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Research Article

EFFECT OF MEDIA CONSTITUENTS ON MICROBIAL ENZYME ACTIVITY

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ABSTRACT

There has been various research activities directed towards evaluating the effect of different carbon and nitrogen sources as well as presence of various divalent metal ions in the media on the yield of enzymes. Each organism or strain has its own special conditions for maximum enzyme production. The aim of the present study was to evaluate the effect of nutritional media components on reductase activity of *Saccharomyces cerevisiae* after cultivating the organism in various media composition. Several carbon sources were investigated for their capacity to support enzyme production. Among the different carbohydrates tried, glucose yielded maximum enzyme productivity. Different nitrogen sources like yeast extract, peptone, tryptone, beef extract and malt extract were considered for the study. The results indicated that the peptone as a source of nitrogen had maximum effect on enzyme expression. While investigating the effect of several metal ions on enzyme production, it was observed that the enzyme expression was maximum when copper was added to the media. During the course of study on the effect of initial pH of the growth media and temperature, pH of 7.0 and temperature of 30 °C was found to have significant effect on enzyme production.

Key words: *Saccharomyces cerevisiae,* Carbon source, pH, Temperature.

INTRODUCTION

Biocatalysts are being employed as an alternative tool in the field of synthetic chemistry and this is mainly due to the fact that they exhibit enantioselectivity and regioselectivity under benign conditions [1,2,3]. Enantiomerically pure secondary chiral alcohols are important chiral building blocks and are useful starting materials for the synthesis of various biologically active compounds and therefore there is a great demand for chiral alcohols.[4] There are number of reports of various chiral alcohols synthesized by biocatalytic methods using whole cell systems [5,6,7]. The reduction of prochiral ketones are brought about by any of the dehydrogenases present in the microbial cell [8].

It is well known that variations in media components in a culture media has a very important role in influencing microbial cell growth and thereby enzyme production [9]. Hence the amount of a particular enzyme expressed by a specific microorganism is directly dependent on the nutrients available in the culture media. Carbonyl reductases are enzymes which are involved in the reduction of ketones. The production of these enzymes are affected by several internal (media composition) and external

(growth conditions) factors. Among them, the composition of the culture media plays a significant role in the expression of enzymes [10]. To achieve high product yields, it is a prerequisite to design an efficient reaction medium that help in rendering the process more economical. The different sources of carbon (glucose, sucrose, maltose, lactose etc.), nitrogen (peptone, tryptone, malt extract etc.) and addition of metal ions (copper, zinc, magnesium, manganese, iron) can significantly affect the production of microbial enzymes. Enzyme activity can also be varied by changing the media pH and incubation temperature, which ultimately affect the yield.

MATERIALS AND METHODS

Chemicals

3-[5-[(4-fluorophenyl)-1,5- dioxopentol]-yl] –4-(S) phenyl oxazolidin-2-one was used as the substrate for media optimization for production of reductase enzyme.

NADH and growth media components were procured from HiMedia Laboratories Pvt. Ltd (Mumbai, India). All other chemicals used were of analytical grade and obtained from standard companies.

Microorganism

Saccharomyces cerevisiae MTCC 174 was selected for the optimization experiment as it gave considerable conversion of the substrate in preliminary screening. The organism was obtained from *MTCC* Chandigarh.

Saccharomyces cerevisiae MTCC 174 was maintained on *YEPD* media containing yeast extract 3.0 g, peptone 10.0 g, dextrose 20 g , agar 20.0 g and distilled water 1000 ml.

The project work was carried out at Dayananda Sagar college of pharmacy, Bangalore in 2011.

Methodology

Inoculum preparation

The organism from the slant culture was subcultured into 200 ml sterile *YEPD* liquid medium and incubated at 30 ºC, 160 rev min^{-1} for 24 h.

Shake flask experiment

The effect of various carbon source, nitrogen source, metal ions, temperature and pH on enzyme activity was studied by inoculating 2 ml of the inoculum to each of 20 ml of different media as mentioned in Table 1. The flasks were incubated at the mentioned temperature and kept at 160 rev min^{-1} for 48 h.

Crude enzyme extract

The cells were harvested by centrifugation at 10,000 rpm for 20 min. The pellet was washed with phosphate buffer pH 7.0 and centrifuged at 12000 rpm for 10 min. The pellet was resuspended in the same buffer and disintegrated using ultra sonicator. The cell debris was removed by centrifugation at 20000 rpm for 1 hr at 4 ºC. The supernatant was used for activity measurement. Reductase activity was determined spectrophotometricaly by measuring the decrease in the absorbance of *NADH* at 340 nm. The reaction mixture contained 4 ml of cell free extract, 20 µl of *NADH* (4.5 mM) and 20 µl of the ketone (10 mM in *DMSO*). Ketone reduction was followed over a time period of 5 min. One unit of enzyme activity is defined as the amount of enzyme required to oxidize 1 µl of *NADH* to *NAD* per minute.

