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Original Research Article

Assessment of acid phosphatase enzyme and influence of potassium iodide on its production in the yeast form of *Sporothrix schenckii*

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ABSTRACT

Background: Sporotrichosis is caused by a dimorphic fungal species, *Sporothrix schenckii* (*S. schenckii*). The enzyme acid phosphatase is pervasive among yeast and yeast like fungi. It has been studied in various fungi like *Aspergillus oryzae*, *Candida albicans* etc. but in *S. schenckii* little is known about enzyme acid phosphatase. The present study depicts the in-vitro influence of Potassium Iodide (KI) on the enzyme acid phosphatase produced by the *S. schenckii* (yeast form).
Methods: A master culture was prepared by incorporating the standard strain of *S. schenckii* in YNB (Yeast Nitrogen Base) medium and was incubated at 37ºC. After preparing the increasing concentrations with KI in YNB medium, 1.0 mL suspension of master culture was inoculated into each bottle and incubated at 37ºC for different time period 6th, 12th, 18th day (early, mid, peak of log period) respectively. After centrifuging, a 5% homogenate was prepared, which was used for acid phosphatase enzyme assay.
Results: The mean acid phosphatase level of control specimen was 20.9±2.01, 50.0±2.25, 45.0±5.10 µg and test specimens was ranged from 14.9±4.89 to 20.2±3.49, 10.2±4.19 to 40.0±6.39 and 10.0±1.81 to 34.7±6.08 µg on day 6, 12 and 18 respectively. The mean value was lower significantly for all the test concentrations as compared to control ($p<0.05$).
Conclusions: The low activity of the enzyme acid phosphatase indicates that KI has inhibitory effect on the growth of *S. schenckii* that has led to decrease in the activity of the enzyme.

Keywords: Acid phosphatase, Potassium iodide, *S. schenckii*, Yeast

INTRODUCTION

Sporotrichosis (mainly cutaneous and lymphocutaneous sporotrichosis) caused by *Sporothrix schenckii* (*S. schenckii*) when it penetrates through the broken skin and differentiates into the yeast form within the infected tissue.[1,2] A number of drugs and other therapies have been recommended for the treatment of sporotrichosis. These include Potassium Iodide (KI), itraconazole, fluconazole, terbinafine, amphotericin B and thermotherapy.[1] KI has been traditionally used in the treatment of sporotrichosis since the early 20th century, with satisfactory results. However, the exact mechanism of action remains unknown.[1,2] The immunological mechanisms involved in prevention and control of *S. schenckii* infections are still not very well understood. However, they probably include both humoral and cellular responses that appear to be triggered by distinct

antigens.[3-5] There is paucity of studies that show the effect of KI on the enzymes of *S. schenckii*. This study is done on the acid phosphatase, one of the enzymes present in the fungus *S. schenckii*. Little is known about pathogenic potential of *S. schenckii* on mammalian host due to paucity of enzymatic information.[6] The enzyme acid phosphatase is ubiquitous among yeast and yeast like fungi.[7] Acid phosphatase has been studied in various fungi. In *Saccharomyces rouxii* and *S. cerevisiae* they were established in exclusive locale in the periplasmic space. In other fungi, acid phosphatase seems to occur at the cell surface.

Acid phosphatase is present in *Aspergillus oryzae*, A. terrus and in blastospores of zoopathogenic *Candida albicans*.[8] So far, the scrutiny of literature goes, there has been no study that shows the influence of KI on this enzyme. It may be helpful to know the one of the mechanisms involved in prevention and control of *S. schenckii* infections. Therefore, it was planned to undertake the present study to determine the acid phosphatase in the yeast form of *S. schenckii* along with the effect of KI on the production of this enzyme in-vitro.

METHODS

This experimental study was conducted in the Department of Microbiology in a tertiary care hospital during the period of February 2015 to January 2016. Institutional Ethical Committee had already approved this study; however, no human and animal subjects were involved in the present study. The standard strain of *S. schenckii* was included in this study. Isolated strains from clinical specimens were not included. A standard strain of *S. schenckii* (ATCC 14284 / MTCC 1359) was procured from Institute of Microbial Technology, Chandigarh, India. A master culture was prepared by doing the subculture of *S. schenckii* from slope of Sabouraud's dextrose agar (SDA) in 50 mL of YNB (Yeast nitrogen base, HiMedia, Mumbai) medium that was incorporated in a screw-capped bottle and was incubated at 37ºC. On day seven, growth of *S. schenckii* in the bottle was adjusted to 90% transmission at 540 nm by adding the appropriate amount of YNB medium as described by Bareja et al.[2] Master culture thus prepared was used for subsequent analysis.

