Research Article

Amylase production by *Streptomyces* species and its application in orange juice clarification

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Abstract

Background: Amylases are enzymes that break down starch and help clarify fruit juices.

Aims: This study focused on screening amylolytic *Streptomyces* spp. isolated from soil samples for their potential for amylase production and fruit juice clarification.

Methods: Select organisms produced amylase, which was assayed by measuring the reducing sugar content of the fermentation medium. The effects of pH, carbon and nitrogen source, as well as agitation and incubation periods, were evaluated to optimize amylase synthesis.

Results: A total of 22 species were isolated, with five—FE4, ELI1, FL2, MS2, and MS5—demonstrating high amylase production ability, which occurred at a pH ranging from slightly acidic to slightly alkaline. Cassava peels supported optimal amylase production in *Streptomyces* spp. A4 (0.834), ELI1 (0.910), and FE4 (0.814 U/mL). The maximum yield of 0.930 U/mL was observed with ELI1 when urea was used as the nitrogen source, at an agitation speed of 100–150 rpm, and peaking on the fourth day of fermentation. It was identified as *S. griseoflavus* ELI_1 using 16S rRNA gene sequencing and submitted to the GenBank with accession number OQ930232. The amylase produced by it was partially purified, markedly increasing its specific activity from 1.50 to 4.56 U/mL. Its ability to clarify orange juice was tested; the turbidity reduced significantly by 16.8% after amylase treatment ($p < 0.05$).

Özet

Dayanak: Amilazlar, nişastayı parçalayan ve meyve sularının arıtılmasına yardımcı olan enzimlerdir.

Amaçlar: Bu çalışma, toprak örneklerinden izole edilen amilolitik *Streptomyces* türlerinin amilaz üretimi ve meyve suyu berraklaştırma potansiyelinin taranmasına odaklanmıştır. Seçilen organizmalar, hücrelerin indirgen şeker içeriği ölçülerek test edilen amilaz üretmiştir.

Yöntemler: Amilaz sentezini optimize etmek için pH, karbon ve azot kaynağı ile çalkalama ve inkübasyon sürelerinin etkileri değerlendirilmiştir.

Bulgular: Toplam 22 tür izole edildi ve bunlardan beş tanesi (FE4, ELI1, FL2, MS2 ve MS5) hafif asidik ila hafif alkali pH aralığında yüksek amilaz üretim kabiliyeti gösterdi. Manyok kabukları, *Streptomyces* spp. A4 (0,834), ELI1 (0,910) ve FE4 (0,814 U/mL) türlerinde optimal amilaz üretimini destekledi. Üre azot kaynağı olarak kullanıldığında, 100-150 rpm çalkalama hızında ve fermantasyonun dördüncü gününde zirveye ulaştığında, ELI1 ile 0,930 U/mL'lik maksimum verim gözlemlenmiştir. 16S rRNA gen dizilemesi kullanılarak *S. griseoflavus* ELI_1 olarak tanımlanmış ve GenBank'a OQ930232 erişim numarasıyla kaydedilmiştir. Bu suş tarafından üretilen amilaz kısmen saflaştırılmış ve spesifik aktivitesi 1,50'den 4,56 U/mL'ye önemli ölçüde artmıştır. Portakal suyunu berraklaştırma kabiliyeti test edilmiş ve amilaz tedavisi

Conclusion: Amylolytic *Streptomyces* spp. were isolated from soil samples, and their amylase yield was optimized. The Amylase of *S. griseoflavus* ELI_1 could optimally clarify orange juice.

sonrasında bulanıklık %16 oranında önemli ölçüde azalmıştır ($p < 0,05$).

Sonuç: Böylece, toprak örneğinden izole edilen amilolitik tür *S. griseoflavus* ELI_1, portakal suyunu en iyi şekilde berraklaştırabildi.

Introduction

Enzymes are essential biocatalysts and find diverse industrial applications (Fasiku et al., 2023). Their enhanced synthesis by easily cultivable and genetically manipulated microorganisms enabled higher yields and bulk production for application to commercial purposes, making them more preferable catalysts over plant- and animal-sourced counterparts (Adrio & Demain, 2014; Liu et al., 2013; Mokrani & Nabti, 2024; Patel et al., 2023), in addition to being more stable and cheaper to produce on a large scale. Different microorganisms produce isozymes with varying properties. Microbial enzymes are non-toxic and biodegradable and can substitute conventional toxic chemical catalysts (Illanes et al., 2012; Khan, 2025; Saravanan et al., 2021). Enzyme-catalyzed processes are more environmentally friendly, economical, and time and energy-efficient than those mediated by traditional methods (Saravanan et al., 2021; Thulasisingh et al., 2024).

