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Biochemical Characterization of Crude Enzyme Obtained from *Penicillium citrinum* NCIM-1398

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Abstract—*Biochemical characterization of endo β-1,4-glucanase activity present in crude enzyme obtained from Penicillium citrinum NCIM-1398 was carried out in the present work. Optimization of temperature stability, pH stability, effect of metal ions and standing time for endo β-1,4-glucanase activity of crude enzyme produced by P. citrinum NCIM-1398 were studied. 55°C was found as optimum temperature, relative enzyme activity was found maximum at pH 5.5 after which enzyme activity decreased gradually. Endo β-1,4-glucanase activity was found to enhance due to the presence of Cu^{++}, Ca^{++} in 1mm concentration and Ni^{++}, Na^{+} with 10mm concentration whereas Hg^{++} revealed inhibitory effect in both concentrations. The effect of different standing time on endo β-1,4-glucanase activity was also studied. The effect of temperature and its effect on the stability of endo β-1,4-glucanase activity was studied. With increasing standing time, enzyme activity decreased and optimum standing time was found as 6 h at 55°C temperature and at initial pH 5.5.*

1. INTRODUCTION

Enzymes are biocatalysts and cornerstone of metabolism. They are produced by living cell to bring about specific bio-chemical reactions. Enzymes are very specific in their action on the substrate. All enzymes are proteinaceous in nature (except ribozymes, called as catalytic rRNA) and may or may not contain a non-protein prosthetic group. Over recent years, the demand of industrial enzymes has increased rapidly because enzymes degrade biomass into simple and nontoxic components. Thus, to meet the increasing requirement of enzymes, mostly new industrial enzymes are produced from microbial origin such as fungal or bacterial source.

Fungi are cultivated using agro-industrial waste products in large-scale fermenters. Public, regulatory and private industrial policies also favour the use of enzymes as substitutes for traditional methods [1].

Enzyme stability is a crucial factor to determine whether application of biocatalysis will be commercially successful. Catalytic proteins loose part of their activity when they are subjected to the action of heat, extreme pH, presence of metal ions in the enzyme vicinity. During the last decades, much research has focused on the improvement of enzymes behavior in the conditions in which they were to be used, and especially on the increase of their thermal stability. The production of heat-resistant enzymes would allow carrying out enzymatic reactions at higher temperatures, and therefore, increasing conversion rates and substrates solubility and reducing the risk of microbial growth and the viscosity of the reaction medium [2]. Heavy metal ions strongly are bound by sulfhydryl groups of proteins [3]. Sulfhydryl binding changes the structure and enzymatic activities of proteins and causes toxic effects evident at the whole organism level [4]. Heavy metal ions like Cd, Cu, Hg, Zn, and Pb in sufficiently high concentrations might kill organisms or cause other adverse effects that change aquatic community structures [5].

Keeping this in view Biochemical characterization of endo β-1,4-glucanase activity present in crude enzyme obtained from *Penicillium citrinum* NCIM-1398 was carried out in the present work. Optimization of temperature stability, pH stability, effect of metal ions and standing time for endo β-1,4-glucanase activity of crude enzyme produced by *P. citrinum* NCIM-1398 were studied.

2. MATERIALS AND METHODS

2.1 Optimization of temperature stability for endo β-1,4-glucanase activity produced by *P. citrinum* NCIM-1398.

Thermo- stability of enzyme endo β-1,4-glucanase activity and xylanase activity was studied at different temperature ranging from 40°C to 90°C with an interval of 5°C for incubation period of 30 min, at initial pH of 5.5±0.2.

2.2 Optimization of pH stability for endo β-1,4-glucanase activity and xylanase activity produced by *P. citrinum* NCIM-1398

Enzyme activity was studied to find out the optimum pH and pH stability of crud endo β-1,4glucanase and xylanase obtained from *P. citrinum* NCIM-1398. Enzymes were

incubated at different pH ranging from pH 4-11 using different buffer viz., citate buffer (pH 3-6) potassium-phosphate buffer (pH 6.0-7.4), sodium phosphate buffer (pH 7.0-8.0), and borate buffer (pH 9-11) keeping other conditions constant as mentioned in section 2.1.

