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Production and Optimization of Amylase Enzyme from *Saccharomyces cerevisiae* by Mangrove Environ

N. Jayalakshmi[1], G. Umamaheswari[2]

P.G & Research Department of Biotechnology, Marudupandiyar College, Vallam, Thanjavur

Abstract: ***In the current investigation studied that the totally 13 yeast colonies were isolated and identified for screening of enzymes production from marine soil yeast. Some of the excellent yeast such as Aureobasidium nulluns, Candida elaebora, C. sake, C. zeylanoides, Cryptococcus hansenii, C.victoriae, Dioszella crocera, Dioszella aurantiace, Leucosporidilla fragaria, L.auscorum, Rhodotorula glacialis, R. larvngis and Saccharomyces cerevisiae were confirmed and determined for the enzyme of amylase, protease and pectinase with specific medium. Among the yeast, the Saccharomyces cerevesiae has been potential strain on the basis of zone formation. Over all three enzymes were tested amylase was greater zone of inhibition when compared to other enzymes. The production of amylase enzymes with optimized pH, temperature, incubation period and nutrient source were altered. But favorable condition of pH is 6, temperature is 20°C, incubation period 8 days and nutrients was 1 mg/1 in carbon, nitrogen and phosphorous source for higher production of amylase enzyme whereas iron content also 0.4 mg/l was excellent performance for execution of amylase enzyme production by solid state fermentation***

Keywords: Amylase, yeast, optimization, fermentation

1. Introduction

In recent years the capability of some yeast species to degrade starch has aroused the interest of several researchers, as the potential value of these microorganisms for certain biotechnological applications, such as the production of single-cell protein or ethanol from a starchy biomass, was recognized (Tubb, 1986). In addition there are current attempts to introduce foreign starch-degrading activity into the non-amylolytic yeast *Saccharomyces cerevisiae* (Tubb, 1986). Most research dealing with microbial amylolytic enzymes has been focused on the enzymes from bacteria and filamentous fungi, several of which have found industrial applications (Fogarty and Kelly 1980). However, far fewer data are available on the amylases from yeasts, although the starch-degrading enzymes of some promising species, such as *Lipomyces kononenkoae, Saccharomycopsis Jibuligera, Schwanniomyces* sp. and *Filobasidium capsuligenum* have been partially characterized (De Mot and Verachtert, 1985 and McCann and Barnett, 1986). Recently the *Candida antarctica* CBS 6678 secretes with β-cyclodextrin as sole carbon source significantly higher levels of amylolytic activity than any of the currently recognized, active starch degrading yeast species (De Mot and Verachtert 1986). This observation urged with further investigation of extracellular amylolytic system of this strain, which was originally isolated from marine described as *Trichosporon oryzae* (Ito *et al.,* 1974). According to recent taxonomic studies strain CBS 6678 is currently accomodated within the genus *Candida* (Barnett *et al.,* 1983 and Kreger-van Rij, 1984).

2. Materials and Methods

Amylolytic activity (Fossi *et al*., 2009)
Amylase producing yeast were screened on Amylase activity medium (AAM) (starch 5g/L; peptone 5g/L, yeast extract 5g/L, $MgSO_4.7H_2O$ 0.5g/L, $FeSO_4.\ 7H_2O$ 0.01g/L; NaCL 0.01 g/L, agar 15g/L) plates. Incubation at 30°C was carried out for 3 days, after which the plates were stained with lugol solution. The colonies forming the largest halo zone were observed.

Protease activity (Strauss *et al*. 2001)
Extracellular protease production was determined on YEPG medium containing 20 g l)1 casein, pH 6. A clear zone around the colony indicated that protease activity was conformed (PrA).

Pectinase activity (Ankin and Anagnostakis 1975)
The secretion of extracellular pectic enzymes was tested on the following medium (g/l) (Pectin -5g, yeast extract-1g, agar- 15g pH 5.0 in 1L distilled water). After cell growth, plates were flooded with hexadecyl trimethyl ammonium bromide (10 g /l) A clear halo around a colony in an otherwise opaque medium indicated degradation of the pectin.

