INFLUENCE OF CARBON SOURCES ON α-AMYLASE PRODUCTION BY BREVIBACILLUS sp. UNDER SUBMERGED FERMENTATION

K.Suribabu[1], T.Lalitha Govardhan[2], K.P.J Hemalatha[3]

[1, 2]*Assistant Professor, PG Department of Microbiology and Research Centre, Dr.Lankapalli Bullayya Post-graduate College, Andhra Pradesh, India, Visakhapatnam-530 013*

[3]*Professor, Department Microbiology, Andhra University, Andhra Pradesh, India, Visakhapatnam-530 003*

Abstract

Numerous marine microorganisms secrete enzymes which can provide new insights and understanding of enzymes. Marine microorganisms have been attracting more attention as source for novel enzymes. Secondary screening is strictly essential in any systematic screening programme which helps in detection of useful bacteria in fermentation processes. Secondary screening also provides information pertaining to the effect of different components of the medium. This is valuable in designing the medium that may be attractive as far as economic consideration is concerned. Natural carbon source, Saccharum officinarum (5%) produced maximum α-amylase while Triticum vulgare (4%) produced very low α-amylase. Synthetic carbon source when supplemented with maltose (1%) and sucrose (4%) regulated higher production of amylase. Starch (2%), dextrose (3%) and galactose (4%) exhibited average effect on production whereas lactose (3%) and mannitol (3%) decreased production. The α-amylase was found to have many applications in the field of starch processing, textile industry, improving shelf life of bread, ethanol production, sewage treatment and effluent treatment.

Keywords**:** *Brevibacillus borostelensis R1, Saccharum officinarum, Triticum vulgare, maltose, sucrose*

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1. INTRODUCTION

Secondary screening is strictly essential in any systematic screening programme which helps in detection of useful bacteria in fermentation processes. It gives an idea about the economic position of the fermentation process involving the use of a newly discovered culture. Thus, one may have a comparative study of the process providing information regarding the enzyme yield potentials of different isolates. Secondary screening also provides information pertaining to the effect of different components of the medium. This is valuable in designing the medium that may be attractive as far as economic consideration is concerned. It determines the optimum conditions for production of enzyme associated with culture [1].

The production of α-amylases by fermentation had been thoroughly investigated and shown to be affected by a variety of physicochemical factors, such as the composition of the growth medium, the type of strain, cell growth, methods of cultivation, inoculum concentration, time of incubation, pH, temperature, salinity, carbon, nitrogen and mineral sources [2-5].

1.1 Carbon Sources: Natural and Synthetic

The addition of different carbon sources affects not only the mode of amylase production but also the rate of carbohydrates metabolized [6]. The addition of carbon source in the form of either monosaccharides or polysaccharides could influence the production of enzymes [7]. Fukumoto [8] initiated a series of studies for economically important alpha-amylase biosynthesis by supplementing various carbon sources. The activity of amylase is enhanced in the presence of different carbon sources such as Oryza sativa [9], Corn [10], Solanum tuberosam [11], Maltose [12], Xylose [13], Starch [14], Lactose [15], Galactose [16], Sucrose and Fructose [17], Glucose [18], Mannitol [19] and Cellulose [20].

Natural carbon sources do not have well defined concentration of the sugar. These sources contain various minerals which may also influence the α-amylase production. Synthetic carbon sources are pure and with defined composition. These sources act as both energy and carbon source. High concentration of these sources may inhibit the production of α-amylase. Simple sugars were used directly whereas the complex sugars were broken down to give simple sugars for utilization.

2. MEDIA AND METERIALS

Brevibacillus borostelensis R1 was cultured in Pikovskaya's medium with additional source of natural and synthetic carbon (1-5%w/v) separately by keeping the physical parameters (Incubation period 24hrs, inoculum size 2%, pH 7.0, temperature 370C and salinity 1%) constant. Samples were incubated in orbital shaking incubator (120rpm) for 24hrs.

