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Expression of enzyme acid phosphatase and effect of potassium iodide on its production in mycelial form of *Sporothrix schenckii*

Rajesh Bareja
Associate Professor, Department of Microbiology, Government Medical College, Badaun, Uttar Pradesh, India

Abstract
Context: Sporotrichosis is a varied disease caused by a single dimorphic fungal species, *Sporothrix schenckii* (*S. schenckii*).
Aims: This study describes the *in-vitro* effect of potassium iodide (KI) on the enzyme acid phosphatase produced by the filamentous form of *S. schenckii*.
Methods and Material: A master culture of *S. schenckii* was prepared in YNB (Yeast nitrogen base) medium and was incubated at 25ºC (mould). KI was added into the YNB medium in increasing concentrations. One mL suspension of master culture was inoculated into each bottle and incubated at 25ºC for different time period, 4th day (early-log period), 9th day (mid-log period), 14th day (peak of growth) respectively. After centrifuging, a 5% homogenate was prepared that was used for acid phosphatase enzyme assay.
Results: The mean acid phosphatase level of control specimen was 25.0 ± 4.53, 39.9 ± 4.07 and 84.1 ± 5.15 µg on day 4, 9 and 14 respectively. The mean acid phosphatase level of test specimens was ranged from 14.0 ± 2.44 (KI 6.4) to 25.0 ± 3.08 (KI 0.05), 10.5 ± 1.61 (KI 6.4) to 44.0 ± 4.44 (KI 0.05) and 8.1 ± 1.62 (KI 6.4) to 80.0 ± 4.68 µg (KI 0.05) on day 4, 9 and 14 respectively.
Conclusions: At the entire test concentrations mean value was lower as compared to control. The low activity of the enzyme acid phosphatase indicates that KI has inhibitory effect on the growth of *S. schenckii* (mould) and has led to decrease in the activity of the enzyme.

Keywords: Acid phosphatase; mould; potassium iodide; *S. schenckii*

Introduction
Sporothrix schenckii (*S. schenckii*), a dimorphic fungus, is distributed worldwide and known as the etiological agent of sporotrichosis. Little is known about pathogenic potential of *S. schenckii* on mammalian host due to paucity of enzymatic information [1]. The enzyme acid phosphatase is ubiquitous among yeast and yeast like fungi [2]. 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* [3]. Potassium iodide (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 [4]. Therefore, it was planned to undertake the present study to estimate the acid phosphatase in the mycelial form of *S. schenckii* and to study the effect of KI on the production of this enzyme *in-vitro*.

Materials and methods
This experimental study was conducted in the department of Microbiology in a tertiary care hospital. 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 in a screw-capped bottle and was incubated at 25ºC. On the seventh day the suspension of YNB medium with *S. schenckii* was adjusted to 90% transmission at 540 nm on the photo-colorimeter as described by Bareja *et al*. [4] 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. Potassium iodide 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 percent 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. 4th day (early-log period), 9th day (mid-log period), 14th 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 25ºC for different time period respectively. The content of respective bottle was centrifuged at 6000 rpm for half an hour on the 4th, 9th, 14th day. 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 was used for subsequent enzyme

assay. The enzyme acid phosphatase was determined by the method of King and Jegatheesam. [5] The phenol in the test was calculated in µg per mg wt. of homogenate by following formula:

Calculation

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

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, 4th day (early-log period), 9th day (mid-log period), 14th day (peak of growth) respectively. On day 4, at blank the mean value was 25.01 µg, which reached at 20.08 µg at 0.4 gram% concentration and finally dropped down to reach to 14.00 µg at 6.4 concentration (Table 1). On day 9, at blank the mean value was 39.91 µg that reached at 23.98 µg at 0.4 gram% concentration and finally dropped down to reach 10.51 µg at 6.4 gram% concentration (Table 2). On day 14, at blank the mean value was 84.10 µg that reached at 55.72 µg at 0.4 gram% concentration and finally dropped down to reach 8.07 µg at 6.4 gram% concentration (Table 3).

On day 4, the mean acid phosphatase level of control specimen was 25.0 ± 4.53 µg. The acid phosphatase level of test specimens was ranged from 14.0 ± 2.44 (KI 6.4) to 25.0 ± 3.08 µg (KI 0.05) [Table 4]. On day 9, the mean acid phosphatase level of control specimen was 39.9 ± 4.07 µg. Among different test concentration mean acid phosphatase levels were ranged from 10.5 ± 1.61 (KI 6.4) to 44.0 ± 4.44 µg (KI 0.05) (Table 4). On day 14, the mean acid phosphatase level of control specimen was 84.1 ± 5.15 µg. For test specimens, mean values were ranged from 8.1 ± 1.62 (KI 6.4) to 80.0 ± 4.68 µg (KI 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 4th to 14th day (Table 5). No deposit obtained at the concentration KI 12.8 gram%.

