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Using fruit peel as a substrate for the isolation, biosynthesis, and optimization of glucoamylase by *Bacillus* sp. under submerged fermentation

Ayesha Kiran[1], Memuna Ghafoor Shahid[1], Najeeb Ullah[2], Abid Sarwar[2], Samraz Tahir[1], Muhammad Nadeem[2], Rubina Nelofer[2], Ayaz Ali Khan[3], Tariq Aziz[4]*, Ashwag Shami[5], Fahad Al-Asmari[6], Fakhria A. Al-Joufi[7]

[1]Department of Botany, Government College University, Lahore, Punjab, Pakistan; [2]Food and Biotechnology Research Center, PCSIR Labs Complex, Gulberg III, Lahore, Pakistan; [3]Department of Biotechnology, University of Malakand Chakdara Dir Lower Khyber Pakhtunkhwa Pakistan; [4]Laboratory of Animal Health Food Hygiene and Quality, University of Ioannina – Arta Campus, Arta, Greece; [5]Department of Biology, College of Science, Princess Nourah bint Abdulrahman University, P.O. Box 84428, Riyadh 11671, Saudi Arabia; [6]Department of Food and Nutrition Sciences, College of Agricultural and Food Sciences, King Faisal University, Al Ahsa, Saudi Arabia; [7]Department of Pharmacology, College of Pharmacy, Jouf University, 72341 Aljouf, Saudi Arabia

***Corresponding Author:** Tariq Aziz, Laboratory of Animal Health Food Hygiene and Quality, University of Ioannina – Arta Campus, Arta, Greece. Email: iwockd@gmail.com

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ORIGINAL ARTICLE

Abstract

Glucoamylase is a fungal enzyme easily obtained by using a fungal strain—*Aspergillus niger*. The aim of this study was to isolate the bacterial strain and culture condition optimization to produce glucoamylase. This optimization was achieved under the submerged fermentation technique where fruit peel was used as substrate. The production of glucoamylase was carried out by using (w/v [weight in volume]) yeast extracts 1%, NaCl (chemical formula for sodium chloride) 0.5%, $ZnSO_4$ (zinc sulfate) 0.5%, K_2HPO_4 (dipotassium phosphate) 0.5, and 1% fruit peel powder as substrate. The optimization of parameters was carried out by one factor at a time technique (OFAT) under submerged fermentation. Different carbon and nitrogen sources were screened for maximum enzyme activity. The physical and chemical parameters such as temperature, pH (potential of hydrogen), inoculum size, and salt concentrations were optimized to achieve the highest production. Based on the biochemical characterizations, the isolated strain was identified as *Bacillus* sp. FBA1 (fructose-bisphosphate aldolase 1). Maximum production of the enzyme was recorded at the 24th hour at 37°C temperature and pH was kept at 7.0. The salts combinations that produced the highest levels of glucoamylase production from *Bacillus* strain FBA1 were (g/L) NaCl 1, $ZnSO_4$ 1.5, $FeSO_4$ (ferrous sulfate) 2, KH_2PO_4 (potassium dihydrogen phosphate) 2, $CaCl_2$ (calcium chloride) 0.1, and $MgSO_4$ (magnesium sulfate) 2. The optimum glucoamylase activity of 2.984 mg/mL/minute for FBA1 was obtained at pH 7 at 37°C. The results showed that fruit peels can be exploited as a potential substrate to produce glucoamylase at optimized conditions by *Bacillus* FBA1.

Keywords: *Bacillus*; fruits peel; submerged fermentation; optimization; glucoamylase

Introduction

Protease and amylase are the most valuable microbial enzymes, and they have been used extensively and in a variety of applications due to their ability to break down organic waste. which include biofuels, agriculture, pharmaceuticals, chemicals, and biotechnological industries (Ullah *et al.*, 2022). Glucoamylase is an extracellular enzyme that is next to protease in ranking among the industrial enzymes' distribution. Glucoamylase comes from a variety of sources, including microbial, animals, and plants. Up until the 1970s, enzymes from plants and animals were thought to be considerable, but today enzymes of microbial sources are gaining more popularity (Cherry *et al.*, 2004). Yeast, mold, and bacteria are some of the microorganisms with a higher capacity to produce glucoamylase. The enzymes obtained from these microbial sources are more beneficial than the enzymes obtained from animals and plants (Najafpour *et al.*, 1994). Because of their shorter lifespan, microbial sources are advantageous for the isolation of glucoamylase. Further, enzymes isolated through these sources can easily be obtained and isolated, and their characteristics altered by biotechnology and genetic engineering techniques.