Natural sources include sagu (Metroxylon sago), wheat flour (Triticum vulgare), rice flour (Oryza sativa), maidha (Triticum vulgare), corn flour (Zea mays), ragi flour (Elusine coracana), jaggary (Saccharum officinarum), barley (Hardeum vulgare), potato (Solanum tuberosam) and chema (Colacashia esculenta). Synthetic sources include maltose (Himedia), xylose (Merck), starch (Merck), lactose (Merck), galactose (Merck), sucrose (Merck), fructose (Merck), dextrose (S.D.Fine-CHEM-Ltd.,), mannitol (Merck) and cellulose (Himedia).

Varying concentrations (1, 2, 3, 4 and 5 % w/v) of ten natural and synthetic carbon sources were added to the 100 ml of Pikovskaya's fermentation medium separately. As synthetic sources need no pretreatment they were added directly into the culture at varying concentrations. However, for natural sources of carbon, they were ground to powder with a mortar and pestle.

Two ml of inoculum of Brevibacillus borostelensis R1 was inoculated to the 100ml of production medium (Pikovskaya's Medium) and incubated in the orbital shaking incubator for 24hrs. After incubation, the medium was subjected to centrifugation at 5,000rpm for 15minutes at room temperature (250C). The supernatant was collected in sterile test tube and the pellet was discarded. Supernatant (0.5 ml) was used for the amylase assay by DNS method [21].Each concentration was assayed in triplicate sets and recorded for statistical analysis. One unit of enzyme activity was defined as the amount of enzyme that releases 1.0 mmol of reducing sugar (maltose) per minute under the assay conditions.

3. RESULTS

Natural carbon sources utilized; Sagu (Metroxylon sago), Wheat flour (Triticum vulgare), Rice flour (Oryza sativa), Maidha (Triticum vulgare), Corn flour (Zeamays), Ragulu (Elusine coracana), Jaggary (Saccharum officinarum), Barley (Hardeum vulgare), Potato (Solanum tuberosam) and chema (Colacashia) were added separately to the production PK medium, at varying concentrations ranging from 1 to 5% maintaining constant physical parameters (Chart -1a-j). Ten synthetic carbon sources used; maltose, xylose, starch, lactose, galactose, sucrose, fructose, dextrose, mannitol and cellulose (Chart -2a-j).

The production of α-amylase was estimated at different concentrations of carbon supplements and the highest production at optimum values were reported in the Table 1. The production was found to be higher in natural carbon supplement PK media as compared with synthetic carbon sources. However, the optimum production 7960.500 ± 0.707 U/ml was found in natural carbon source of Jaggary (Saccharum officinarum) at 5%.

Table 1 The highest production of α-amylase at optimal concentrations of carbon sources (10): Natural and Synthetic

Natural Carbon Sources	% of Carbon Sources	Amylase activity (U/ml)
Sago (Metroxylon sago)	2	7080.50 ± 0.50
Wheat flour (Triticum vulgare)	5	2261.00 ± 1.41
Rice flour (Oryza sativa)	4	1980.50 ± 0.70
Maidha (Triticum vulgare)	4	1341.75 ± 0.35
Corn flour (Zeamays)	3	3661.00 ± 1.414
Ragulu (Elusine coracana)	5	1901.000 ± 1.414
Jaggary (Saccharum officinarum)	5	7960.500 ± 0.707
Barley (Hardeum vulgare)	3	2480.500 ± 0.707
Solanum tuberosam (Potato)	4	2420.500 ± 0.707
Chema (Colacashia)	5	3000.500 ± 0.707
% of Synthetic Carbon Sources		
Maltose	1	3680.50 ± 0.71
Xylose	5	2241 ± 1.41
Starch	2	2061.50 ± 2.12
Lactose	3	1441.50 ± 2.12
Galactose	4	2361.00 ± 1.41
Sucrose	4	2820.75 ± 1.06
Fructose	4	4321 ± 1.41
Dextrose	3	2161 ± 1.41
Mannitol	3	1280.50 ± 0.70
Cellulose	1	2721.00 ± 1.41