Table 1: Expression of acid phosphatase in *S. schenckii* (mould) on 4th day

Conc. of KI (gram %)	I	II	III	IV	V	Mean (µg)
Control/ Blank	25.2	26.78	31.42	22	19.66	25.01
0.05	21.82	24.16	26.52	29.6	23.1	25.04
0.1	20.24	17.8	22.68	24.22	15.22	20.03
0.2	22.72	24.3	26.1	20.2	16.78	22.02
0.4	20.52	24.48	21.72	18.32	15.4	20.08
0.8	18.5	14.56	18.42	21.34	18.28	18.22
1.6	20.76	16.28	21.1	22.32	19.68	20.02
3.2	18.24	22.76	19.5	17.32	14.2	18.40
6.4	14.24	16.1	14.66	9.82	15.2	14.00

Table 2: Expression of acid phosphatase in *S. schenckii* (mould) on 9th day

Conc. of KI (gram %)	I	II	III	IV	V	Mean (µg)
Control/ Blank	40.32	37.5	45.24	41.9	34.62	39.91
0.05	44.36	40.24	48.34	38.86	48.4	44.04
0.1	40.62	44.24	32.5	47.2	35.62	40.03
0.2	35.72	37.24	31.42	41.24	29.1	34.94
0.4	24.34	19.56	27.32	18.6	30.1	23.98
0.8	30.62	32.32	28.76	35.24	23.14	30.01
1.6	35.46	39.32	31.36	42.12	30.22	35.69
3.2	20.42	23.56	16.12	18.2	21.46	19.95
6.4	10.48	8.2	10	11.4	12.5	10.51

Table 3: Expression of acid phosphatase in *S. schenckii* (mould) on 14th day

Conc. of KI (gram %)	I	II	III	IV	V	Mean (µg)
Control/ Blank	85.24	80.54	77.36	87.24	90.16	84.10
0.05	80.4	73.62	78.48	81	86.6	80.02
0.1	72.8	76.32	78.3	67.48	65.24	72.02
0.2	65.4	63.24	75.1	62.86	59.36	65.19
0.4	56.4	59.86	51.24	50	61.12	55.72
0.8	42.46	44.26	50.16	48.46	35.56	44.18
1.6	33.4	28.56	24.12	33.8	30.22	30.02
3.2	9.12	14.24	12.5	12.46	10.6	11.78
6.4	10.34	7.24	8.16	6	8.62	8.07

Table 4: Observations of mean acid phosphatase in *S. schenckii* (mould) on different time intervals (n=5 for each concentration)

Conc. of KI (gram %)	Day 4			Day 9			Day 14		
	Mean (µg)	SD	"p"	Mean (µg)	SD	"p"	Mean (µg)	SD	"p"
Control	25.0	4.53		39.9	4.07		84.1	5.15	
0.05	25.0	3.08	1.000	44.0	4.44	0.572	80.0	4.68	0.630
0.1	20.0	3.63	0.112	40.0	6.03	1.000	72.0	5.59	0.002
0.2	22.0	3.64	0.582	34.9	4.80	0.373	65.2	5.95	<0.001
0.4	20.1	3.44	0.119	24.0	4.93	<0.001	55.7	4.99	<0.001
0.8	18.2	2.41	0.014	30.0	4.52	0.008	44.2	5.73	<0.001
1.6	20.0	2.30	0.112	35.7	5.08	0.548	30.0	3.96	<0.001
3.2	18.4	3.12	0.018	20.0	2.88	<0.001	11.8	1.97	<0.001
6.4	14.0	2.44	<0.001	10.5	1.61	<0.001	8.1	1.62	<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* (mould) at different concentrations

Conc. of KI (gram %)	Day 4 to Day 9			Day 4 to Day 14			Day 9 to Day 14		
	Mean Change (µg)	SD	"p"	Mean Change (µg)	SD	"p"	Mean Change (µg)	SD	"p"
Control/ Blank	14.90	3.30	0.001	59.10	9.62	<0.001	44.19	8.33	<0.001
0.05	19.00	6.39	0.003	54.98	5.87	<0.001	35.98	4.58	<0.001
0.1	20.00	6.21	0.002	52.00	5.84	<0.001	31.99	9.13	0.001
0.2	12.92	5.57	0.007	43.17	3.63	<0.001	30.25	8.26	0.001
0.4	3.90	7.25	0.296	35.64	6.22	<0.001	31.74	5.81	<0.001
0.8	11.80	4.75	0.005	25.96	5.66	0.001	14.16	4.08	0.001
1.6	15.67	5.65	0.003	9.99	3.98	0.005	-5.68	4.49	0.047
3.2	1.55	3.82	0.416	-6.62	2.36	0.003	-8.17	3.35	0.006
6.4	-3.49	3.44	0.086	-5.93	2.12	0.003	-2.44	2.16	0.065