Optimization studies of yeast

Effect of pH
Yeast inoculated culture medium containing enzymes was incubated at pH of 5, 6 and 7. The pH of culture medium was adjusted using 0.1N HCl and 0.1N NaOH solution.

Effect of Temperature (°C)
The assessment of enzyme production was carried out at different temperature ranging from 10, 20 and 30°C altered. After incubation the culture medium was filtered and analyzed for percentage variation

Effect of different nutrient sources
The Nutrient source optimized in carbon sources (glucose) nitrogen sources (peptone extracts), phosphorous source (Dipotassium phosphate) and trace element (zinc sulphate) were optimized with potential fungal species. After

incubation the culture medium was filtered and analyzed for percentage variation

3. Result and Discussion

Several reviewers (Steele and Stowers 1991; Bull *et al*., 1992) still stress the fact that, although advances in genetics and microbial physiology having a strong impact on enzyme production, screening programmes for the selection of micro-organisms able to produce bioactive molecules continue to be an important aspect of biotechnology.

In the present research suggests that the isolation and screening of enzyme production for amylase, pectinase and protease were determined. Among the enzyme production of amylase was high production when compared to pectinase and protease. The name of the yeast *Aureobasidium nulluns, Candida elaebora, C. sake, C. zeylanoides, Cryptococcus hansenii, C.victoriae, Dioszella crocera, D. aurantiace, Leucosporidilla fragaria, L. auscorum, Rhodotorula glacialis, R. larvngis* and *Saccharomyces cerievisiae* were tested by zone measurement by the YM medium. Among the ten yeast, *Saccharomyces cerevisiae* was high zone of measurement (22mm) followed by *C. sake* (18mm) observed for amylase production. In the case of pectinase enzyme screening *Saccharomyces cerevisiae* (14mm) followed by *Dioszella crocera* (13mm) was recorded. Whereas pectinase enzyme production was high in the yeast of *Saccharomyces cerevisiae* then second was *Aureobasidium nulluns, Dioszella crocera, D. aurantiace* and *Leucosporidilla fragaria* was each (10mm) zone measurement observed (Table-1).

The production of yeast proteases has so far been studied mainly for their implications in the beer and wine industry (Bilinski and Stewart 1990; Dizy and Bisson 2000; Strauss *et al*., 2001). Only a few yeast proteases have been studied for alternative potential applications (Ray *et al.,* 1992; Poza *et al*., 2001). Pectin enzymes for industrial uses have so far been produced by moulds and bacteria (Sakai *et al.*, 1993), the pectolytic activity of yeasts has, however, been studied with contrasting results (Charoenchai *et al*., 1997; Blanco *et al*., 1999; Strauss *et al*., 2001).

Optimization of growth condition is a prime step in using microorganisms in fermentation technology (Kathiresan and Manivannan, 2006). Optimization of enzyme production by using different yeast was analyzed. The *Saccharomyces cerevisiae* has high production with excellent zone of measurement observed. According to the pH, the pH 6 was more favour when compared to 5 and 7 pH. As per earlier report (Aiba *et al*., 1983) the high temperature may inactivate the expression of gene responsible for the starch degrading enzyme. Most of the starch degrading bacterial strain revealed a pH range between 6.0 and 7.0 for normal growth and enzyme production (Gupta *et al*., 2003). The temperature also altered 10, 20 and 30 ^{o}C was introduced for the production of enzyme by potential yeast, the 20^{o}C temperature high production. Whereas incubation period was 4, 8 and 12 days changed, but 8 days incubation period was higher production of amylase enzyme recorded (Table-2). Sujeeta *et al*. (2017) reported that the amylase activity of yeast isolates increase from 2.08 to 9.36 U/ml as the incubation time increase from 24 to 48 hours at 30°C.