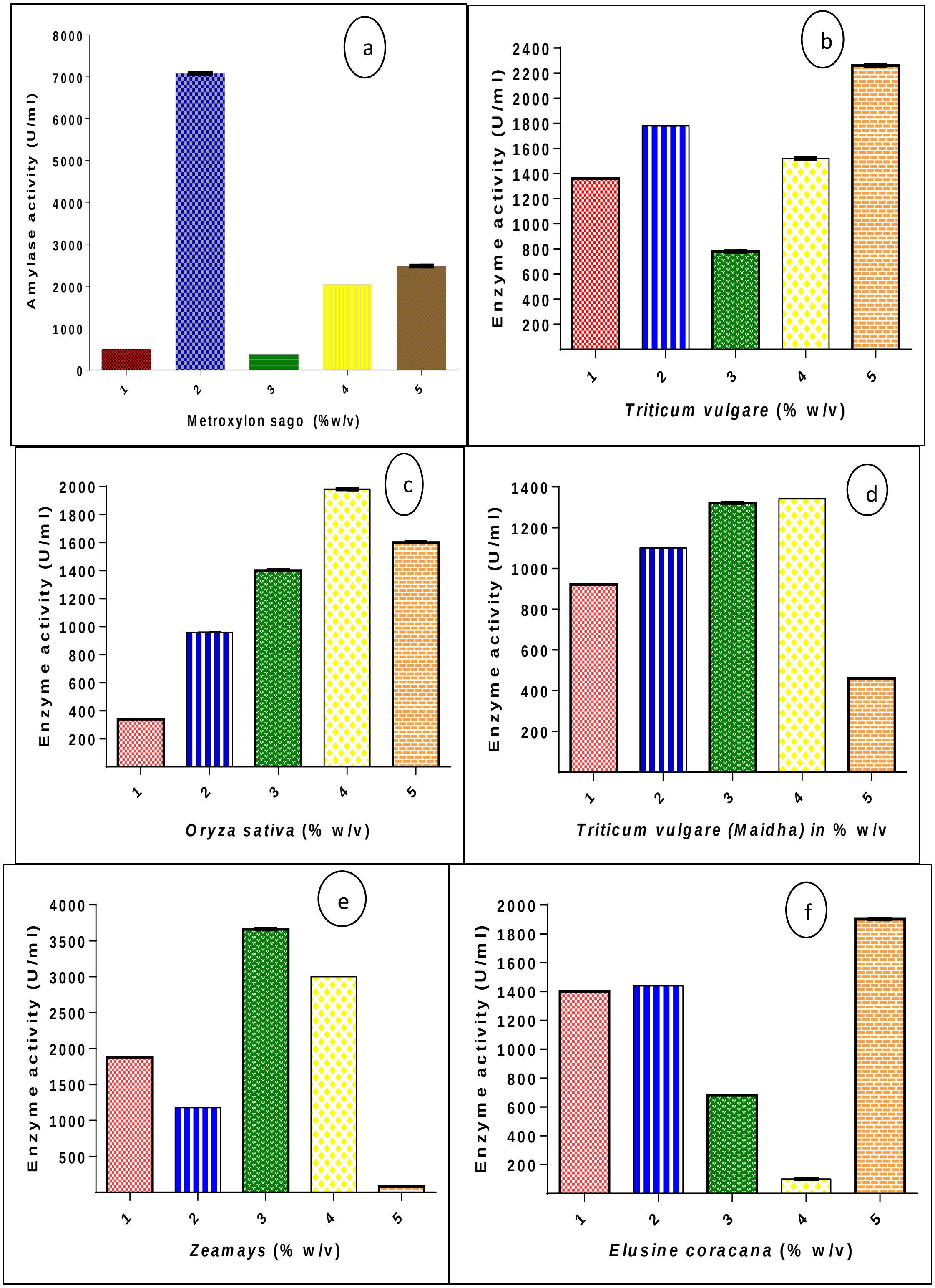


Chart -1: a-f. Effect of different concentrations of natural carbon sources on the production of α-amylase by B. borstelensis R1 at different concentrations: a, Metroxylon sago; b, Triticum vulgare; c, Oryza sativa; d, Triticum vulgare; e, Zeamays and f, Elusine coracana.

