COMPARATIVE STUDIES ON SUCROSE AND GLUCOSE SUGARS ON L-ASPARAGINASE PRODUCTION, USING *STREPTOMYCES* SPECIES

Supported by tetfund

Casmir B. **Asikhia**[1], Courage G. **Dimowo**[2]
Department of Microbiology, University of Benin, P.M.B 1154, Benin City, Edo State, Nigeria.
Email: courage.dimowo@uniben.edu.

Received: September 14, 2023 **Accepted:** November 28, 2023

Abstract: Asparaginase is an enzyme that is used as a medication; it has been particularly studied for its applications as a chemotherapeutic agent in the treatment of human cancer. The aim of this study was to compare Sucrose and Glucose sugars in L-asparaginase activity using *Streptomyces* species. Starch casein agar was used in the isolation of *Streptomyces* species. L-asparaginase production was by using Czapek dox medium supplemented with sucrose and glucose sugar. Maximum enzyme activity was achieved when Czapek Dox medium was supplemented with sucrose as carbon source. The lowest optical density was 0.135 on day zero, while the peak optical density observed was 1.48 ± 0.0243 on day day 5. The lowest enzyme activity was 5.241± 0.1390 µmol/min/ml on day zero while the highest enzyme activity observed was 80.120 ± 0.1385 µmol/min/ml on day 5. Conclusively, the medium supplemented with sucrose, had higher optical density and l-asparaginase activity compared to glucose in this enzyme production.

Keywords: Activity, Asparaginase, Carbon, Chemotherapeutic, Density, Glucose and Sucrose.

Introduction

Microbial L-asparaginases have been particularly studied for their applications as a chemotherapeutic agent in the treatment of human cancer. It could either be produced as an intracellular or extracellular enzyme using appropriate nutrient medium. Extracellular L-asparaginase are produced by few actinomycetes, while cell bound l-asparaginase were reported by few actinomycetes such as *Streptomyces karnatakensis* and *Streptomyces albidoflavus* (Deshpande *et al.,* 2014).

Some effective carbon sources for the production of microbial metabolites such as enzymes are sucrose, glucose fructose (Shanthipriya *et al.,* 2015). In previous research it has being stated that out of nine carbon sources that can be used in L-asparaginase production, glucose was the favorite followed by sucrose (Poorani *et al.*, 2009).

Microbial growth describes an increase in cell number. Provided with the right conditions, (temperature, pH, water availability/activity) microbes can multiply very quickly. While growth for multicellular organisms is typically measured in terms of increase in size of a single organism, microbial. Growth can be measured by the increase in population either by measuring the increase in cell number or the increase in overall cell mass (Dimowo *et al.,* 2021). The production of secondary metabolites (L-asparaginase) using *Streptomyces* species is greatly influenced by various fermentation parameters such as available nutrients, pH, temperature, agitation, mineral salts, metal ions, precursors, inducers and inhibitors. pH is the most essential parameters control in enzyme secretions because microbes are sensitive to the concentrations of hydrogen ions present in the fermentation process (Bibbs 2005; Geshava *et al.*, 2005). In this study, comparative studies on sucrose and glucose sugars on l-asparaginase production, using *Streptomyces* species were carried out.

Materials and Methods

Isolation of Microorganism (*Streptomyces* species)

Isolation and enumeration were carried out by serial dilution and pour plate method. Serial dilution was carried out by taking 25 g of soil sample and mixed with 225 ml of sterile distilled water. The mixture was agitated for 1 hr at 1000 rpm using a water bath to enable the dislodge of microorganisms. Serial dilution was carried out and 1 ml sample was transferred to a sterile labeled petri plate and the molten agar was poured onto it and it was allowed to solidify. The plates were inverted and incubated at 37 °C for 3 to 4 days (Luhana *et al.*, 2013)

Purification of Isolates

Pure culture of *Streptomyces* species were obtained by using the streak plate method, here a sterile straight wire was used to pick the isolates and inoculated on a fresh Petri dish containing solidified starch casein agar. The pure cultures were streaked carefully in a zig zag pattern. The culture were further incubated for 3 to 4 days at 37 °C (Dimowo and Omonigho 2017)

Identificaton of isolates: Identification of isolated *Streptomyces* species was carried out according to Bergey's Manual of Determinative Bacteriology (Bergey and Holt 2000). Colony morphology, Spore arrangement, Gram staining reactions were used to identify the isolates.