2.3 Effect of metal ions on endo β-1,4-glucanase activity of *P. citrinum* NCIM-1398

The effect of different cations (Na^{+}, Ca^{++}, Cd^{++}, Co^{++}, Cu^{++}, Fe^{++}, Hg^{++}, Mn^{++}, Mg^{++}, Ni^{++}, Pb^{++}, Zn^{++}) were carried out. Enzymes were incubated in the presence of cations in two different concentrations (1mM and 10mM) of each cation. Cations were mixed with the enzymes at room temperature and kept for one hour keeping other conditions constant as mentioned in the section 2.1. Effect of each cations on endo β-1,4-glucanase activity obtained from *P. citrinum NCIM*-1398 were studied.

2.4 Standing time of endo β-1,4-glucanase activity of *P. citrinum* NCIM-1398.

Efferent of different standing time on endo β-1,4-glucanase activity obtained from *P. citrinum* NCIM-1398 were studied. Enzymes were incubated at different standing time (half an h,1 h, 2 h, 4 h, 6 h,12 h, 24 h) while other parameters were remained constant as mentioned in section 2.1.

2.5. Statistical analysis

All experiments were carried out in triplicate and experimental results were presented as a mean of ± standard deviation of three identical values.

3. RESULTS AND DISCUSSION

3.1 Optimization of temperature stability of endo β-1,4-glucanase activity produced by *P. citrinum* NCIM-1398.

Table1 showed the effect of temperature and its effect on the stability of endo β-1,4-glucanase *P. citrinum* NCIM-1398. The study shows that enzyme activity increased linearly up to temperature 55°C after that the activity of enzymes decreased. From the above finding it may be concluded that 55°C was the optimum temperature for both the enzymes obtained from *P. citrinum* NCIM1398.

Tables 1: Temperature stability of endo β-1,4-glucanase and activity produced by *P. citrinum* NCIM-1398.

Temperature, °C	*P. citrinum* NCIM-1398
	Endo β-1,4-glucanase
40	75.16
45	91.82
50	100.00
55	**103.51**
60	100.94
65	89.67
70	88.58
75	73.16
80	41.09
85	33.28
90	27.10

Assay Conditions:

Incubation time, Min	=30
Temperature, °C	=varied
Initial pH	=5.5±0.1
Substrate con.	=2% (w/v) carboxymethyl cellulose (CMC) in 50 Mm citrate buffer

Thermostability is an intrinsic property of enzyme and determined by its primary structural protein. Generally, thermostable enzyme exhibits enhanced conformational rigidity [6]. The enhanced intrinsic stability of thermostable enzymes is the cumulative effect of hydrogen bonds, ion -pair interaction and hydrophobic interaction [7]. Cardoso *et al.* 2003 [8] and Carmona *et al.*, 2005 [9] also reported similar results for *Acrophialophora nainiana* and *Aspergillus versicolor* respectively that both these organisms exhibits maximum xylanse activity at 55°C.

3.2 Optimisation of pH stability of endo β-1,4-glucanase and xylanase activities produced by *P. citrinum* NCIM-1398.

It was observed in Table 2 that relative enzyme activity was maximum at pH 5.5 after which enzyme activity decreased gradually for endo β-1,4-glucanase obtained from *P. citrinum* NCIM-1398 respectively, maximum activity of endo β-1,4-glucanase was observed at pH 6 after which it declined.

Tables 2: pH stability of endo β-,4-glucanase and xylanase activities produced by *P. citrinum* NCIM-1398.

pH	*P. citrinum* NCIM-1398
	Endo β-1,4-glucanase
4.0	81.57
4.5	82.04
5.0	100.00
5.5	**101.50**
6.0	103.18
6.5	74.84
7.0	74.56
7.5	50.51
8.0	47.24
8.5	35.55
9.0	32.09
9.5	30.87
10.0	27.60
10.5	22.92
11.0	19.93

Assay Conditions:

Incubation time, Min	=30
Temperature, (^{o}C)	=55±2.0
Initial pH	=2% (w/v)
Substrate con.	=varied
	=carboxymethyl cellulose in 50 Mm citrate buffer

Hence, it may be concluded that pH 5.5 was optimum for both the enzymes obtained from *P. citrinum* NCIM-1398. At the high acidic and alkaline pH change in enzyme activity may be due to changes in secondary or tertiary structure of cellulase and destruction of active site as well [10]. The ionic characteristics of important acidic and basic functional groups in the active site of enzyme which are essential for the catalytic activities of the endoglucanase is affected by change in pH [11]. Juwon and Emmanuel (2012) [12] reported the affinity of cellulase for substrates is affected by the change in pH especially when the active site has been altered leading to a decreased affinity for the substrate. This may be responsible either for the decline in either side of the pH optima or the stability of enzyme itself. This leads to considerable de-naturation and subsequent inactivation of enzyme. Similar results were reported for *Schizophyllum commune* that xylanase activity was stable at a pH range of 4.0–7.0 with optimum activity at pH 5.5 [13].