YNB medium was prepared and dispensed in 50 mL aliquots into 150 screw capped (160 mL capacity) bottles. KI was added into the YNB medium in increasing concentrations in such a way so as to have final concentrations of 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4 and 12.8 gram% of the medium. One bottle of YNB without KI was served as a control.

Out of 150 bottles, 50 bottles each were used for three different days, i.e. 6th day (early-log period), 12th day (mid-log period), 18th day (peak of growth). Out of 50 bottles, 5 bottles were used as control (without KI) and rest were used for various concentration of KI. To reduce the error 5 bottles were used for each concentration of KI. One mL suspension of master culture was inoculated into each bottle and incubated at 37ºC for different time period respectively. The content of respective bottle was centrifuged at 6000 rpm for half an hour on the 6th, 12th, 18th day respectively. The deposit thus obtained was suspended in 5.0 mL citrate buffer saline (0.15 mol/L Sodium chloride, 0.015 mol/L Sodium citrate, pH 7) and was centrifuged again at 6000 rpm for half an hour. The process was repeated twice to ensure proper washing. The deposit thus obtained was taken, dried in the folds of filter paper, weighed and was crushed finely in a tissue homogenizer. A 5% homogenate was prepared from each weighed tissue in ice-cold distilled water and used for subsequent enzyme assay.

The enzyme acid phosphatase was determined by the method of King and Jegatheesam.[9] Set up four new test tubes (15 mL), marked as 'T', 'S', 'C' and 'B' for test, standard, control and blank respectively. Dispensed 1.0 mL of citrate buffer and 1.0 mL of substrate in two tubes, T and C. Placed them in a water bath at 37°C for 3 minutes.

Dispensed 0.1 mL of the homogenate in T and incubated both the tubes at 37°C for 1 hour. Dispensed 1.0 mL of 0.5 N-sodium hydroxide in both tubes to stop the reaction and mixed well. In the tube C dispensed 0.1 mL of the homogenate. In the tube S, mixed together 1.1 mL of buffer, 1.0 mL diluted phenol standard and 1.0 mL of 0.5 N-sodium hydroxide. In the tube B, mixed together 1.1 mL of buffer, 1.0 mL of water and 1.0 mL of 0.5 N-sodium hydroxide. To all the tubes dispensed 1.0 mL of 0.5 N-sodium bicarbonate, 1.0 mL of amino-antipyrine solution and 1.0 mL of potassium ferricyanide solution. Mixed well after each addition. The reddish brown colour thus produced was measured against the blank at 520 nm. The amount of phenol present in the standard tube was 10 µg. Thus, the phenol in the test was calculated in µg per mg wt. of homogenate by following formula:

$$Total\ Acid\ Phosphatase = \frac{T - C}{S - B} \times 10 \times \frac{Vol.of\ homogenate}{0.1ml} \times \frac{1}{Wt.of\ tissue}$$

Statistical analysis

The statistical analysis was done using SPSS (Statistical Package for Social Sciences) Version 15.0 statistical Analysis Software. ANOVA (Analysis of Variance) test was used to compare the within group and between group variances amongst the study groups. Dunnett's "t"-Test was used for comparing each experimental mean with the control mean.

RESULTS

The enzyme acid phosphatase was determined for three different days, 6th day (early-log period), 12th day (mid-

log period), 18th day (peak of growth) respectively. On day 6, with increasing concentration a declining trend of acid phosphatase was seen. At blank the mean value was 20.90 µg which reached at 15.06 µg at KI 0.2 gram% and finally dropped down to reach 14.92 µg at KI 0.8 gram% concentration (Table 1). On day 12, a zigzag pattern of change in acid phosphatase level was observed with starting value of 49.98 µg at blank showing a decline at KI 0.05 and 0.1 gram% concentration and an incline (40.02µg) followed by decline to reach at 10.15 µg at KI 0.8 gram% concentration (Table 2). At 18th day too, a zigzag pattern of change in acid phosphatase level was observed with starting value of 44.96µg which reached to 10.00µg at KI 0.8 gram% concentration showed numerous inclining and declining trends (Table 3).On day 6, mean acid phosphatase level of control specimen was 20.9±2.01 µg. The mean acid phosphatase levels of test specimens were ranged from 14.9±4.89 (KI 0.8 gram%) to 20.2±3.49 µg (KI 0.05 gram%). There was no statistically significant difference in mean acid phosphatase levels as compared to control was seen for all the concentrations (Table 4).

Table 1: Expression of enzyme acid phosphatase in *S. schenckii* (yeast) on 6th day.

Conc. of KI (gram%)	I	II	III	IV	V	Mean(µg)
Control/ Blank	22.18	23.46	20.52	18.2	20.14	20.90
0.05	17.34	21.54	16.38	20.4	25.12	20.15
0.1	23.1	18.46	15.76	22.82	20.14	20.05
0.2	15.2	19.54	13.24	11.26	16.1	15.06
0.4	12.3	19.68	9.42	19.1	14.78	15.05
0.8	18.42	10.2	9.64	20.62	15.72	14.92

Table 2: Expression of enzyme acid phosphatase in *S. schenckii* (yeast) on 12th day.