Production of l-asparaginase:

Isolates were screened using the rapid plate method; positive isolate for L-asparaginase production was used. Production of L-asparaginase was carried out in Erlenmeyer flask containing Czapek Dox broth supplemented with L-asparagine (1g/L), 30 g Sucrose, 30 g Glucose and a control containing no carbon source as production medium for 7 days at 120 rpm and at 28 ºC. A 5 % inoculum was used. Enzyme activity was determined every 48 hr, by withdrawing 5 ml of sample. The cell free supernatant was used for estimating the extracellular enzyme activity (El – Naggar *et al.*, 2018).

Assay for l-asparaginase:

Enzyme assay was carried out at intervals. 5ml of the production medium was withdrawn using a sterile pipette and carefully dispensed into a bottle using cuvette, the

optical density was checked spectrophotometrically at 600 nm. The sample was then centrifuged at 120,000 rpm. The supernatant was then used for the assay. 0.5 ml enzyme solution was added to 0.9 ml buffer (pH 8.5) and the amino acid L-asparagine. It was incubated at 37 °C for 10 mins, thereafter, 0.5 ml of TCA was added to stop the reaction and precipitate the different protein molecule. The sample was then centrifuged thereafter, 50 µl of supernatant was collected and placed in a clean tube. 2000 µl of distilled water was added to the supernatant with 250 µl of Nessler's Reagent and 250 µl NaOH solution was added and the solution was left for 15 mins. Using the spectrophotometer, the result was read at 600 nm. Ammonia standard curve was prepared. L-asparaginase Activity was calculated (Dimowo *et al.*, 2021).

Results / Discussion

The results of optical density of *Streptomyces* species in L-asparaginase production are presented in Table 1. Sucrose, had the highest optical density of 1.48^{c} ± 0.0243 and Glucose had the least optical density of 0.686^{b}±0.0193 on day 5. Sucrose had the highest optical density of 0.984^{b} ± 0.0031 on day 2 of production and glucose had the least optical density of 0.261^{a} ± 0.0520. The isolate reached death phase on day 5 of production (Fig. 1).

Table 1: Optical Density of *Streptomyces* species in L-asparaginase production

SOURCE	DAY 0 Mean ± S.D	DAY 3 Mean ± S.D	DAY 5 Mean ± S.D	DAY 7 Mean ± S.D
CONTROL	0.166^{a}±0.0115	0.603^{b}±0.0012	1.544^{c}±0.0010	0.512^{b}±0.0010
SUCROSE	0.135^{a}±0.0063	0.984^{b}±0.0031	1.495^{c}±0.0243	0.844^{b}±0.0017
GLUCOSE	0.081^{a}±0.0514	0.261^{a}±0.0520	0.686^{b}±0.0193	0.653^{b}±0.0010

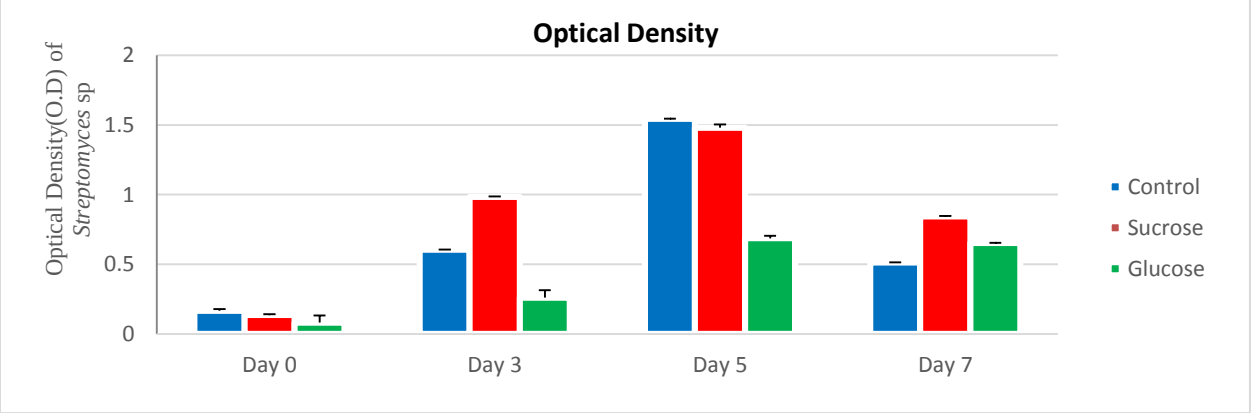

Figure 1: Optical density of *Streptomyces* sp. grown in CZAPEK Dox medium supplemented with sucrose and glucose. Values are in mean ± s.d