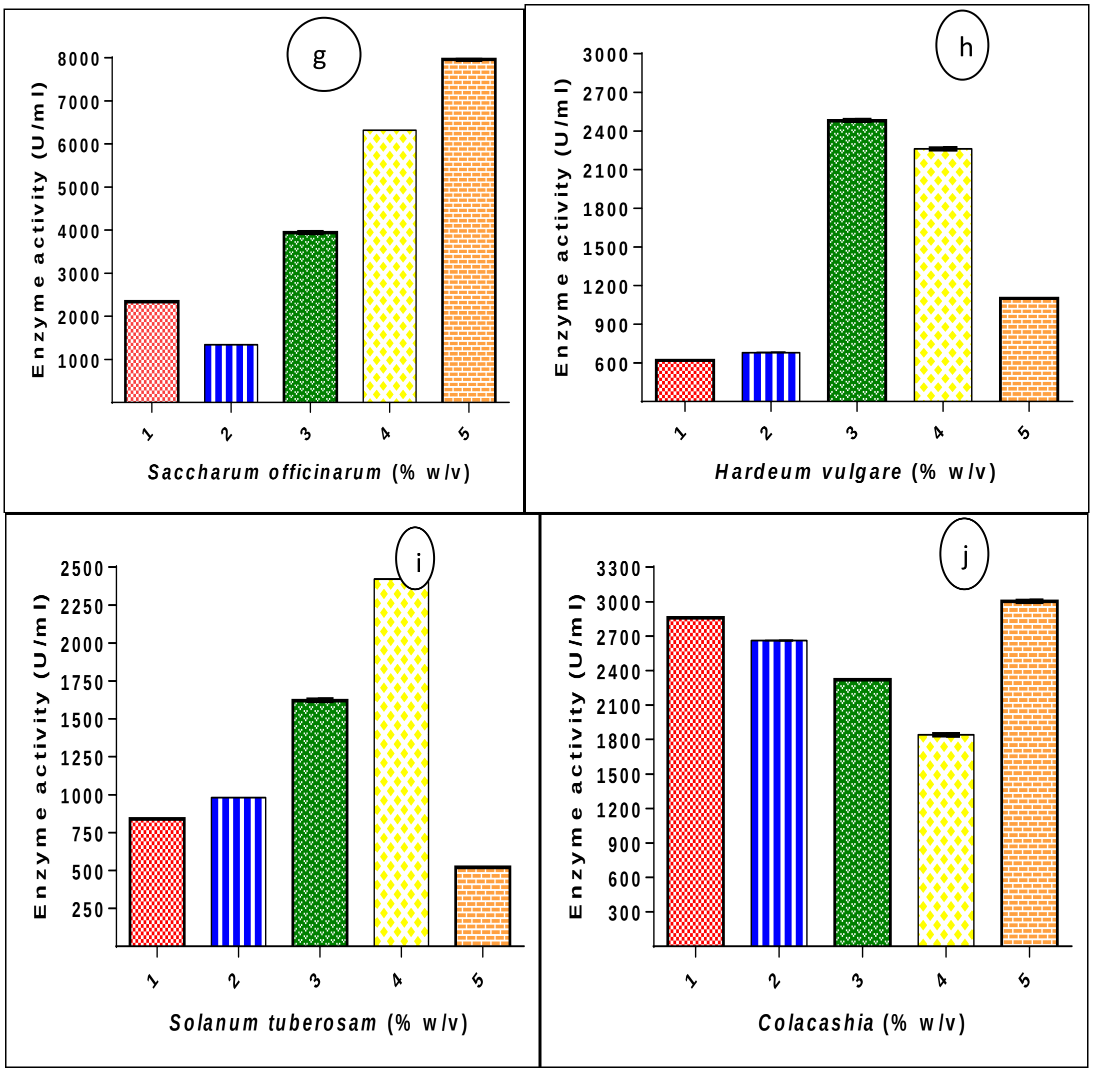
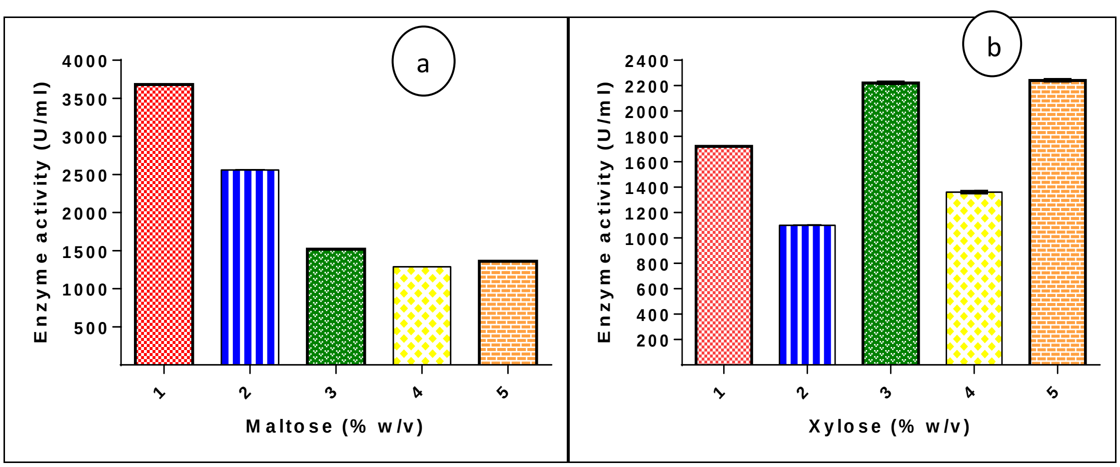