Amylolytic enzymes catalyze the hydrolytic cleavage of α-1,4-glycosidic bonds in starch molecules to produce low molecular weight sugars, like maltose, dextrin, and glucose, which have many applications, including for detergent production, brewing, baking, and the paper and textile industries (Fasiku et al., 2020; Gómez-Villegas et al., 2021; Li et al., 2012). The ability of microbes to produce amylases has been widely documented (Fasiku et al., 2020; Oyenado & Omoruyi, 2024; Vojnovic et al., 2024), including that of *Streptomyces* spp. (Al-Dhabi et al., 2020; El-Sayed et al., 2024; Rathore & Singh, 2021). Optimizing key physiological parameters and culture conditions, such as inoculum size, pH, agitation, and temperature, is crucial for maximizing amylase productivity (Arifeen et al., 2024; Oussadi & Kitouni, 2015).

The benefits provided by fruit juices have led to a continuous increase in their consumption among health-conscious individuals (Kahraman et al., 2017). However, undesirable turbidity, haze formation, and sedimentation in fruit juice-based food products can deter their purchase. One of the many factors influencing the turbidity of fruit juices is the starch content. Although an initial centrifugation step can eliminate starch to a significant extent, typically, ~5% remains. Enzymes play a crucial technological role in clarifying juices, with amylase being a key enzyme involved in starch breakdown, which enhances filterability, post-extraction yield, and clarity of the juice (Bamigboye et al., 2022; Casas et al., 2025; Oliveira et al., 2018).

Amylases derived from *Streptomyces* spp. are highly valued for their properties of extracellular secretion, broad substrate specificity, and stability and activity across a broad temperature and pH range, which are critical for industrial applicability (Barman & Dkhar, 2024). Additionally, alkaliphilicity and resistance to metal ions and detergents, which facilitate elevated amylase production and yield under cost-effective fermentation conditions, are desirable properties (Ali et al., 2023; Suthar et al., 2024). The yield can be enhanced significantly under optimal production parameters such as carbon and nitrogen sources, incubation period, aeration, etc. (Fahmy, 2022). The ability of amylase to effectively hydrolyze starch and related polysaccharides responsible for turbidity and viscosity of fruit juices makes them desirable biocatalysts (Bamigboye et al., 2022).

Given the rising demand for industrial-use enzymes with greater efficiency, there remains a need to explore novel *Streptomyces* species for amylase production. This study aimed to clarify fruit juice using amylase produced by *Streptomyces* species isolated from soil.

Materials and Methods

Soil from five locations in Ibadan, Nigeria, was sampled. They include the botanical garden (7.4558° N, 3.8965° E), dump site (7.3776° N, 3.8738° E), mechanical site (7.4408° N, 3.9080° E), palm oil production facility (7.3740° N, 3.8398° E), and the Department of Microbiology, University of Ibadan (7.3776° N, 3.9471° E). The samples were collected from depths of 2–10 cm from the soil surface with a spatula, placed in sterile zip-lock bags, and immediately transported to the laboratory. The samples were then mixed to obtain a composite, passed through a sieve, and air-dried overnight at room temperature.

Isolation of *Streptomyces* spp.

Streptomyces species were isolated from soil samples on inorganic salt starch agar using the pour plate method. The samples were first serially diluted. The culture medium employed was International *Streptomyces* Project (ISP) Medium No. 4. It was composed of 10g/L starch, 1g/L K_2HPO_4, 1g/L $MgSO_4$, 1g/L NaCl, 2g/L $(NH_2)_4SO_4$, 2g/L $CaCO_3$, 0.001g/L $FeSO_4$, 0.001g/L $MnCl_2$, 0.001g/L $ZnSO_4$, and 20g/L agar-agar. The components were mixed in required amounts, the pH was adjusted to 7.2, and the prepared medium was steam-sterilized. It was then supplemented with 50 μg/mL nystatin and 30 μg/mL rifampicin, cooled down, and poured into plates containing 1 mL aliquots of each serial dilution. The agar was allowed to solidify, and the plates were incubated at 28 °C for 5 days (Kharel et al., 2010).

Identification of *Streptomyces* Isolates

Subculturing involved repeated streaking to obtain pure isolates, which were then characterized by Gram staining, spore formation ability, biochemical tests (oxidase, citrate, catalase, and starch hydrolysis), and macroscopic examinations of colony color and pigment formation.