Table 2 explains the determination of L-asparaginase activity; Glucose had enzyme activity of 65.566^{b} ± 0.3784 µmol/min/ml, while Sucrose had highest values of 80.120^{c} ± 0.1385 µmol/min/ml L-asparaginase activity respectively on day 5 of production. Mean values with similar superscripts within each column are not significantly different. This showed that there were no significant difference in L-asparaginase activity for all the isolate on day 0.

Table 2: L - asparaginase activity (µmol/min/ml) of *Streptomyces* species.

SOURCE	DAY 0 Mean ± S.D	DAY 3 Mean ± S.D	DAY 5 Mean ± S.D	DAY 7 Mean ± S.D
CONTROL	5.636^{a}±0.1820	12.514^{b}±0.1390	29.151^{a}±0.1384	17.545^{b}±0.4809
SUCROSE	$5.24^{a}1$±0.1390	6.818^{a}±0.0910	80.120^{c}±0.1385	22.605^{c}±0.1390
GLUCOSE	6.696^{a}±0.1390	12.514^{b}±0.1390	65.566^{b}±0.3784	15.666^{a}±0.1390

The growth of *Streptomyces* in the course of producing L-asparaginase followed a normal growth pattern, with Lag phase, growth/exponential phase, stationary phase and death phase. From figure 1, it can be deduced that *Streptomyces* species maintained a reasonable level of lag phase where the organism tend to adjust to environmental factors and the medium. The highest growth level reached stationary phase on day 5 and a decline /death phase after day 5 to 7. According to Shanthipriya *et al.,* (2015), at stationary phase the growth rate and death rate are equal. The stationary phase is the phase at which growth ceases, but cells remains metabolically active and produce secondary metabolites. The proteins synthesized at this phase are necessary as they confer viability to the organisms. Sucrose and glucose are some of the most effective carbon sources for the production of L-asparaginase. Simple sugar glucose is useful for production of good yield of L-asparaginase. Out of nine carbon sources that can be used in L-asparaginase production, glucose is the favourite followed by sucrose (Poorani *et al.,* 2009).

In this study, the two carbon sources used were sucrose and glucose sugars, along with a blank sample to act as Control. They were supplemented in modified Czapek Dox medium to determine their impact on L-asparaginase activity and optical density of the isolate *Streptomyces* species. Compared with the carbon sources tested, L-asparaginase activity was high in presence of Sucrose. Comparable results were also obtained in the medium with no Carbon source present (Control).

In observation of the optical density, production medium supplemented with sucrose had the highest absorbance reading of 1.495^{c}±0.0243 at 600 nm on day 5 and the lowest absorbance reading of 0.135^{a} ± 0.0063 on day 0. Compared to the medium supplemented glucose with its highest absorbance reading of 0.686 on day 5 with the lowest absorbance reading of 0.081 on day 0. It therefore means that the organism was able to utilize sucrose more in carrying out its metabolic activities. In contrast with experiments carried out in previous studies by Dimowo *et al.,* (2021), considering the optical density, *Actinosynnema mirum* supplemented with glucose had absorbance reading of 1.965 ± 0.001 at 600 nm on day 6. Its reducing sugar concentration reduced from 47.88 ± 0.16 mg/ml to 6.77 ± 0.06 mg/ml; it means therefore that the organism was able to utilize glucose to carry out its metabolic activities. Also, *Streptomyces nobilis* maintained a gradual growth level from day 0 to day 3 and a short stationary phase. Day 5 marked the beginning of the death phase L- asparaginase activity of the different isolates increased from day 0 to day 2, meaning that the organisms were able to utilize the amino acid during the exponential phase. The peak of this enzyme activity was on day 2 for all the marine actinomycetes, *Streptomyces avermitilis* had the highest value of 20.99 ± 0.58 µmol/min/ml and the lowest being *Streptomyces nobilis*.