Chart -1: g-j. Effect of different concentrations of natural carbon sources on the production of α-amylase by B. borstelensis R1 at different concentrations: g, Saccharum officinarum; h, Hardeum vulgare; I, Solanum tuberosam and j, Colacashia.



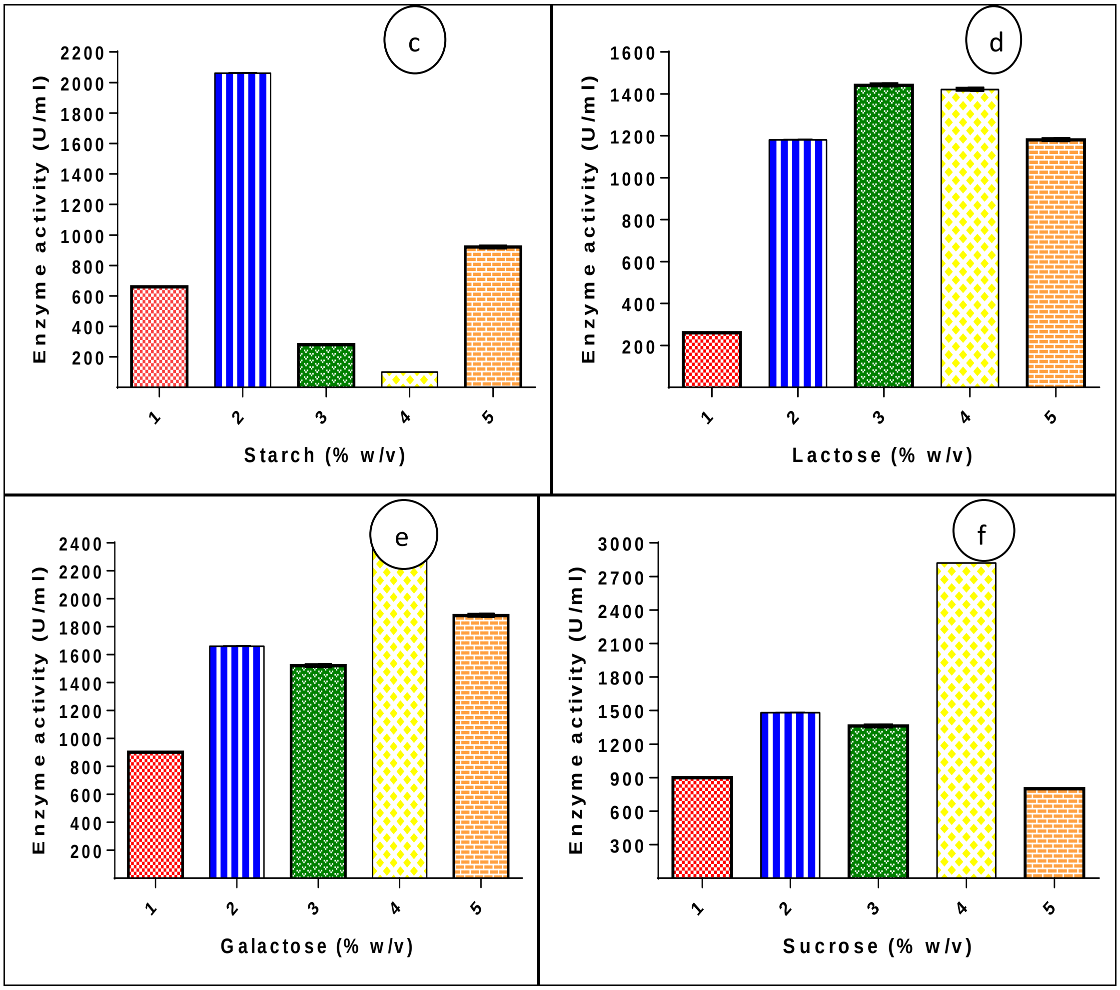
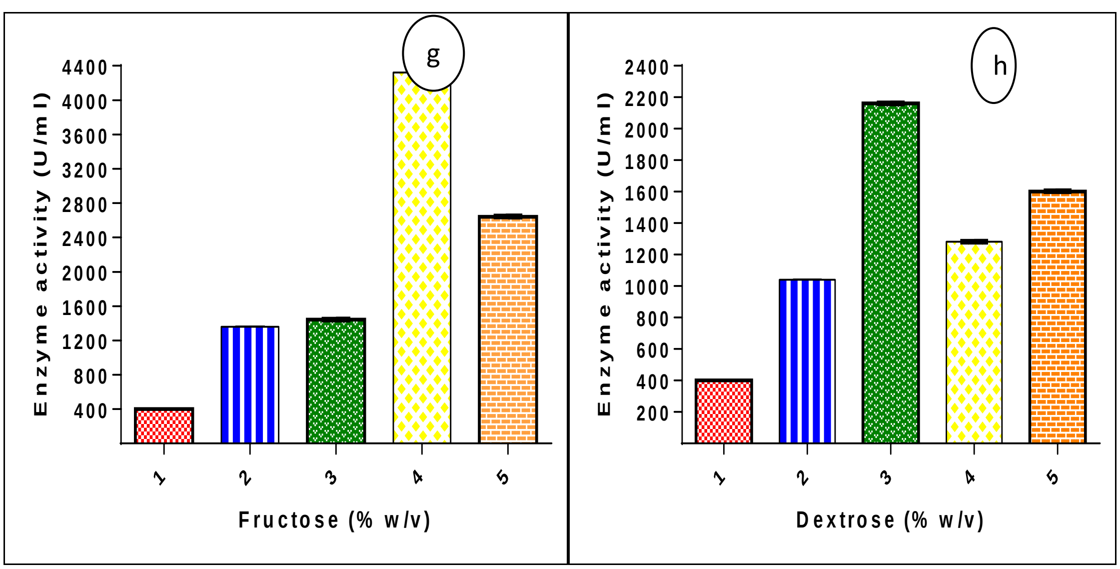