Paired 't'-test used

Discussion

Acid phosphatases have been reported to occur in fungi, such as, *Aspergillus*, *Fusarium*, *Penicillium* and *Neurospora* [6-9]. Garrison and Arnold observed maximal activity of 9-units/g dry weight at pH 4.2 in mycelial phase of *S. schenckii* [10]. Arnold *et al*. also studied acid phosphatases of *S. schenckii*. They studied the activity of acid phosphatase in mycelial phase of *S. schenckii* in gel electrophoresis [11]. 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 mycelial form of *S. schenckii* were studied. The activity of enzyme acid phosphatase was estimated on 4th, 9th and 14th day of incubation for mycelial form respectively that included various phases of growth, early log, mid log and exponential phase. On day 4, mean acid phosphatase level of control specimen was 25.0 ± 4.53 µg. However there was continuous decrease in mean acid phosphatase but a statistically, significant difference in mean acid phosphatase levels as compared to control was observed only at KI 0.8, KI 3.2 and KI 6.4 gram% respectively ($p<0.05$). At all these concentrations mean value was lower as compared to control (Table 4). On day 9, mean acid phosphatase level of control specimen was 39.9 ± 4.07 µg. A significant difference between test groups as compared to control group was observed at concentrations KI 0.4, 0.8, 3.2 and 6.4 gram% respectively. At all these concentrations, mean phosphatase levels of test groups were lower as compared to controls (Table 4). On day 14, mean acid phosphatase level of control specimen was 84.1 ± 5.15 µg. Mean value of test specimens showed a continuous and significant decline as compared control for all the concentrations except KI 0.05 gram% (Table 4). Grover and Thakur studied the activity of enzyme acid phosphatase in *S. schenckii* in mycelial phase. They concluded that there was no adverse effect of various concentration of KI on the production of enzyme acid phosphatase in early log phase and mid log phase. However, in the exponential phase of the growth, there was decrease in the enzyme production with concentration of 0.8% and 3.2% of KI [12]. In the present study, all the three phases of *S. schenckii*, mean value of test specimens showed a continuous and significant decline in the activity of enzyme acid phosphatase as compared to control (Fig 1).

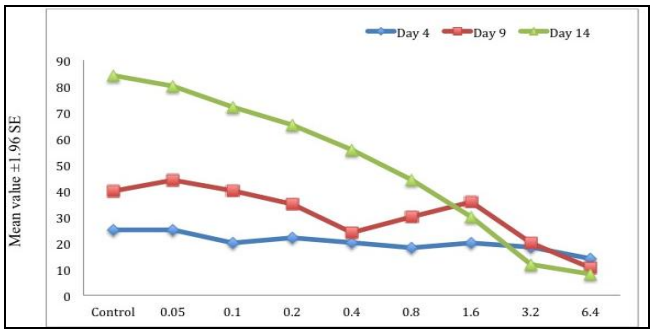

Fig 1: Expression of mean acid phosphatase in *S. schenckii* (mould) on different time intervals

By comparing all the three days, in general there was an increase in the activity of enzyme with increase in duration of incubation 4th to 14th day. By doing the comparison between day 4 and day 9, an increase in mean acid phosphatase levels of control as well as all test concentrations except KI 6.4gram% was observed. This change was significant statistically too for control as well as all the test concentrations except KI 0.4, KI 3.2 and KI 6.4 gram%. For KI 6.4 gram% a decline in mean value was observed but it was not significant statistically ($p=0.086$) (Table 5). When compared day 4 and 14, a significant increase in mean acid phosphatase levels ($p<0.05$) was observed for control as well as all the test concentrations except KI 3.2 and 6.4 gram%. For KI 3.2 and 6.4 gram%, a significant decline in acid phosphatase levels was observed ($p=0.003$) (Table 5). Between day 9 and day 14, a

significant increase in mean acid phosphatase levels was observed for controls and all the test groups upto KI 0.8 gram% ($p<0.05$). From concentrations KI 1.6 to 6.4 gram%, the change was negative but was significant statistically only for KI 1.6 and 3.2 gram% only. For KI 6.4 gram%, though the change was negative yet it was not significant statistically ($p=0.065$) (Table 5).

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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