Conc. of KI (gram %)	I	II	III	IV	V	Mean (µg)
Control/ Blank	53.4	48.26	50.14	47.68	50.46	49.98
0.05	24.36	25.74	29.1	21.2	25.14	25.10
0.1	18.24	25.46	26.68	30.1	29.3	25.95
0.2	39.28	32.18	35.78	47.2	45.68	40.02
0.4	33.82	30.12	21.48	26.2	38.42	30.00
0.8	10.46	8.32	7.1	7.6	17.28	10.15

Table 3: Expression of enzyme acid phosphatase in *S. schenckii* (yeast) on 18th day.

Conc. of KI(gram %)	I	II	III	IV	V	Mean(µg)
Control/ Blank	48.64	37.32	43.28	50.36	45.2	44.96
0.05	17.28	20.62	19.46	18.5	24.5	20.07
0.1	27.14	36.54	30.12	21.86	34.24	29.98
0.2	28.24	37.12	35.46	29.48	43.2	34.70
0.4	33.46	17.5	20.36	28.16	25.46	24.98
0.8	12.22	7.46	9.26	11.1	10	10.00

Table 4: Expression of mean acid phosphatase in *S. schenckii* (yeast) at different time intervals (n=5 for each concentration).

Conc. of KI(gram %)	Day 6			Day 12			Day 18		
	Mean(µg)	SD	p	Mean(µg)	SD	p	Mean(µg)	SD	p
Control/Blank	20.9	2.01		50.0	2.25		45.0	5.10	
0.05	20.2	3.49	0.997	25.1	2.84	<0.001	20.1	2.76	<0.001
0.1	20.1	3.08	0.995	26.0	4.71	<0.001	30.0	5.81	<0.001
0.2	15.1	3.12	0.068	40.0	6.39	0.013	34.7	6.08	0.014
0.4	15.1	4.39	0.068	30.0	6.57	<0.001	25.0	6.32	<0.001
0.8	14.9	4.89	0.060	10.2	4.19	<0.001	10.0	1.81	<0.001

Significance of difference as compared to control (Dunnett's t-test has been used)

Table 5: Comparison of change in mean acid phosphatase levels in *S. schenckii* (yeast) at different concentrations.

Conc. of KI (gram %)	Day 6 to Day 12			Day 6 to Day 18			Day 12 to Day 18		
	Mean change (µg)	SD	p	Mean change (µg)	SD	p	Mean change (µg)	SD	p
Control/Blank	29.09	2.49	<0.001	24.06	6.67	0.001	-5.03	4.95	0.085
0.05	4.95	5.17	0.099	-0.08	1.89	0.926	-5.04	3.54	0.034
0.1	5.90	6.22	0.101	9.92	8.01	0.050	4.02	7.50	0.297
0.2	24.96	8.66	0.003	19.63	5.29	0.001	-5.32	9.01	0.257
0.4	14.95	7.23	0.010	9.93	8.29	0.055	-5.02	7.18	0.193
0.8	-4.77	5.74	0.137	-4.91	3.49	0.035	-0.14	4.29	0.944

Paired 't'-test used.

On day 12, mean acid phosphatase level of control specimen was 50.0±2.25 µg. For test specimen mean levels were ranged from 10.2±4.19 (KI 0.8 gram%) to 40.0± 6.39 µg (KI 0.2 gram%). For all the concentrations, the difference was statistically significant ($p<0.05$) as compared to control (Table 4). On day 18, mean acid phosphatase level of control specimen was 45.0±5.10 µg. For test specimens, mean values were ranged from 10.0±1.81 (KI 0.8 gram%) to 34.7±6.08 µg (KI 0.2 gram%). The mean value was lower significantly for all the test concentrations as compared to control ($p<0.05$) (Table 4). By comparing all the three days, in general there was an increase in the activity of enzyme with increase in duration of incubation 6th to 18th day (Table 5). No deposit obtained at the concentration KI 1.6, 3.2, 6.4 and 12.8 gram%.

DISCUSSION

The enzyme acid phosphatase is ubiquitous among yeast and yeast like fungi.[7] Acid phosphatases have been reported to occur in fungi, such as, Aspergillus, Fusarium, Penicillium and Neurospora.[10-13] Garrison and Arnold observed maximal activity of 3 units/g at pH 4.0 in yeast phase of *S. schenckii*.[14] Arnold et al, also studied acid phosphatases of *S. schenckii*.[15] They studied the activity of acid phosphatase in yeast phases of *S. schenckii* in gel electrophoresis. Neither they mentioned the different phases of growth i.e. lag, log, and stationary phase nor studied the effect of KI with varied concentration on it.