3.3 Effect of metal ions on endo β-1,4-glucanase activity produced by *P. citrinum* NCIM-1398.

It was also observed that the activity of endo β-1,4-glucanase obtained from *P. citrinum* NCIM-1398 was enhanced by $Cu^{++,}$ Ca^{++} with 1mm concentration and

Tables 3: Effect of metal ions on endo β-1,4-glucanase activity of *P. citrinum* NCIM-1398.

Metal ions	Relative endo β-1,4-glucanase activity, %	
	1 mM	10 mM
Na^{+}	101.82	118.41
Ca^{++}	115.96	100.19
Cd^{++}	111.88	101.01
Co^{++}	111.34	78.71
Cu^{++}	**129.01**	**67.83**
Fe^{++}	98.83	133.36
Hg^{++}	95.29	50.71
Mn^{++}	64.84	76.54
Mg^{++}	89.59	108.62
Ni^{++}	**44.72**	**269.03**
Pb^{++}	96.11	85.78
Zn^{++}	107.80	93.66

Assay conditions:

Incubation time, min	=30
Temperature, ^{o}C	=55±2.0
Initial pH	=5.5±0.1
Substrate con.	=2% (w/v) CMC in 50 Mm citrate buffer

Ni^{++}, Na^{+} with 10mm concentration (Table 3). Hg^{++} has inhibitory effect on endo β-1,4-glucanase obtained from both the organisms. The inhibitory effect of Hg^{++} ion might be related to its binding with thiol groups, tryptophan residue, or the carboxyl group of amino acid residues in the enzyme [14]. This inhibitory or inducing effect of metal ions may be due to conformational change in the active site within the enzyme. Gautam *et al.* 2018 [15] reported similar finding for *S. commune* ARC-11 that K^{+}, Na^{+}, and Zn^{++} at 10 mM concentration enhance xylanase activity, whereas both concentration of 1 and 10 mM of Hg^{++} strongly inhibited the xylanase activity.

3.4 Effect of standing time on endo β-1,4-glucanase activity produced by *P. citrinum* NCIM-1398

Table 4 shows the effect of different standing time on endo β-1,4-glucanase activity produced by *P. citrinum* NCIM-1398 at 55^{o}C temperature and at initial pH 5.5. It was observed that with increasing standing time, enzyme activity decreased and optimum temperature for endo β-1,4-glucanase activity produced by *P. citrinum* NCIM-1398 was 6 h.

Tables 4: Effect of standing time on endo β-1,4-glucanase activity obtained from *P. citrinum* NCIM-1398.

Time (Hrs.)	Relative endo β-1,4-glucanase activity, %
0.5	100.00
1	96.82
2	91.41
4	82.95
6	**73.86**
12	60.97
24	43.25

Assay conditions:

Incubation time, Min	=varied
Temperature, ^{o}C	=55±2.0
Substrate con.	=2% (w/v) CMC in 50 Mm citrate buffer
Initial pH	=5.5±0.1

Similar results have been reported for *S. commune* ARC-11 by Gautam *et al*. 2018 [15] that enzyme remains stable for longer time 180 min at a temperature of 55^{o}C.

4. CONCLUSION

Enzyme used in the present study was found to be stable in wide range of temperature and pH. Metal ions such as Cu^{++}, Ca^{++} in 1mm concentration and Ni^{++}, Na^{+} with 10mm concentration was found to enhance endo β-1,4-glucanase activity whereas Hg^{++} revealed inhibitory effect in both concentrations. Relative Endo β-1,4-glucanase activity was found around 73% after 6 hours of standing time. Thus, the attributes of enzyme make it suitable for different industrial applications.

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