RESULTS AND DISCUSSION

Effect of media condition on production of enzyme

Effect of Carbon Source: Five different carbohydrates (glucose, mannitol, maltose, sucrose and lactose) were used in five different media and the results are tabulated in Table No 2. Soni et.al [11] have evaluated the effect of several carbon sources on carbonyl reductase activity of *C.vishwanathii.* They have found maximum activity when mannitol was used as the carbon source where as Singh et.al [12] reported that glucose was the most suitable carbon source for maximum production of carbonyl reductase by *M. koreensis.* The results of the present study agree with both the reports that glucose is the preferred carbon source by the organism for the production of the enzyme, followed by mannitol, which are simple sugars. Disaccharides had comparatively less effect on enzyme production.

Effect of Nitrogen source

Source of nitrogen is one of the important factor that affect the growth and metabolites of microorganisms. Variation in the nitrogen source can affect the metabolic processes of the cell significantly. The effect of five different organic source of nitrogen was considered for the study (Table No 3). The data collected during the study indicated that the addition of organic nitrogen did not have positive effect on the enzyme production. Similar results has been observed by Singh et. al [13] in their study on the effect of different nitrogen sources on carbonyl reductase activity of *M.koreenis.* The species of *B.cenocepacia* also exhibited similar results when 3-[5-[(4-fluorophenyl)-1,5, dioxopentol]-yl] –4-(S) phenyl oxazolidin-2-one was used as the ketone in the study [12]. Among the different nitrogen sources used, peptone showed maximum effect compared to other nitrogenous compounds.

Effect of Metal ions

The presence of metal ions in the culture media greatly affects the metabolic activity of the microbial cell. The metal ions act as co-factors in various enzymatic reactions. They are important regulators of enzyme production, therefore the effect of some divalent ions was considered for the study (Table No 4). Among the various metal salts used, addition of copper sulphate to the media had a moderate effect on enzyme activity, whereas addition of ferric ion had the least effect. Carbonyl reductase activity of *C.vishwanathii* [11] has been reported to have increased when Ca^{2+} was added to the growth media and considerably decreased when Fe^{2+} and Zn^{2+} were included individually to the medium. On the contrary the reductase activity of *B.cenocepacia*[12] has been reported to have been enhanced on addition of Fe^{3+++} salt to the media and addition of Ca^{2+} and Mg^{2+} had mild effect. Zn^{2+} was found to be the metal ion which promoted enzyme activity in case of *M. koreensis* [13]. These findings clearly indicate that the effect of metal ions on reductase activity depend on the type of microbial cell used as biocatalyst in bioconversion reactions.

Effect of pH

The optimum pH for growth of fungi usually falls in the acidic range. The effect of medium pH ranging from 6 to 8 on the enzyme induction was studied. The maximum enzymatic activity was found at pH 7 and there was no significant difference in enzyme activity in acidic pH but there was marked decrease when the pH was maintained at 8 (Table No 5). The study on the effect of media pH on the activity of carbonyl reductase of *C. vishwanathii* revealed sufficient enzyme activity over a broad range of pH which the authors attribute to the presence of multiple carbonyl reducases acting on the substrate [11]. In case of carbonyl reductase of *M. korneesis* used in the reduction of 4-fluroacetophenone, the optimum pH was 7.0 [13], which is in accordance with the result obtained in the present study.

Effect of Temperature

The incubation temperatures of the microbial cells affect some of its metabolic activities. The cells grown at different temperatures did not show any marked difference in the activity of the enzyme involved. The maximum effect was observed at 30 °C and the least was found at 40 °C (Table No 6). The optimum temperature for the production of enzyme involved in the reduction of the same molecule by *B.cenocepacia*[12] has been reported to be in the range of 35-37 °C which is comparable to the result obtained in the present study for the reduction using *S. cerevisiae.*

CONCLUSION

Biocatalytic synthesis of chiral compounds has been found to be an important alternative route in the synthesis of several enantiopure drug intermediates. The biocatalysis can be made more productive and efficient by enhancing the enzyme activity in the selected microbial system by varying media components and growth conditions. The present study supports the fact that the variation in growth media in terms of composition and condition does affect the enzyme productivity.