In the present study, the expression of acid phosphatase and the effect of KI with increasing concentration on yeast form of *S. schenckii* were studied. The activity of enzyme acid phosphatase was estimated on 6th, 12th, and 18th day of incubation for yeast form respectively that included various phases of growth i.e. early log, mid log and exponential phase. On day 6, mean acid phosphatase level of control specimen was 20.9±2.01 µg. The acid phosphatase levels of test specimens were ranged from 14.9±4.89 (KI 0.8 gram%) to 20.2±3.49 µg (KI 0.05 gram%). Statistically, no significant difference in mean acid phosphatase levels as compared to control was observed for all the concentrations (Table 4). On day 12, mean acid phosphatase level of control specimen was 50.0±2.25 µg. For test specimen mean levels were ranged from 10.2±4.19 (KI 0.8 gram%) to 40.0±6.39 µg (KI 0.2 gram%). For all the concentrations difference from control was significant statistically ($p<0.05$). On day 18, mean acid phosphatase level of control specimen was 45.0±5.10µg. For test specimens, mean values were ranged from 10.0±1.81 (KI 0.8 gram%) to 34.7±6.08 µg (KI 0.2 gram%). For all the test concentrations, mean value was significantly lower as compared to that of control ($p<0.05$) (Table 4).

By comparing all the three days, in general there was increase in the mean acid phosphatase level with increase in duration of incubation 6th to 12th day in control and test concentrations followed by a decrease in control as well as test concentrations except KI 0.1 on day 14 (Table 4,5). Between day 6 to 12, control group showed a mean change of 29.09±2.49 µg, statistically, this change was significant. Among different test groups, the extent of change ranged from -4.77±5.74 (KI 0.8 gram%) to 24.96±8.66 µg (KI 0.2 gram%). Among test groups, statistically, this change was significant for concentrations of KI 0.2 and KI 0.4 gram% only. Between day 6 to 18, control group showed a mean change of 24.06±6.67 µg, statistically, this change was significant. Among different test groups, the extent of change was ranged from -0.08±1.89 (KI 0.05 gram%) to 19.63±5.29 µg (KI 0.2 gram%). Among test groups, statistically, this change was significant for concentrations of KI 0.1, 0.2 and 0.8 gram% only (Table 5). Between day 12 to 18, control group showed a mean change of -5.03±4.95 µg, statistically, this change was not significant. Among different test groups, the extent of change was ranged from -5.32±9.01 (KI 0.2 gram%) to 4.02±7.50 µg (KI 0.1 gram%). Among test groups, statistically, this change was not significant for any of the concentrations (Table 5).

There was sharp incline in the mean acid phosphatase level of control specimen between day 6 and day 12 i.e. mid log phase, but after that a gradual decline was observed i.e. exponential phase. The same observations were observed for increasing concentrations of KI also

except KI 0.1 gram%. From concentration of KI 0.2 gram% and onward, there was an incline in the activity of mean acid phosphatase upto day 12 that was followed by a decline in the enzyme activity (Table 4,5 and Figure 1).

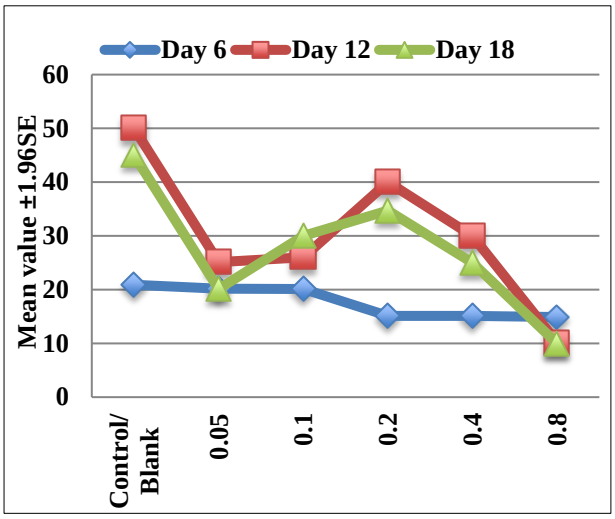

Figure 1: Expression of mean acid phosphatase in *S. schenckii* (yeast) at different time intervals.

CONCLUSION

In this study, an increase in mean acid phosphatase levels of control as well as all test concentrations was observed but at all these concentrations mean value was lower as compared to control. It indicates that KI has inhibitory effect on the growth of *S. schenckii* and this has led to decrease in activity of enzyme acid phosphatase. This effect along with other defense mechanisms of the body may be the mode of action of KI in the treatment of sporotrichosis.

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