Glucoamylase is a fungal enzyme and easily obtained by using a fungal strain—*Aspergillus niger*. On an industrial scale, glucoamylase can be produced by the members of genus *Aspergillus, Rhizopus*, and *Bacillus* (Bennett, 1998). Although filamentous fungi are a preferred choice to obtain maximum yield on an industrial scale, bacilli bears an edge due to it belonging to a house of thermostable proteins (Bajpai and Bajpai, 1989). Bacilli can also produce enzymes with desirable properties. Almost all species of genus *Bacillus* have the potential to produce different enzymes (Pretorius *et al.*, 1986). The most important stains of *Bacillus* widely used for the production of enzymes are: *Bacillus licheniformis* (Fogarty and Kelly, 1990), *B. amyloliquefaciens, B. stearothermophilus* (Wind *et al.*, 1994), *B. subtilis* (Kumar, 2009), and *B. megaterium*.

Bacteria are an ideal organism for industrial production due to their faster rate of development and simplicity in genetic modification. The cost-effectiveness of the process would increase with the use of thermostable glucoamylase in the starch business due to the possibility of it producing more starch with prolonged enzymatic catalysis (Sauer *et al.*, 2000). The generation of glucoamylase is greatly influenced by the types and concentrations of the carbon and nitrogen sources (Fadel *et al.*, 2020). Behera *et al.* (2021) studied the impact of adding carbon, nitrogen, and phosphorus on the synthesis of glucoamylase and demonstrated that the medium in solid-state fermentation required the addition of urea, maltose, wheat bran, and potassium biphosphate to produce the most enzymes. The type and concentration of nutrients that are available to the organism affect how an enzyme is produced. Other nutrients may be similarly crucial to the composition of the medium as a sufficient carbon supply. According to Ellaiah *et al.* (2002), solid-state fermentation can produce glucoamylase using urea as a nitrogen source. According to Behera *et al.* (2021), adding yeast extract at a very low concentration to a medium containing starch and $(NH_4)_2SO_4$ (ammonium sulfate) enhances the production of glucoamylase enzyme by *A. niger* during the submerged fermentation technique. The medium, including starch, wheat bran, $(NH_4)_2SO_4$, FeSO4 (ferrous sulfate), $MgSO_4$ $7H_2O$ (magnesium sulfate heptahydrate), and 0.1N HCl (hydrochloric acid with a normality of 0.1), was said to produce the most glucoamylase.

The induction of the glucoamylase production can be significantly influenced by the pH (potential of hydrogen) and temperature levels (Bertolin *et al.*, 2003; Mishra and Debnath, 2002). Instead of keeping pH at a constant value, there is the possibility of manufacturing glucoamylase under different pH values by using the batch cultivation method. This enzyme was most active at 65°C temperature and 5.0–5.5 pH range (Mamo and Gessesse, 1999), and at 45°C, glucoamylase enzyme produced by *A. niger* was stable. Nearly 80% of the enzyme's activity was lost within 75 minutes after the enzyme began to inactivate at 60°C (Ramadas *et al.*, 1996) It turns out that today, glucoamylase is one of the most crucial enzymes in the food industry due to the fact that it is utilized to make glucose syrup from liquid starch (Dale and Langlois, 1940; Nguyen *et al.*, 2002; Nigam and Singh, 1995). Moreover, it is used to produce numerous fermented foods for commercial use in the baking, juice, beverage, pharmaceutical (Fogarty and Kelly, 1990; Hesseltine, 1965), and in some cases, textile, leather, and detergent sectors (Vishnoi *et al.*, 2020).

Food wastes can be used as feedstock in bioproducts to produce enzymes like glucoamylase due to their high protein and carbohydrate content. This is a creative waste management method. In many biotechnological processes, glucose serves as the main carbon source. Nevertheless, depending on where they come from, fruits and vegetables waste are abundant in moisture, carbohydrates, and more. Some fruits and vegetables waste also contain significant amounts of proteins, lipids, and natural colorants, and occasionally antioxidants and other beneficial components, in addition to hydration and carbs. A growing interest in the generation of biochemical from food waste has been observed recently (Lam *et al.*, 2013). Today, submerged fermentation is used for the synthesis of enzymes more all over the world; this method has a lot of potential for producing biocatalysts effectively. It is a low-cost technique that produces a lot of output per volume of the reactor and an easy subject for culture management and downstream operations.

Additionally, when crude extracts are applied directly and when the purity of the enzymes is not required, this technique is employed for the production and usage of enzyme (Abdollahi *et al.*, 2021; Shoji *et al.*, 2007). Submerged fermentation has several advantages over traditional solid-state fermentation, one of which is that 90% of all commercial enzymes are extracted from microbes that have been optimized and genetically modified using submerged fermentation (Panpatte and Jhala, 2019)

The development of the genetically engineered microbial strains, the optimization of growth settings, and the choice of suitable microbial strains can all help in increasing and optimizing the production of required enzymes and other primary and secondary metabolites. Further optimization of growth media ingredients helps in improving the effectiveness of the cultivation process (Kumar *et al.*, 2007). The main objective of this study was to isolate glucoamylase producing strain from soil samples and the production of glucoamylase under submerged fermentation using potato peel as substrate.