Screening for Amylolytic Activity

Streptomyces spp. were screened for amylase production based on starch hydrolysis. The microorganisms were inoculated into ISP4 medium and incubated at 28 °C for 72 h. The plates were then flooded with Gram's iodine solution. Dark blue coloration is observed when iodine reacts with starch. Amylolytic organisms formed a clear hydrolysis zone, unlike non-amylolytic ones (Abd-Elhalem et al., 2015).

Inoculum Preparation

First, 50 mL of ISP4 broth was dispensed into a 250 mL Erlenmeyer flask, sterilized, and allowed to cool. The broth was inoculated with a loopful of the microbial culture and incubated at 37 °C in a rotary shaker for 24 h.

Amylase Assay

For this, 1 mL of each inoculum was aseptically transferred into individual 250 mL Erlenmeyer flasks containing 50 mL of ISP-4 broth (fermentation medium; pH 7.0), and the microbes were grown aerobically at 28 °C for 96 h with agitation at 120 rpm. Subsequently, the medium and its contents were centrifuged at 5,000 rpm for 20 min. The supernatant was collected and used as crude amylase. Amylase activity was quantitatively assessed, employing the 3,5-dinitrosalicylic (DNS) method (Gusakov et al., 2011) with slight modifications. A 0.2 mL aliquot of the crude amylase was added to 0.5 mL of 0.1 M sodium phosphate (pH 7.0) containing 1% starch solution. The reaction mix was then incubated at 35 °C for 30 min, and then, the reaction was terminated by adding 1.0 mL of the DNS reagent. The reaction mix was placed in a boiling water bath for 10 min. The OD_{540} was measured with a UV-Vis spectrophotometer (Gusakov et al., 2011). Amylase production was ascertained by extrapolating the absorbance values to a standard graph, and the best amylase producers were selected for further studies.

Effect of Culture Parameters on Amylase Production

The effects of diverse cultivation conditions on amylase productivity were assessed using 50 mL media (Krishna et al., 2015). Buffers of various pH values—5.0, 6.0, 7.0, 8.0, and 9.0—were used to prepare ISP4 media and assess the influence of pH on amylase production. The impacts of different carbon (C) (soluble starch, mannitol, cassava peels, wheat bran, and sucrose at 10 g/L) and nitrogen (N) (urea, potassium nitrate, yeast extract, peptone, and ammonium sulfate at 10 g/L) sources on amylase production were examined. Each medium was inoculated with selected *Streptomyces* spp. and incubated at 28 °C for 96 h at 120 rpm in a rotary shaker. The effect of agitation on bacterial growth and amylase yield was estimated by culturing select *Streptomyces* spp.

in ISP4 media at 28 °C on a rotary shaker set to different rpm: 100, 150, and 200 rpm for 96 h. To study the impacts of incubation time, the isolates were inoculated into ISP4 media and cultured on a rotary shaker for 2, 4, 6, and 8 days. Amylase productivity under each fermentation condition was determined via amylase assay.

Partial Purification and Specific Activity Determination

The stock inoculum of the highest amylase-producing isolate was added to the best production medium and cultured under optimal conditions. The enzyme produced was recovered after incubation: 100 mL of the cell-free crude extract was brought to 40% saturation with ammonium sulfate for 30 min and constantly stirred until the salt was completely dissolved. The solution was incubated at 4 °C for 24 h and then centrifuged at 5,000 rpm for 20 min. The precipitated pellet was resuspended in 0.1 M sodium phosphate buffer (pH 7.0), poured into a dialysis tube, and sealed. The tube was filled with four times the volume of the suspension contained and dialyzed for 18 h. The partially purified amylase was quantified by applying Lowry's method (Lowry et al., 1951), and the OD_{660} was measured against a blank solution on a UV-Vis spectrophotometer. The specific activity of the enzyme was determined using the formula-

$$\text{Specific activity} = \text{Enzyme activity (U/mL)/Protein concentration (mg/mL)}$$

Fruit Juice Clarification Ability

Sweet orange (*Citrus sinensis*) fruits were purchased from Ajibode, Ibadan, and washed thoroughly under running water. Their juice was extracted aseptically, and 10 mL aliquots were dispensed into sterile test tubes, sealed with sterile non-absorbent cotton wool, and pasteurized by heating at 85 °C for 3 min (Kareem & Adebowale, 2007). Then, 1 mL of amylase was aseptically dispensed into each test tube, while the one added with distilled water served as a blank. The contents were aliquoted at onset (0 h) and then at 30 min. They analyzed for clarity by spectrophotometrically measuring the transmittance (%) at 660 nm.