However, in this study the isolated *Streptomyces* supplemented with sucrose had a small increase in growth level from Day 0 to Day 3. An exponential increase was observed on day 5, and a short stationary phase was observed. The death phase occurred after Day 5 down to day 7. L-asparaginase activity of sucrose and glucose showed increase from Day 3 to Day 5, although glucose had no lag phase in enzyme activity. The peak of enzyme activity was on day 5 for all carbon sources tested, sucrose had the highest enzyme activity of 80.120^{c}±0.1385 µmol/min/ml while glucose had the lowest enzyme activity of 65.566^{b}±0.3784 µmol/min/ml. A unit of L-asparaginase is the amount of enzyme that can breakdown or catalyze 1 µmole of L-asparagine to aspartic acid and ammonia (Dimowo *et al.,* 2021).

In this study, all the parameters (Enzyme activity, Optical density) examined showed a considerable impact on L-asparaginase production by the isolate, *Streptomyces* sp. highest enzyme activity was achieved when Czapek Dox medium was supplemented with sucrose as carbon source. The peak Enzyme Activity observed was 80.120 ^{c}±0.1385 µmol/min/ml on day 5, as shown in figure 1. During the enzyme production process, the highest Optical Density observed was 1.495^{c}±0.0243 on day 5, as shown in figure 2.

The present work was aimed at comparing sucrose and glucose for the activity of L-asparaginase and not the isolation or characterization of the enzyme. Moreover the work does not include investigation of the anticancer effect of L- asparaginase.

Conclusion

While both carbon sources used in this experiment showed significant effect on L-asparaginase production by *Streptomyces* species, sucrose was shown to be the most favourable of the two carbon sources. This study shows that sucrose produced more enzymes, has more enzymatic activity and a higher Optical density when it was supplemented in Czapek Dox medium for the production of L-asparaginase using *Streptomyces* species. The control (sample without an external carbon source) produced similar result as the medium supplemented with sucrose. However, environmental and chemical factors as well as period of incubation and medium used could influence the outcome of the experiment.

Acknowledgement

We sincerely appreciate the Department of Microbiology, University of Benin, Benin City, Nigeria.

Conflict of Interest

There were no conflict of interest during and after this study.

References

Bergey D H I and Holt, J G. 2000. *Bergey's manual of determinative bacteriology*. 9th edn Philadelphia, Lippinott Williams and Wilkins

Bibb M J, 2005. Regulation of secondary metabolism in *Streptomycetes*. *Curr. Opinion in Microbiol.*, (8)**:** 208-215.

Deshpande N, Choubey P. and Agashe M. 2014. Studies on optimization of growth parameters for L-asparaginase production by *Streptomyces ginsengisoli*. *The Sci. World J.* (9) 2: 1-6

Dimowo C G, Omonigho S E, Oshoma C E and Oyedoh O P. 2021. Evaluating the Concept of Reducing Sugar (Glucose) and Growth in L-Asparaginase Production Using Some Selected Marine Actinomycetes. *Afri. Scien.*, (3): 105-112.

Dimowo C G. and Omonigho S E. 2017. Microbiological analysis of Vegetables sold in open markets in Benin City. *Nig. J. Life Sc.* **7** (1):169-176.

El – Naggar N E, Deraz S F. and El. Ewasy, S M. 2018. Production of *L*-asparaginase by newly isolated *Streptomyces* species from Egyptian soils using biotechnology approach. *Pharm. and Toxicol.* **19** (51):1-15.

Geshava V, Ivanova V B, Gesheva R. 2005. Effect of nutrients on the production of AK- 111-81 macrolide antibiotic by *Streptomyces hygroscopicus*. *Microbiol. Res.*, **110** (3): 243-248.

Luhana K K, Dave, A A, Patel, K U 2013. Production, purification and characterization of extracellular L-asparaginase (anti cancerous enzyme) from *Aspergillus niger*. *Int J of Chem Appl* **2** (3): 14 – 25, 2013.

Mishra A, Das M D. 2002. Survival and applications. *J. of Appl. Biochem. and Biotechnol.* **102** (18)**:** 193–199.

Poorani E, Saseetharan M K and Dhevagi, P. 2009. L-asparaginase production and molecular identification of marine *Streptomyces* species strain EPD 27". *Int. J of Integ Biol*. **7:**150–155.

Shanthipriya A, Koteswara R V, Girisham S, Reddy SM 2015. Factors influencing L-asparaginase production by three thermophilic caprophilous fungi. *Res. in Pharma. Sci*. **5** (5):12-1248

.