In the current research analysis that the nutrient content was manipulated for amylase enzyme by *Saccharomyces cerevisiae*. The nutrient glucose source as a carbon source with different concentration altered. The 1.0 mg/l was higher production when compared to other concentration of glucose. Peptone as a nitrogen source with 1.0mg/l was high production of enzyme. Similarly peptone is the best nitrogen source for amylase production for both organisms though bacto-tryptone supported the maximum growth. Though Adenosine mono phosphate supported the maximum growth, the amylase production is the maximum for K_2HPO_4. Maximum growth and amylase production for both C1 and C2 occurred in presence of calcium ion (Chandrima, 2010). The α- Amylase is known to be a calcium metalloenzyme having at least one calcium ion associated with its molecule. Enhanced bacterial growth and enzyme production may be the result of increased availability of calcium ions (Hewitt and Solomons, 1996).

Dipotassium phosphate was altered from 0.5, 1.0, 1.5 mg/l as phosphorus content here also 1.0mg/l was excellent production of amylase enzyme, whereas iron content was altered of 0.2, 0.4 and 0.6 mg/l treated. The 0.2 mg/l was more suitable for production of amylase enzymes (Table-3).

4. Conclusion

The marine yeasts are comparatively few and that this group of marine mycota is still poorly understood. Different kinds of immobilized enzymes reactors and multiphase reaction systems have greatly influenced the processes that require catalysis by amylase. The marine derived *S.cereivisiae* was highest fermentative activity among the many yeast isolates and species isolated and allied to the enzyme production from various biomass. The enzyme from the marine yeast strains has so many unique properties and many potential applications in biotechnology.

Table 1: Isolation and identification of yeast from Muthupet mangrove soil

S. No	strain code	Name of the yeast	Screening of enzymes (mm)		
			Amylase	Protease	Pectinase
1	NJJUM 1	*Aureobasidium nulluns*	12	08	10
2	NJJUM 2	*Candida elaebora*	16	10	07
3	NJJUM 3	*C. sake*	18	10	07
4	NJJUM 4	*C.zeylanoides*	12	09	06
5	NJJUM 5	*Cryptococcus hansenii*	08	07	06
6	NJJUM 6	*C. victoriae*	09	08	09
7	NJJUM 7	*Dioszella crocera*	09	08	09
8	NJJUM 8	*D. aurantiace*	11	13	10
9	NJJUM 9	*Leucosporidilla fragaria*	9	10	10
10	NJJUM 10	*L. muscorum*	11	10	06
11	NJJUM 11	*Rhodotorula glacialis*	10	09	07
12	NJJUM 12	*R. larvngis*	08	07	09
13	NJJUM 13	*Saccharomyces cerevisiae*	22	14	17

Table 2: Effect of pH, temperature and incubation period on the amylase production using *Saccharomycetes cerevisiae*

Incubation period		4th day			8th day			12th day		
Temperature (°C)		10	20	30	10	20	30	10	20	30
pH	5	0.868	0.952	1.103	1.072	1.121	1.109	0.896	1.007	0.972
	6	0.917	0.926	0.981	1.038	2.041	2.079	1.008	1.101	1.920
	7	0.526	0.542	0.560	0.581	0.592	0.643	0.498	0.507	0.508

Table 3: Effect of nutrient source on the production of Amylase enzymes by *Saccharomycetes cerevesiae*

S.No	*Nutrient source*	*Concentration mg/ml*	*Production of enzymes (IU/ml)*
1	Glucose	0.5	0.872
		1.0	0.965
		1.5	0.754
2	Peptone	0.5	0.943
		1.0	0.976
		1.5	0.854
3	Dipotassium phosphate	0.5	0.654
		1.0	0.743
		1.5	0.654
4	Zinc sulphate	0.5	0.543
		1.0	0.432
		1.5	0.343

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