Percentage clarity was calculated as follows:

$$\% \text{ Clarity} = \frac{Tt - Tc}{Tc} \times 100\%$$

T_t = Transmittance of the test solution.

T_c = Transmittance of the control solution.

Molecular Species-Level Identification of the Isolate

DNA was extracted per the procedure described by Haque et al. (2022). The 16S rRNA gene was PCR-amplified using a GeneAmp 9700 PCR System Thermal cycler (Applied Biosystems Inc., USA). The reaction mixture contained 1 μL of 10 mM dNTPs mix, 3 μL of 25 mM $MgCl_2$, 10 μL of 5x GoTaq colorless reaction mix (Promega, WI, USA), 1 μL of each primer (10 pmol), 0.3 U of Taq DNA polymerase (Promega), 8 μL of DNA template, and 42 μL of sterile distilled water. The primers used were 1525R: 5'- AAGGAGGTGATCCAGCC-3' and 27F: 5'- AGAGTTTGATCMTGGCTC AG-3'. The amplicons were

sequenced on a 31310xl Genetic Analyzer (Applied Biosystems, MA, USA), utilizing a BigDye Terminator V3.1 Cycle Sequencing Kit (Applied Biosystems). All gene data were analyzed using MEGA 11 (Kumar et al., 2018; Tamura & Nei, 1993).

Statistical Analysis

All experiments in this work were carried out in duplicate. The mean and standard error of the mean of amylase activities were graphically represented. One-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was used to determine the statistical significance of the differences in mean values at $p < 0.05$.

Results

Isolation, Quantitative Screening for Amylase, and Presumptive Identification of *Streptomyces* spp.

In total, 45 pure isolates were obtained from the soil samples. Preliminary identification indicated only 22 isolates as presumptive amylolytic *Streptomyces* spp. The enzyme yields of these isolates were quantified, based on which, five *Streptomyces* spp. with maximum amylase production were selected. These included: MS2, A1, FE4, A4, and ELI1, demonstrating activities of 0.654, 0.767, 0.787, 0.806, and 0.878 U/mL, respectively. All of them were citrate-positive, but catalase- and oxidase-negative. They could ferment glucose, fructose, and sucrose, as well as hydrolyze starch (Table 1).

Effects of Culture- and Nutrition-Associated Parameters on the Levels of Amylase Produced by the Five Isolates

The ANOVA test was conducted, and the means of amylases were plotted. The effect of pH on the five *Streptomyces* spp. to produce amylase is presented in Figure 1. A pH of 6.0 supported amylase production in ELI1 at 0.89 U/mL, which varied significantly ($p < 0.05$) from the yields of the other species. At a pH of 7.0, amylase production by FE4, MS2, A1, and A4 was 0.80, 0.658, 0.808, and 0.816 U/mL, respectively.

Adding cassava peels to the culture medium as a carbon source supported amylase production in A4, ELI1, and FE4 at 0.834, 0.91, and 0.814 U/mL. The amylase yield of ELI1 differed significantly ($p < 0.05$) when compared with the other four. However, starch as a carbon source supported amylase production to the maximum extent by A1 at 0.84 U/mL and MS2 at 0.750 U/mL (Figure 2). As N sources, peptone supported maximum amylase production by FE4 (0.818 U/mL); urea by ELI1 (0.930 U/mL) and A4 (0.884 U/mL); and KNO_3 by A1 (0.864 U/mL) and MS2 (0.802 U/mL). The highest yield detected was by ELI1, which did not vary significantly ($p > 0.05$) from that of A4 with urea (Figure 3).

The effects of different agitation speeds—100, 150, and 200 rpm—on amylase production are presented in Figure 4. At an agitation speed of 100 rpm, amylase production by A1, A4, and MS2 was 0.864, 0.880, and 0.840 U/mL; at 150 rpm, it was 0.980 and 0.920 U/mL by ELI1 and FE4, respectively.

Table 1. Cultivation and biochemical characteristics of five selected *Streptomyces* spp. isolated from soil samples.

Isolate code	Colony color	Soluble pigment	Cell morphology	Gram stain	Spore formation	Citrate	Oxidase	Catalase	Starch hydrolysis	Glucose	Fructose	Sucrose	Mannitol	Lactose	Galactose
Streptomyces sp. A1	Cream	ND	Cocci	+	+	+	-	-	+	+	+	+	+	+	+
Streptomyces sp. A4	Cream	ND	Cocci	+	+	+	-	-	+	+	+	+	+	+	+
Streptomyces sp. FE4	Gray	Gray	ND	+	+	+	-	-	+	+	+	+	+	+	+
Streptomyces sp. ELI1	Gray	Gray	ND	+	+	+	-	-	+	+	+	+	+	+	+
Streptomyces sp. MS2	White	White	Cocci	+	+	+	-	-	+	+	+	+	+	+	+

+ = positive; − = negative; ND = not determined.