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Table 1: Composition of nutrient media

Media Components Media	Yeast Extract g	Peptone G	Carbon source g					Nitrogen source g			Metal ions mM				D.H$_2$O L	Temp °C	pH
			Dextrose	Mannitol	Sucrose	lactose	Maltose	Malt Extract	Beef Extract	Tryptone	$ZnSO_4$	$CuSo_4$	$FeCl_3$	$CaCl_2$			
1	3	10	20	-											1	30	7.0
2	3	10		20											1	30	7.0
3	3	10			20										1	30	7.0
4	3	10				20									1	30	7.0
5	3	10					20								1	30	7.0
6	13		20												1	30	7.0
7		13	20												1	30	7.0
8			20					13							1	30	7.0
9			20						13						1	30	7.0
10			20							13					1	30	7.0
11	3	10	20								1				1	30	7.0
12	3	10	20									1			1	30	7.0
13	3	10	20										1		1	30	7.0
14	3	10	20											1	1	30	7.0
15	3	10	20												1	25	7.0
16	3	10	20												1	35	7.0
17	3	10	20												1	40	7.0
18	3	10	20												1	30	6.0
19	3	10	20												1	30	6.5
20	3	10	20												1	30	7.5
21	3	10	20												1	30	8.0

Table 2: Effect of Carbon source on enzyme activity

S. No	Carbon source	Enzyme activity 1x 10^{-2} μm/min
1	Glucose	0.302
2	Mannitol	0.250
3	Sucrose	0.100
4	Lactose	0.180
5	Maltose	0.120

Table 3: Effect of nitrogen source on enzyme activity

S. No	Nitrogen Source	Enzyme activity 1x 10^{-2} μm/min
1	Beef Extract	0.15
2	Peptone	0.17
3	Yeast Extract	0.14
4	Tryptone	0.12
5	Malt Extract	0.08

Table 4: Effect of metal ions on enzyme activity

S.No	Metal salt	Enzyme activity 1x 10^{-2} μm/min
1	Zinc Sulphate	0.24
2	Copper Sulphate	0.36
3	Calcium Chloride	0.18
4	Ferric Chloride	0.16
5	Magnesium Sulphate	0.26

Table 5: Effect of pH on enzyme activity

S. No	pH	Enzyme activity 1x 10^{-2} μm/min
1	6.0	0.257
2	6.5	0.280
3	7.0	0.302
4	7.5	0.160
5	8.0	0.096

Table 6: Effect of incubation temperature on enzyme activity

S. No	Temperature in °C	Enzyme activity 1x 10^{-2} μm/min
1	25	0.240
2	30	0.302
3	35	0.280
4	40	0.170

REFERENCES

1. Ogawa J and Shimizu S. Industrial microbial enzymes: their discovery by screening and use in large-scale production of useful chemicals in Japan. Curr Opin Biotech. 2002;13: 367-375.
2. Ishige T, Honda K and Shimizu S. Whole organism biocatalysis. Curr Opin Chem Biol. 2005; 9:174-180.
3. Marino ST, Buczek DS, Huggins DA, Krywult BM, Sheehan CS, Nguyen T. Choi N, Parsons JG, Griffiths PG, James IW, Bray AM, White JM and Boyce RS. Synthesis of Chiral Building Blocks for Use in Drug Discovery Molecules. 2004; 9: 405-426.
4. Xiao ZJ, Zong MH and Lou WY. Highly enantioselective reduction of 4-(trimethylsilyl)-3-butyn-2-one to enantiopure (R)-4-(trimethylsilyl)-3-butyn-2-ol using a novel strain Acetobacter sp. CCTCC M209061. Bioresource Technol. 2009; 100:5560-5565.
5. Abas FZ, Uzir MH and Zahar MHM.. Effect of pH on the biotransformation of (R)-1-(4-bromo-phenyl)-ethanol by using Aspergillus niger as biocatalyst.J Appl Sci. 2010;10(24):3289-3294.
6. Matsuda T, Yamanaka R and Nakamura K. Recent progress in biocatalysis for asymmetric oxidation and reduction. Tetrahed Asym. 2009;20:513-557.
7. Singh A, Basit A and Banerjee UC. Burkholderia cenocepacia: a new biocatalyst for efficient bioreduction of ezetimibe intermediate. J Ind Microbiol Biotechnol. 2009;36:1369-1374.
8. Rodrigues JAR, Moran PJS, Conceicao GJA and Fardelone LC. Recent Advances in the Biocatalytic Asymmetric Reduction of Acetophenones and α, β- Unsaturated Carbonyl Compounds. Food Technol Biotechnol. 2004;42(4): 295-303.
9. Ibrahim HM and Elchidir EE. Response surface method as an efficient tool for medium optimization. Trends in applied sciences research. 2011;6(2):121-129.
10. Soni P, Singh M, Kamble AL and Banerjee UC. Response surface optimization of the critical medium components for carbonyl reductase production by Candida viswanathii MTCC 5158. Bioresource Technol. 2007;98: 829-833.
11. Soni P, Prasad GS and Banerjee UC. Optimization of physicochemical parameters for the enhancement of carbonyl reductase production by Candida viswanathii. Bioprocess and Biosyst Eng. 2006;29(3):149-156.
12. Singh A, Basit A and Banerjee UC. Burkholderia cenocepacia: a new biocatalyst for efficient bioreduction of ezetimibe intermediate. J Ind Microbiol Biotechnol. 2009;36:1369-1374.
13. Singh A, Christi V and Banerjee UC. Production of carbonyl reductase by Metschnikowia koreensis. Bioresour Technol. 2011;102(22):10679-10685.