Material and Methods

Source of bacterial strain

Soil sample for the isolation of bacterial strain for the production of glucoamylase was collected from selected site in PCSIR Laboratories Complex in Lahore, Pakistan.

Isolation of glucoamylase producing Bacillus strain

A 1 g soil sample was weighed and transferred into 9.0 mL sterile saline. Serial dilutions were prepared by transferring 1.0 mL from $l0^{-1}$ dilution in to separate tubes of up to 10^{-6}. The first four test tubes were discarded, and then the fourth and fifth dilutions were processed under heat shock at 80^{o}C for 15 minutes. This step ensures that if there is any bacterial stain, then it must be of genus *Bacillus.* Further, 0.1 mL of the sample was transferred to sterilized plates and starch agar medium composition (starch 10 g/L, peptone 5g/L, NaCl [chemical formula for sodium chloride] 2.5 g/L, MgSO4 [magnesium sulfate] 2.5 g/L, and agar 15 g/L) were added. The inoculated plates were incubated at 37^{o}C for 24 hours. After incubation, different colonies appeared, after which the morphologically contrastively colonies were further purified by streaking on nutrient agar medium plates for isolation of pure colonies.

Screening of glucoamylase strain

The pure stains were further streaked on the starch agar medium and incubated for 24 hours. Then the iodine solution was flooded on petri plates with bacterial colonies for the identification of bacteria. When iodine reacts with starch, a dark brown color appears. Thus, starch hydrolysis gives a clear zone around the bacterial colony.

Identification of glucoamylase producing strain

Biochemically and morphologically, the strain was identified by the recommended protocol of Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 1994) and Diagnostic Microbiology (Betty *et al.*, 2002).

AgroIndustrial waste collection

Agroindustrial waste in the form of fruit peels (i.e., apples, bananas, pomegranates, and oranges) were collected as substrate from the vegetable and fruit markets of Lahore.

These peels were dried in an oven for 72 hours and then the dried leaves were ground and the powdered extract of the peel were stored for further use in the production of glucoamylase.

Production medium

The production of glucoamylase was carried out by using (w/v [weight in volume]) yeast extract 1%, NaCl 0.5%, $ZnSO_4$ (zinc sulfate) 0.5%, K_2HPO_4 (dipotassium phosphate) 0.5%, and 1% fruit peel powder as substrate. The initial pH of the medium was adjusted as 7.0 of the fermentation medium. Initially, the inoculums were added (2% v/v [volume per volume]) and incubated at 37°C for 48 hours. After incubation, the fermented broth was centrifuged at 9000 rpm for 10 minutes with the supernatant used as crude enzyme.

Production of glucoamylase

Different chemicals and physical parameters were optimized. Optimization of fermentation conditions was carried out in a stepwise manner for maximum enzyme production from *Bacillus* sp. FBA1 (fructose-bisphosphate aldolase 1). Selection of components, including carbon, nitrogen, and mineral components were based in batch fermentation on a one factor at a time (OFAT) methodology.

Screening of carbon and nitrogen sources

Different carbon sources—maltose, galactose, glucose, fructose, and fruit peels—and nitrogen sources—beef extract, peptone, yeast extract, urea, ammonium

sulphate, ammonia solution—were screened to obtain suitable carbon and nitrogen source for glucoaylase production.

Optimization of different physiochemical parameters

The optimization of parameters was carried out by the OFAT under submerged fermentation. The effects of various parameters were studied independently.

The effect of various pH on the productivity of glucoamylase enzyme

Various pH such as 3, 4, 5, 6, 7, and 8 were maintained using 1N NaOH (sodium hydroxide solution with a normality of 1) and 1N HCl solution with the help of pH meter in the fermentation medium to observe the maximum yield of glucoamylase enzyme. Comparative optimization was being observed, with each bacterial strain of six replicates.

The effect of various incubation temperature on the productivity of glucoamylase enzyme

Fermentation medium for the optimization of temperature was placed at various incubating temperature—30°C, 35°C, 40°C, and 45°C—for 24 hours in a shaking incubator using submerged fermentation to observe the maximum yield of glucoamylase enzyme. Comparative optimization was being observed, with each bacterial strain of six replicates.