The yield of ELI1 differed significantly ($p < 0.05$) from those of the other four species at all three agitation speeds. During the 8-day incubation period, amylase production started on day 2 and gradually rose till day 4 before declining (Figure 5). Maximum and minimum yields recorded on days 4 and 8 were 0.890 and 0.680 U/mL for A4, 0.866 and 0.640 U/mL for A1, and 0.910 and 0.780 U/mL for MS2, respectively. The highest yield recorded was with ELI1 at all fermentation periods and varied significantly ($p < 0.05$) when compared with the other four *Streptomyces* species.
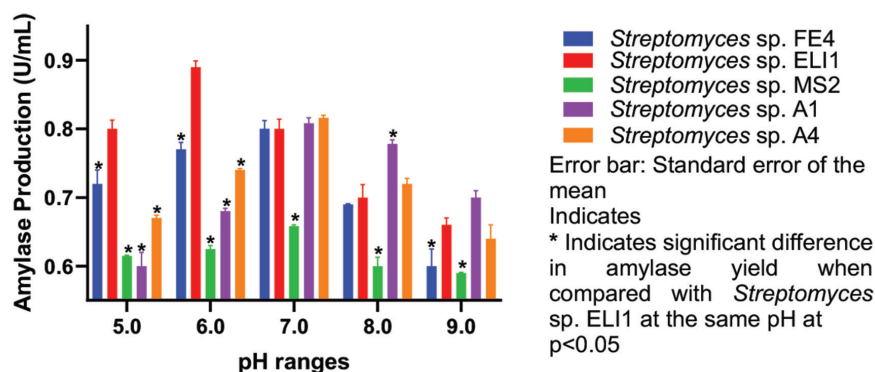

Figure 1. Effect of pH on amylase production by the five selected *Streptomyces* species.
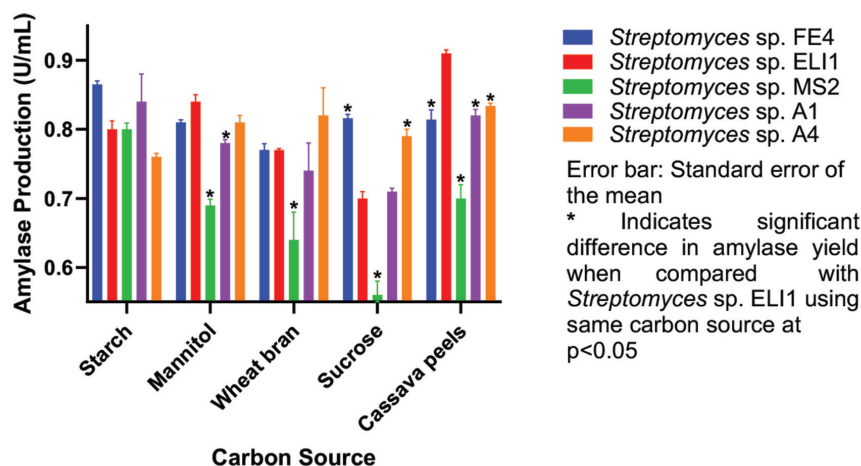

Figure 2. Effect of carbon source on amylase production by the five selected *Streptomyces* species.
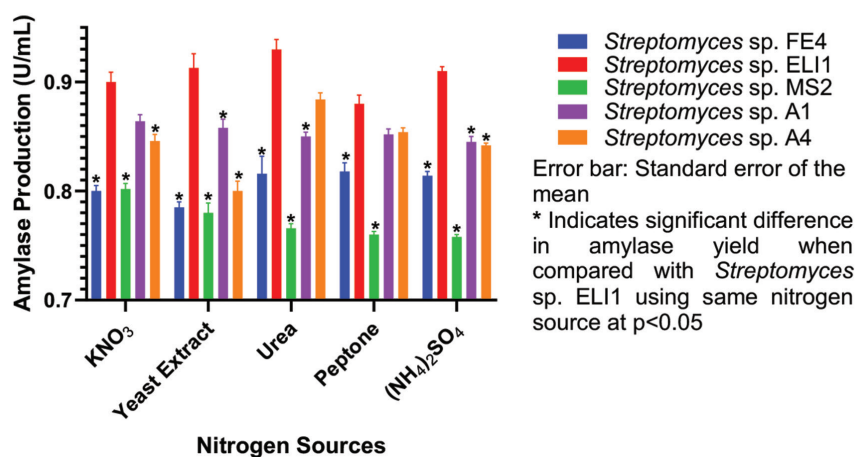

