International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

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 growth responses of yeast on exposure to arsenic stress. Yeast strain was taken from NARC Pakistan. Few drops of different concentrations of sodium arsenide (1mg/L, 2mg/L, 3mg/L, 4mg/L, 5mg/L, 6mg/L, 7mg/L, 8mg/L, 9mg/L and 10mg/L) were applied to yeast grown on CLED media to determine 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 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 soil and over all plant growth. Hence strong competent measures are required to overcome arsenic issue.

Key words: Yeast, Arsenic, CLED, Well Method, Spread Method and 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 effect 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 day by day increasing arsenic contamination in soils due to multiple types of anthropogenic activities, this study was designed to analyze growth activity of *Saccharomyces cerevisiae* (Yeast) under sodium arsenide stress of varying concentrations also to determine if yeast is resistant to this arsenic compound or not based on its growth. As soil is the most important growth media of plants, it is worth noting that soil microbes which promote plant growth symbiotically are resistant to arsenic poisoning or not because in otherwise case 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 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 media 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]. Current study planned is therefore aimed to check the growth response of yeast under arsenic stress.

2. Materials and Methods

Current study was done at Botany department, Nusrat Jahan College Rabwah Pakistan to determine growth responses of yeast on exposure to arsenic stress. For all purpose sterilized apparatus has been used. Three methods were used i.e., spread, well and disc method. Yeast strain was taken from NARC Pakistan. Few drops of different concentrations of sodium arsenide (1mg/L, 2mg/L, 3mg/L, 4mg/L, 5mg/L, 6mg/L, 7mg/L, 8mg/L, 9mg/L and 10mg/L) were applied to yeast grown on CLED media.

2.1 CLED Media Preparation

CLED medium was prepared by weighing 36 g of solid CLED agar on weighing machine, mixed it in 1000 ml distilled water. Dissolved it by heating with continuous shaking with a sterilized glass stirrer and boiled it for 1 minute for proper cessation. Cooled at 50°C and poured it in sterilized disposable labeled petri plates. Waited for them to solidify 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 inoculation of yeast on solidified media. For this purpose, the drops of varying concentration of sodium arsenide 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 arsenide was given in well with 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 help of page puncture. Then these discs were placed in solutions of sodium arsenide of varying concentrations for a specific time to let the concentration of sodium arsenide be infused into discs. Meanwhile 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 sizes were seen in well diffusion method and disc diffusion method. These inhibitory zones were measured using measuring scales respectively.

3. Results

3.1 Well Diffusion Method

According to 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 effecting yeast growth.

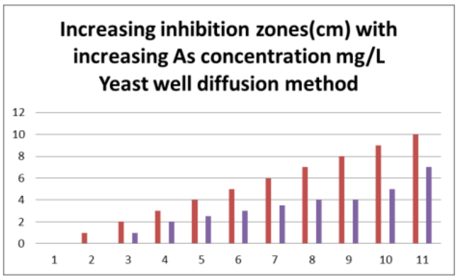


Figure 1: Represents an increase in inhibition zones of yeast growth with increasing sodium arsenite concentrations through well diffusion method

3.2 Disk Diffusion Method

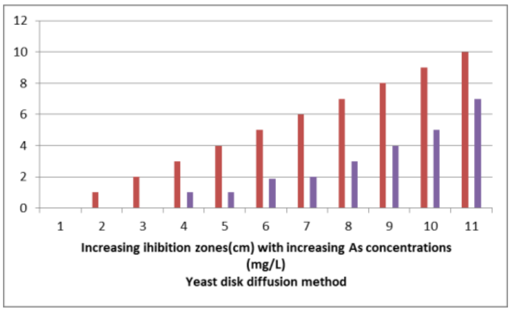


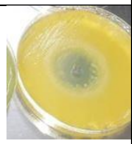
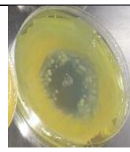
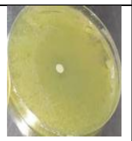
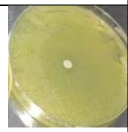
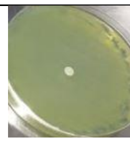
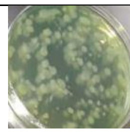
Figure 2: Represents an increase in inhibition zones of yeast growth with increasing sodium arsenite concentrations through disk diffusion method

Yeast survives and resists sodium arsenite stress upto 3mg/L, beyond this level of stress yeast growth is inhibited as sodium arsenite concentration increases (Figure 2). In accordance to results of figure 2 it can be considered that if sodium arsenite is present in solid form in soil it will harm microbes of its nearby region more pronouncedly as compared to farther regions.

3.3 Spread Method

In spread method as all levels of sodium arsenite stress were equally mixed in CLED media in respective petri plates yeast growth retardation begins at very low concentration (1 mg/L) and this growth retarding activity enhances with increasing 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										

4. Discussions

This present study has shown that yeast cannot tolerate higher levels of sodium arsenite contamination in soil. According to results of well diffusion method, yeast growth is getting declined as arsenic contamination increases from 3mg/L and as this concentration keeps on increasing yeast growth keeps retarding. Similarly in case of disk diffusion method, yeast growth starts retarding from 4mg/L contamination level of sodium arsenite. As arsenic level will increase yeast growth inhibition rate will also keep on increasing. Spread method showed more intense results as compared to both disk and well diffusion method. As in this case yeast started inhibiting its growth from very lowest level of contamination (1mg/L) and as contamination increased, enlarged growth inhibited patches were seen. All these above mentioned methods can be correlated to natural soil environment in such a way if sodium arsenite is present in water dissolved form it will diffuse more rapidly and affect more prominently as compared to sodium arsenite present in solid form because solid form will take more time to disperse and mix with soil or surrounding water. While if sodium arsenite is in liquid form and is equally distributed in all regions of soil it will be more detrimental. Our results are in strong accordance to the work of (3) whose work suggested that arsenic pollutions harms plant survival. In view of results of our study it can be said that plants are affected by arsenic pollution in two ways, first way is direct effect on plants second is indirect effect on plants. Indirect effect is by harming soil microbial lives which are symbiotically beneficial for plants. Hence this research paper directs attention to all who are concerned to devise such agricultural methods that can overcome arsenic contamination preventing plants and soil micro flora and fauna from harms of this heavy metal.

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