The effect of various inoculum size on the productivity of glucoamylase enzyme

For the optimization of inoculum size, different cementation of inoculum medium—10 mL, 15 mL, 20 mL, and 25 mL—was poured in to the fermentation medium and placed for 24 hours in a shaking incubator using submerged fermentation to observe the maximum yield of glucoamylase enzyme. Comparative optimization was being observed, with each bacterial strain of six replicates.

The effect of various KH_2PO_4 on the productivity of glucoamylase enzyme

Different concentration of KH_2PO_4 (potassium dihydrogen phosphate) were measured—0.1, 0.5, 1, 1.5, 2, and 2.5—and added while keeping the other concentration of fermentation ingredients the same, and then placed for 24 hours in a shaking incubator using submerged fermentation to observe the maximum yield of glucoamylase enzyme. Comparative optimization was being observed, with each bacterial strain of six replicates.

The effect of various $MgSO_4$ $7H_2O$ on the productivity of glucoamylase enzyme

Different concentration of $MgSO_4$ were measured—0.1, 0.5, 1, 1.5, 2, and 2.5—and added while keeping the other concentration of fermentation ingredients the same and then placed for 24 hours in a shaking incubator using submerged fermentation to observe the maximum yield of glucoamylase enzyme. Comparative optimization was being observed, with each bacterial strain of six replicates.

The effect of various $FeSO_4$ H_2O on the productivity of glucoamylase enzyme

Different concentration of $FeSO_4$ H_2O (ferrous sulfate monohydrate) was measured—0.1, 0.5, 1, 1.5, 2, and 2.5—and then added while keeping the other concentration of fermentation ingredients same. Fermentation medium were placed for 24 hours in a shaking incubator using submerged fermentation to observe the maximum yield of glucoamylase enzyme. Comparative optimization was being observed, with each bacterial strain of six replicates.

The effect of various $CaCl_2$ on the productivity of glucoamylase enzyme

Different concentration of $CaCl_2$ (calcium chloride) were measured—0.1, 0.5, 1, 1.5, 2, and 2.5—and added while keeping the other concentration of fermentation ingredients same. Then the medium was placed for 24 hours in a shaking incubator using submerged fermentation to observe the maximum yield of glucoamylase enzyme. Comparative optimization was being observed, with each bacterial strain of six replicates.

The effect of the various concentration of NaCl on the productivity of glucoamylase enzyme

Different concentration of NaCl were measured—0.1, 0.5, 1, 1.5, 2, and 2.5—and added while keeping the other concentration of fermentation ingredients the same. Then the medium was placed for 24 hours in a shaking incubator using submerged fermentation to observe the maximum yield of glucoamylase enzyme.

The effect of various ZnSO4 on the productivity of glucoamylase enzyme

Different concentration of $ZnSO_4$ was measured—0.1, 0.5, 1, 1.5, 2, and 2.5—and added while keeping the other concentration of fermentation ingredients same. Then the medium was placed for 24 hours in a shaking incubator using submerged fermentation to observe the maximum yield of glucoamylase enzyme. Comparative optimization was being observed, with each bacterial strain of six replicates.

Analytical procedure

Enzyme assay

A 1 mL supernatant was taken from each flask in to sterilized test tubes with same labelling, and 1 mL starch

solution was added in it. The test tubes were placed in an incubator at 37°C for 25 minutes. After incubation, 1 mL of DNS (dextrose normal saline) were added in to each test tube and placed in boiling water for 5 minutes; then 1 mL of distilled water was added in the supernatant samples.

In a 250 mL beaker, 60 mL distilled water was taken, and 1 g DNS was added. This solution was stirred until a clear solution of pale-yellow color was obtained. Then 30 g of potassium sodium titrate was added slowly and stirred well until no crystals remained. Meanwhile, 2N NaOH (sodium hydroxide solution with a normality of 2) was prepared by dissolving 1.6 g of NaOH pellets in 20 mL distilled water. Then, 2N NaOH was added in to the potassium sodium titrate solution and stirred continuously. The final volume was raised to 100 mL and stored in a dark bottle at 4°C.

Statistical analysis

Different parameters were statistically analyzed by one-way ANOVA for comparisons to record the data by using standard error and standard deviation in order to reach the accurate conclusion. Duncan's multiple range tests were used to compare the significant differences in the mean values at $P \leq 0.05$. (Steel and Torrier, 1996).

Results

The two main barriers to the effective application of any technique in the enzyme industry are the overall cost of enzyme manufacturing and downstream processing. Enzymes are typically produced when using any microbial organism with various fermentation procedures, such as solid-state and submerged. The separation of the necessary enzyme is expensive when employing a solid-state fermentation method for glucoamylase synthesis in comparison to a liquid phase system.

Optimization of fermentation condition for maximum glucoamylase production from *Bacillus* sp.