Figure 3. Effect of nitrogen source on amylase production by the five selected *Streptomyces* species.
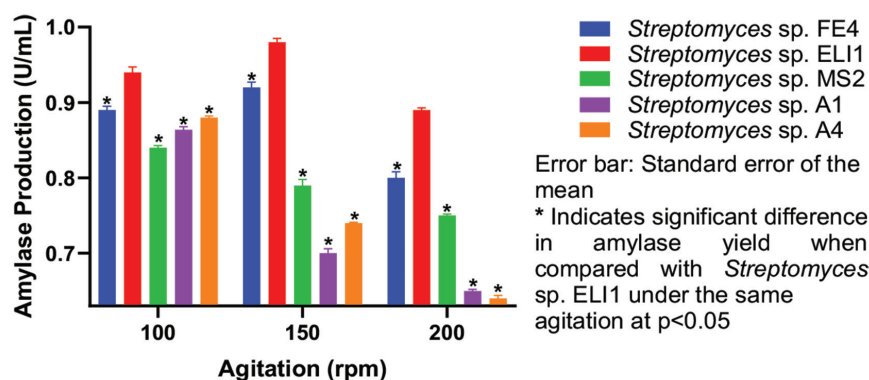
Figure 4. Effect of agitation on amylase production by the five selected *Streptomyces* species.
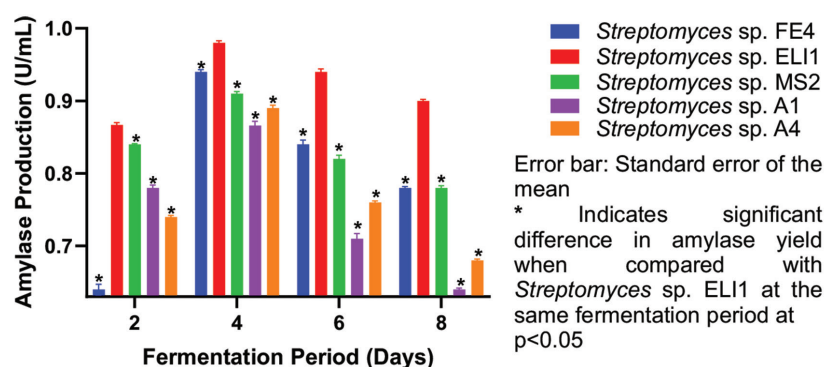

Figure 5. Effect of fermentation day on amylase production by the five selected *Streptomyces* species.

Partial Purification of Amylase

Streptomyces sp. ELI1 was selected due to its consistently high amylase production, and the enzyme synthesized by it was partially purified. Table 2 summarizes the purification steps. Ammonium sulfate precipitation increased the specific activity from 1.500 to 2.930 U/mL, and further to 4.560 U/mL by dialysis. However, the protein content of the crude declined from 0.653 mg/mL to 0.434 mg/mL after ammonium sulfate precipitation and further to 0.317 mg/mL post-dialysis.

Clarification of Orange Juice Using Amylase

The fruit juice clarifying potential was determined based on the increased transmittance of juice, which was 16.8% within 30 min of incubation with the amylase produced by ELI1. The value differed significantly from the initial one ($p < 0.05$); the amylolytic activity was 3.234 U/mL. This result confirmed the breakdown of starch, one of the factors responsible for turbidity.

Molecular Identification of the ELI1 Isolate

The best amylase producer among the five selected *Streptomyces* sp., ELI1, was identified at the molecular level as *S. griseoflavus* ELI_1. The sequence of the gene encoding amylase was submitted to the National Center for Biotechnology Information GenBank

(Accession number: OQ930232). It was closely related to that of *S. griseoflavus* LMG 19344 with accession number: NR042291 (Figure 6).

Discussion

Different amylolytic species of *Streptomyces* were isolated from the soil samples collected in Ibadan, Nigeria. They were identified based on biochemical and morphological characteristics. Their amylolytic ability was determined based on the size of the zones of hydrolysis observed around the colonies growing on starch agar. The isolation of amylase-producing *Streptomyces* spp. from soil samples has been reported (Ali et al., 2023; Saadoun et al., 2023; Shu et al., 2025), confirming that soil is a repository of amylase-producing microbes.

