective petri plates and this growth retarding activity enhanced with increase in sodium arsenite stress levels (Table 1).

Table 1: Images of inhibition zones of yeast growth in different methods in response to different levels of sodium arsenite stress

Method	1 mg/L	2 mg/L	3 mg/L	4 mg/L	5 mg/L	6 mg/L	7 mg/L	8 mg/L	9 mg/L	10 mg/L
Well Diffusion										
Disc Diffusion										
Spread Diffusion										

The result of the present study clearly demonstrates that the yeast cannot tolerate higher levels of sodium arsenite contamination in soil. According to the results of well diffusion method, yeast growth got declined as arsenite contamination increased above 3 mg/L. Similarly, in case of disk diffusion method, yeast growth retarded above 4 g/L contamination level of sodium arsenite. Spread method showed more prominent results as compared to the both disk and well diffusion methods. In this method, yeast inhibition was recorded from even the lowest level of contamination (1mg/L). All studied methods show that diffusion of sodium arsenite in dissolved form is much more in dissolved form as compared to the solid form (as solid form takes more time to disperse and to diffuse into soil or surrounding water). Our results are in strong agreement with the previous studies which shows that arsenic pollution severally harms the plant survival [3, 6-15]. The results of the present study show that plants are affected by arsenic pollution in two ways: firstly, directly affecting plants and secondly, indirectly affecting the plants by harming the soil microbial lives which are symbiotically beneficial for plant growth. The results of the present study clearly highlights that arsenic consumption in agricultural fields should be strictly banned to save plants and soil micro flora and fauna.

4. Conclusions

Following important conclusions can be withdrawn from the present study:

- Arsenic strongly inhibited the yeast growth.
- In dissolved form, arsenic is more toxic to yeast.
- Arsenic contamination of agricultural field is a serious issue which needed to be overcome.

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