The two main barriers to an effective application of any technique in the enzyme industry are the overall cost of enzyme manufacturing and downstream processing. Enzymes are typically produced when using any microbial organism with various fermentation procedures, such as solid-state and submerged. The separation of the necessary enzyme is more expensive when employing a solid-state fermentation method for glucoamylase synthesis in comparison to a liquid phase system (Imran *et al.*, 2012; Negi and Banerjee, 2009).

Since this process is frequently used for industrial-scale enzyme synthesis and also simple to isolate the required product from the entire fermentation broth, it is frequently utilized when working with bacterial species. Therefore, submerged batch fermentation approach was used for the synthesis of glucoamylase by *Bacillus* strain FBA1. The fermentation conditions were produced using the OFAT technique.

Effect of various pH on the production of glucoamylase

Figure 2 shows the effect of pH on the glucoamylase production from *Bacillus* sp. The pH of the medium was increased from 3.0 to 8.0. The production of enzyme was observed as maximum (0.073 mg/mL/minute for *Bacillus* strain) at 7.0 pH. When the pH of the medium was

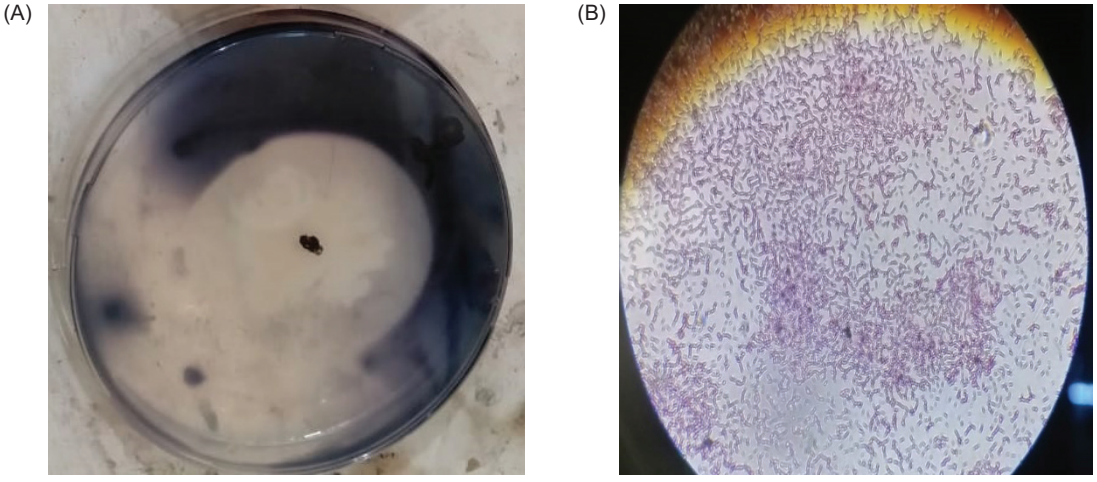

Figure 1. (A) Iodine test and (B) microscopic identification of *Bacillus* sp.

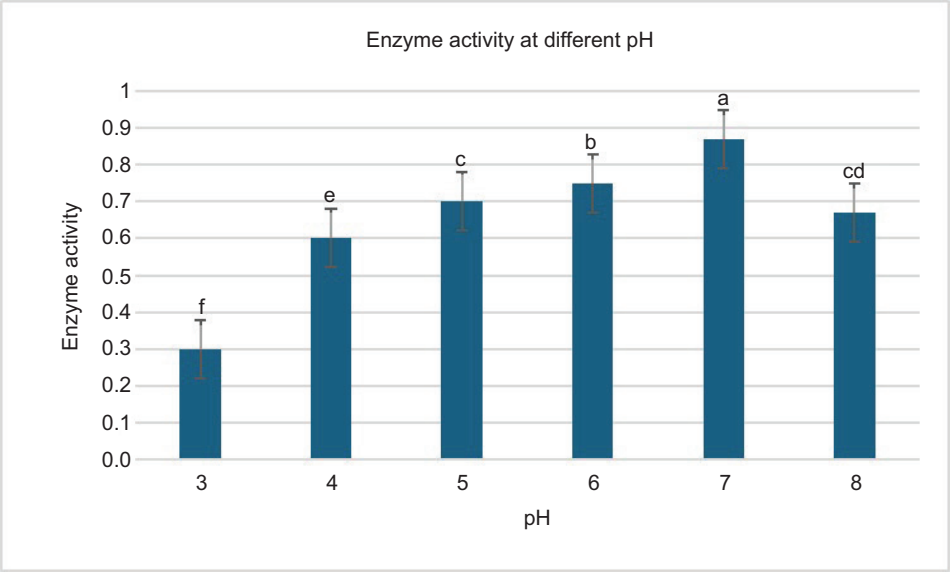

Figure 2. Optimization effect of pH.

affected from the pH value of 7.0, the yield of the enzyme reduced. At 3.0 pH, the production of the enzyme was low (0.03 mg/mL/minute), and at 8.0 pH, the glucoamylase production was low (0.07 mg/mL/minute). Therefore, optimized pH for the production of enzyme was 7.0 by *Bacillus* strain.