The production and stability of enzymes are sensitive to pH (Kizhakedathil & Subathra, 2021). Among the different pH levels studied, *S. griseoflavus* ELI_1 produced the highest quantity of amylase at pH 6. This result is similar to that reported by Saad et al. (2021), using *Bacillus licheniformis* WF67. However, Olakusehin and Oyedeji (2021) reported that the maximum yield with *Aspergillus flavus* S2-OY was at pH 5.

Table 2. Step-wise purification of amylase produced by *Streptomyces* sp. ELI1.

	Activity	Volume	Protein (mg/mL)	Total protein	Total activity	Specific activity (U/mL)	Recovery (%)	Purification fold
Crude	0.980[c]	100	0.653[a]	65.3[a]	98.00[a]	1.500[c]	100.00[a]	1.00[c]
Ammonium sulfate precipitation (40%)	1.272[b]	50	0.434[b]	21.7[b]	63.60[b]	2.930[b]	64.89[b]	1.95[b]
Dialysis	1.447[a]	10	0.317[c]	3.17[c]	14.47[c]	4.560[a]	14.76[c]	3.04[a]

Mean values with various alphabetical superscripts along the column indicate statistically significant differences at $p < 0.05$.
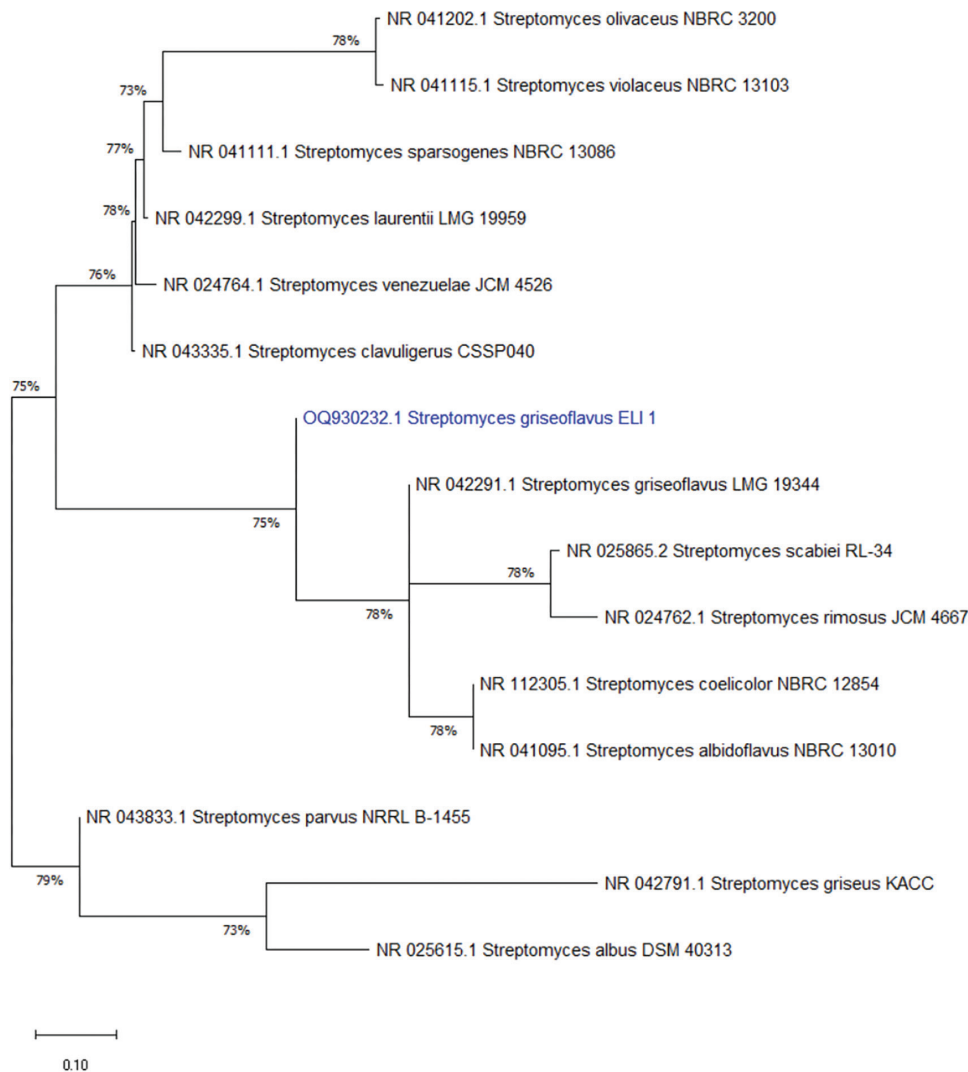

Figure 6. Phylogenetic tree indicating *S. griseoflavus* ELI_1 and other closely related microorganisms.