Chart -2: a-f. Effect of different concentrations of synthetic carbon sources on the production of α-amylase by B. borstelensis R1: a, Maltose; b, Xylose; c, Starch; d, Lactose; e, Galactose and f, Sucrose.



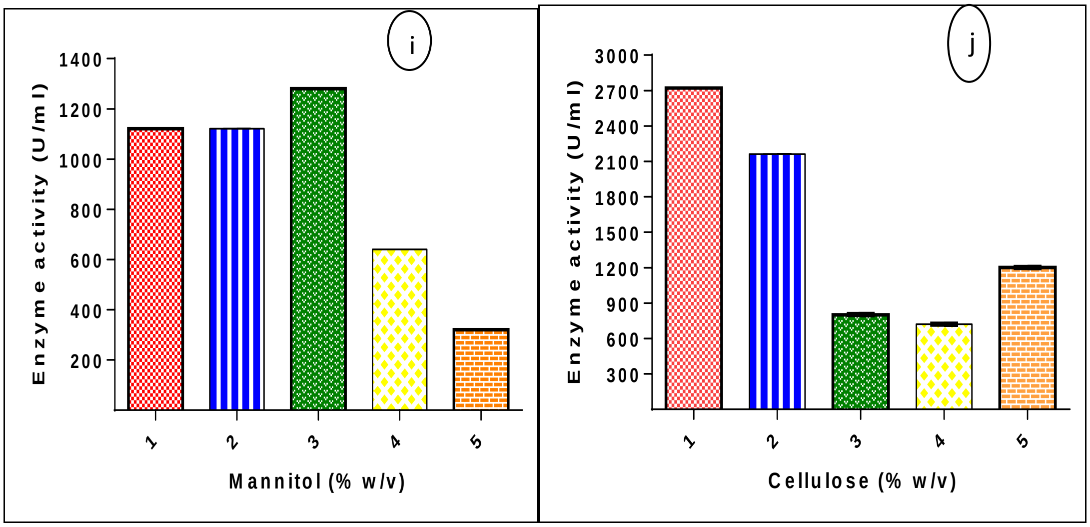


Chart -2: g-j. Effect of different concentrations of synthetic carbon sources on the production of α-amylase by B. borstelensis R1: g, Fructose; h, Dextrose; i, Mannitol and j, Cellulose.

4. DISCUSSIONS

The production of α-amylase was found to be highest in natural carbon supplement PK medium than synthetic carbon sources. The optimum production was found in natural additive of *Saccharum officinarum* (5%) when compared with other sources. In *Triticum vulgare* (4%) the production was the least. The production of amylase was found to be maximum in *B.borostelensis* R1 by the addition of 2% *Metroxylon sago* as reported by Yang & Liu [22]. *Triticum vulgare* add-on in optimized production with *Pencillium citrinum* HBF62 was reported by Djekrif-Dakhmouche *et al.*, [23].

In the synthetic carbon source supplement maltose (1%) and sucrose (4%) showed apex production of amylase, starch (2%), dextrose (3%) and galactose (4%) exhibited moderate effect on production whereas lactose (3%) and mannitol (3%) demonstrated production depletion. Narang & Satyanarayana [24] reported maltose as a provoking additive in some *Bacillus sps.* Different concentrations of maltose was reported by (1%) [25, 26], 0.5% [27]. In contrast maltose suppressed the production of α-amylase in *Bacillus coagulans* [28]. Sucrose as stimulant was described by Babu and Satyanarayana [29]. Starch in *Bacillus sp.* was found to be better augment to enhance amylase production as inquire into by Sodhi *et al.*, [30]. Starch supplement (2%) in PK medium gave maximum production in our studies. Similar findings were observed by Aditi *et al.*, [31]. Dextrose exhibited enhanced amylase production by *Bacillus sp.* in the circulate of Narang & Satyanarayana [24].Optimal production of amylase in different concentrations of dextrose 0.075% and 1.0% was examined by Salva & Moraes [32] and Lin *et al.*, [33] respectively. Lactose in *Bacillus sp.* was found to be enhancing the production as investigated by Hillier *et al.*, [34]. Lactose (3%) in PK medium has produced maximum production. In close relation with our studies Arunava *et al.*, [35] and Hamilton *et al.*, [36] reported lactose 2% and 4% respectively. Carlsen & Nielsen [37] had showed accelerating effect of mannitol in the amylase production in *Aspergillus oryzae.*

5. CONCLUSIONS

Natural carbon source Saccharum officinarum (5%) produced maximum α-amylase while Triticum vulgare (4%) produced very low α-amylase. Synthetic carbon source when supplemented with maltose (1%) and sucrose (4%) regulated higher production of amylase. Starch (2%), dextrose (3%) and galactose (4%) exhibited average effect on production whereas lactose (3%) and mannitol (3%) decreased production.

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BIOGRAPHIES

Dr. K. Suribabu, *Assistant Professor, PG Department of Microbiology and Research Centre, Dr.Lankapalli Bullayya PG College, Andhra Pradesh, India,Visakhapatnam-530 013,* ***ksuribabu_sda@yahoo.com***

Dr. T.Lalitha Govardhan, *Associate Professor, PG Department of Microbiology and Research Centre, Dr.Lankapalli Bullayya Post-graduate College, Andhra Pradesh, India, Visakhapatnam-530 013,* ***drlalithagovardhan@gmail.com***

Dr. K.P.J Hemalatha, *Professor, Department Microbiology College of Science and Technology, Andhra University, Visakhapatnam- 530 003, Andhra Pradesh, India,* ***hemalathakpj@gmail.com***