Effect of incubation temperature

In the production of an enzyme temperature plays an important role. The effect of incubation temperature on glucoamylase synthesis was observed for both *Bacillus* sp. was incubated in fermentation broth at different temperatures. The Figure 3 depicts the effect of temperature on the production of glucoamylase. A noticeable difference in the production temperature for both the stains was observed. The initial temperature of the medium was varied from 30°C–45°C. Initially, at 30°C the glucoamylase production was extremely low i.e., 0874mg/mL/min

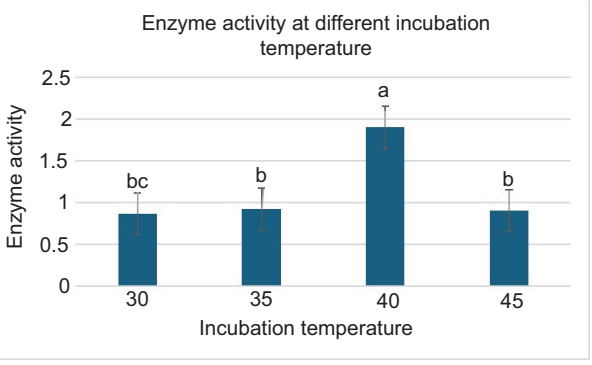

Figure 3. Optimization effect of temperature.

(FBA1 strain) When the temperature was raised up to 40°C, the glucoamylase production was also increased. The production of enzyme was observed maximum (1.9mg/mL/min) at 40°C temperature. After this temperature, glucoamylase production decreased due to increase in the temperature and at 45°C the production of the enzyme drastically decreased i.e., 0.913mg/mL. Therefore, 40°C was optimized incubation temperature for the production of glucoamylase enzyme by *Bacillus* strain.

The development of the bacteria and the synthesis of the enzyme are both significantly influenced by the choice of the optimal temperature. In this study, the impact of incubation temperature on enzyme production by FBA1 strains was examined. It was discovered that the maximal glucoamylase production occurred at 40°C. The synthesis of enzymes ultimately decreased as the incubation temperature was further raised. The biomass eventually ran out to create a substantial amount of enzyme since the growth of the bacteria was temperature-dependent, and a change in the ideal temperature slowed down the growth rate of *Bacillus* FBA1. The results of other researchers were different from this study—they reported 30°C as optimum incubation temperature for the production of glucoamylase (Mamo and Gessesse, 1999; Mishra and Debnath, 2002).

Effect of various inoculum sizes

The Figure 4 shows the effect of different inoculum sizes on the glucoamylase production by *Bacillus* sp. in the shaking fermentation. For inoculation, a 16-hour-old vegetative inoculum was used at different levels 10 mL,

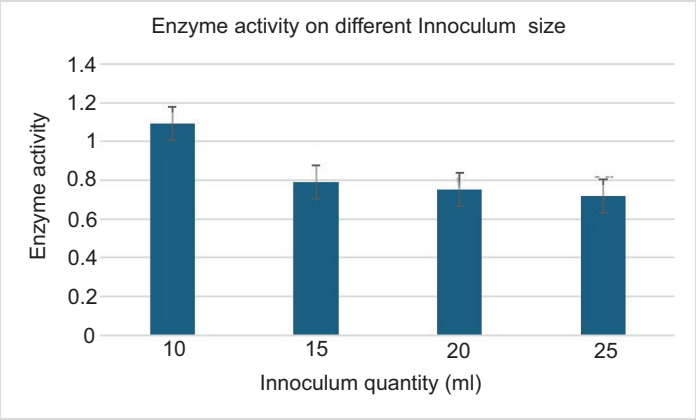

Figure 4. Optimization effect of inoculum size.

15 mL, 20 mL, and 25 mL (v/v). The enzyme production decreased with an increase in the size of inoculum from 10 mL to 25 mL. At 10 mL, the inoculum size increased with the maximum amount of glucoamylase produced (1.092 mg/mL/minute) after 24 hours.

When the size of inoculum was further raised, the production of glucoamylase decreased, although the maximum yield of the enzyme was produced after 24 hours at 10 mL inoculum size. Hence, 10 mL inoculum of *Bacillus* sp. strain produced maximum amount of the enzyme.

The effect of nitrogen and carbon sources

A medium containing organic nitrogen sources stimulated both the growth of bacteria and enzyme secretion as compared to inorganic nitrogen sources. Of the various nitrogen sources tested in this study, significant growth and enzyme yield was achieved in the medium containing yeast extract (1.955 mg/mL/minute) for *Bacillus* and almost negligible enzyme production when inorganic nitrogen sources were used (Table 1). All the carbon sources stimulated glucoamylase production in FBA1 stain of *Bacillus* sp., and fructose proved to be a suitable inducer. The highest amount of enzyme units (1.970 mg/mL/minute) was observed by *Bacillus* in 24 hours of incubation (Table 1).

Effect of micronutrients

Bacillus strain was tested for the ability to secrete glucoamylase using a variety of micronutrients, including KH_2PO_4, $MgSO_4$, $CaCl_2$, $FeSO_4$, and NaCl.). Each salt has a unique impact on the generation of enzymes. $MgSO_4$, $CaCl_2$, KH_2PO_4, $FeSO_4$, $ZnSO_4$, and NaCl concentrations were all altered. The salt combinations that produced the highest levels of glucoamylase secretion from *Bacillus* strain were (w/v %) KH_2PO_4 2, NaCl 1, $MgSO_4.7H_2O$ 2, $ZnSO_4$ 1.5, $CaCl_2$ 0.1, and $FeSO_4.7H_2O$ 2 in the production medium (Table 1).

Discussion

The secretion and stability of the generated enzyme were mostly controlled by the pH of fermentation medium. While the bacterial culture was growing and fermenting, macro- and micronutrients were being consumed, and numerous metabolites or byproducts were created along with the required enzyme. The pH change was ultimately caused by all these components. Depending on the type of microbial source employed, glucoamylase can be generated in both acidic and basic conditions (Fadel *et al.*, 2020). According to Nigam and Singh (1995), *LactoBacillus amylovorus* produced the most glucoamylase at pH 6.0. However, archaeal species like *Thermoplasma acidophilum, Picrophilus torridus*, and *P. oshimae* are also among the stains that were modified to produce enzyme at very high acidic pH levels (Adhiyanto *et al.*, 2021).

The development of the bacteria and the synthesis of the enzyme are both significantly influenced by the choice of the optimal temperature. In this study, the impact of incubation temperature on enzyme production FBA1 strains was examined, and the result showed that the maximal glucoamylase production occurred at 40°C. The synthesis of enzymes ultimately decreased as the incubation temperature was further raised. The biomass eventually ran out to create a substantial amount of enzyme since the growth of the bacteria was temperature-dependent, and a change in the ideal temperature slowed down the growth rate of *Bacillus* FBA1. The results from other researchers were different from this study, as they reported 30°C as optimum incubation temperature for the production of glucoamylase (Mamo and Gessesse, 1999; Mishra and Debnath, 2002).

Fogarty and Kelly (1990) isolated glucoamylase from *C. themohdroysulfuricum* and studied the effect of different

Table 1. Effect of various macro- and micronutrients for the production of glucoamylase.

KH_2PO_4 Concentrations (w/v %)	Enzyme Activity mg/ml	min ± SD
1	0.1	0.673±0.0012[a]
2	0.5	0.563±0.0082[c]
3	1	0.667±0.0053[ab]
4	1.5	0.2667±0.0892[b]
5	**2**	**0.72±0.0155[ac]**
6	2.5	0.5273±0.0017[b]
NaCl Concentrations		
1	0.1	0.453±0.0155[b]
2	0.5	0.545±0.0017[ab]
3	1	**0.653±0.0082[ac]**
4	1.5	0.427±0.0055[a]
5	2	0.5433±0.002[b]
6	2.5	0.533±0.033[d]
$MgSO_4.7H_2O$ Concentrations		
1	0.1	0.295±0.002[a]
2	0.5	0.696±0.002[c]
3	1	0.637±0.008[b]
4	1.5	0.543±0.001528[ad]
5	**2**	**0.865±0.002[ac]**
6	2.5	0.857±0.007[b]
$ZnSO_4$ Concentrations		
1	0.1	0.467±0.082[a]
2	0.5	0.317±0.019[b]
3	1	0.867±0.053[d]
4	**1.5**	**1.0317±0.0002[c]**
5	2	1.023±0.001155[d]
6	2.5	0.963±0.067[ab]
$CaCl_2$ Concentrations		
1	0.1	**1.622±0.055[ac]**
2	0.5	1.253±0.0089[b]
3	1	1.502±0.005[cb]
4	1.5	1.56±0.0015[b]
5	2	1.307±0.05[c]
6	2.5	1.34±0.0115[d]
Nitrogen source		
1	Peptone	1.752±0.0014[a]
2	Yeast Extract	**1.955±0.0014[b]**
3	Urea	1.747±0.0017[c]
4	Beef Extract	1.624±0.0015[ad]
5	Ammonium chloride	0.426±0.0012[ab]
6	Ammonium sulphate	0.326±0.00066[c]
Carbon source		
1	Glucose	1.514±0.0014[a]
2	Fructose	**1.977±0.001[ac]**
3	Galactose	1.945±0.0006[b]
4	Maltose	0.347±0.0035[a]
5	Sucrose	0.265±0.0557[c]
6	Fruit Peels	1.47±0.008[ab]
$FeSO_4.7H_2O$ Concentrations		
1	0.1	1.465±0.001[a]
2	0.5	1.859±0.0008[b]
3	1	2.144±0.001[b]
4	1.5	2.043±0.001[d]
5	**2**	**2.179±0.001[ac]**
6	2.5	2.017±0.0011[ab]

carbon sources on its production. From their findings, it was concluded that the soluble starch from potato showed a comparatively high titer of glucoamylase. Furthermore, Kumar *et al.* (2007) also enhanced glucoamylase production using a more increased concentration of soluble starch (5.0%). Other researchers have also supplemented the growth medium with a variety of starch and varied the concentration to further enhance the production of enzyme (Kumar, 2009). Similarly, Arifeen *et al.* (2024) isolated the amylase producing the *Bacillus ichenoformis* strain from garden soil of *Lichi chinesis* and used rice polish as substrate. Helal *et al.* (2021) reported that when peptone is added in combination with the yeast extract, optimum enzyme was produced by *Rhizopus* sp., whereas when these two components were used separately, they produced comparatively less amount of enzyme. Similarly, Helal *et al.* (2021) also used a mixture of peptone and yeast extract for the optimum production of enzyme. However, when both these complex nitrogenous sources were used they induced excessive proteolytic enzyme secretion which ultimately leads to the hydrolysis of various amylotic enzymes (Vishnoi *et al.*, 2020).

The production of enzymes is mostly Ca^{+2} (calcium ions) dependent because most of the α-amylase enzymes are considered as metalloenzymes. The studies reveal that when $CaCl_2$ is added in the production media, a higher yield of enzymes were observed (Zare *et al.*, 2021). In this study, different concentrations of $CaCl_2$ (0.1 g, 0.5 g, 1 g, 1.5 g, 2 g, 2.5 g) were added in the production media, and a maximum of glucoamylase production was observed in 0.1 g for *Bacillus*. Similar results were also reported by Kumar *et al.* (2007) for the production of thermostable α-amylase from *Bacillus subtilis* KIBGE HAR—a newly isolated strain.

An increase in the production of enzymes might be due to the fact that calcium plays a vital role in the physiology and metabolism of bacteria. It also provides stability

to the desired enzyme against the proteases, which was also produced during the process of fermentation (Manfredini *et al.*, 2021). When the concentration of calcium increased more than 0.1 grams, decline in the enzyme production was clearly observed. It could be because of bacterial growth that ultimately affect the production of enzyme (Manfredini *et al.*, 2021).

Conclusion

The aim of this study was to isolate an economical source for the production of glucoamylase enzyme that could be industrially desirable. Glucoamylase, a cost-effective enzyme, was produced from *Bacillus* sp. using the submerged fermentation. Fruit peels were used as substrate as they are budget- and eco-friendly carbon source for the production of enzyme. The maximum production of glucoamylase was obtained from a cost-effective thermostable *Bacillus* FBA1 strain. The optimum pH and temperature for the production of glucoamylase from *Bacillus* FBA1 were 7.0 and 37°C. Ideal incubation period for the growth of bacteria were 24 hours with a 10 mL inoculum size. Glucoamylase activity were 2.984 mg/mL/minute with 5 g fruit peel as a carbon source and yeast as nitrogen source for 24 hours at pH 7 and 37°C temperature.

Data Availability Statement

All the data generated in this research work has been included in the manuscript.

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Author Contributions

Conceptualization: Memuna Ghafoor Shahid; methodology: Ayesha Kiran; software: Najeeb Ullah; validation: Abid Sarwar; formal analysis: Samraz Tahir; investigation: Muhammad Nadeem; resources: Rubina Nelofer; data curation: Fahad Al-Asmari; writing—original draft preparation: Ayesha Kiran; writing—review and editing: Fakhria A. Al-Joufi; visualization: Ashwag Shami; supervision: Tariq Aziz; project administration: Ayaz Ali Khan; funding acquisition: Tariq Aziz.

Conflicts of Interest

The authors declare no conflicts of interest.

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