The type of C source is critical for media formulation, as it determines the growth rate of microorganisms and affects the production of metabolites such as amylase, etc. (Kizhakedathil & Subathra, 2021). In this study, the type of C source used in the fermentation medium markedly affected amylase production. The highest amylase production was recorded when agro-waste (cassava peels) was employed. Similarly, Iram et al. (2020) reported that the best yield of amylase was observed when grape fruit peels, as an agro-waste, were used. Adebare et al. (2021) utilized cassava peels for the production of amylase by *Aspergillus niger*. Varying types of agro-wastes have been used for amylase production (Abd-Elhalem et al., 2015; Iram et al., 2020; Olakusehin & Oyedeji, 2021; Sahu et al., 2024), as they are rich in carbon, with a few also containing a small proportion of N. The use of agrowastes as a C source can help reduce amylase production costs.

Nitrogen affects the synthesis of amino acids and thus, microbial growth. N can serve as a secondary energy source and influences enzyme production (Kizhakedathil & Subathra, 2021). The absence of many types of amino acids and other N sources in the production medium has been linked to remarkable effects on amylase biosynthesis (Singh et al., 2016). Peptone supported amylase production by *Streptomyces* FE4. Kavitha and Vijayalakshmi (2010) reported enhanced amylase production by *Streptomyces tendea* TK-VL-33 when peptone was utilized. Fahmy (2022) also reported better yield with peptone compared to yeast extract, ammonium sulfate, potassium nitrate, and urea.

Another critical parameter affecting microbial enzyme production is agitation, which influences O_2 distribution. Oxidative reactions utilize O_2 as a terminal electron acceptor to provide energy for various cellular activities. Agitation speed also affects morphology and subsequent biosynthetic activity (Ibrahim et al., 2015). In this study, a speed of 100 rpm optimized amylase production by A1, A4, MS2, and FE4, while 150 rpm was the best for ELI1. However, a further increase in speed reduced amylase yield, most likely due to the adverse effects, such as mechanical and oxidative stress, that were induced in cells (Prabakaran & Pugalvendhan, 2009). The maximum amylase production was by ELI1 at 150 rpm, aligning with the findings of Ahmed et al. (2015) and Niyomukiza et al. (2023) in *Penicillium notatum* and *Bacillus subtilis* W3SFR5, respectively.

Metabolite production, growth rate, and culture characteristics of microbes are influenced by the incubation period (Chowdary et al., 2018; Fasiku & Wakil, 2022). Earlier reports indicated better amylase production by *Streptomyces* spp. on day 4 (Singh et al., 2020), which is consistent with the results of this study. Further prolongation suppressed amylase production, which could be due to cells predominantly being in the decline phase of growth, leading to nutrient depletion and the accumulation of toxins in the culture medium, ultimately reducing growth and consequently enzyme production (Baysal et al., 2003).

Suspended colloidal or insoluble compounds such as starch, pectin, hemicellulose, cellulose, proteins, and other cell-wall fragments released during fruit processing cause fruit juices to become turbid (Sharma et al., 2017; Wang et al., 2022). Turbidity may be desirable in naturally cloudy juices or undesirable in juices intended to be clear, i.e., depending on the product (Sharma et al., 2017). In this work, the turbidity of fruit juice decreased after treatment with the amylase produced by *S. griseoflavus* ELI_1. Fruit juice clarity is one of the properties that determines consumer interest (Bamigboye et al., 2022), which led to a search for cost-effective and efficient clarification processes. In this work, the clarity of orange juice was enhanced by the amylase produced by ELI1. Starch is one of the compounds that contribute to fruit juice turbidity and post-concentration haze formation, as it can induce gel formation and membrane fouling, thereby decreasing the filtration rate (Hossain et al., 2024). Amylase helps reduce turbidity by breaking down the starch, clearing haziness, turbidity,

and cloudiness, thereby improving the quality and shelf life of the juice (Hossain et al., 2024). Clarification of grape (Sondhi et al., 2021), orange (Bamigboye et al., 2022; Sondhi et al., 2021), apple (Hossain et al., 2024, Roheen et al., 2024), pear (Liviu et al., 2022), and banana (Shwe & Win, 2019) juice has been reported.

Study Limitations

This study was limited to soil samples collected from a single geographic region, which may not represent the full diversity of amylolytic *Streptomyces* spp. present in other environments. The enzyme was only partially purified, and characterization was restricted to basic assays without detailed kinetic or structural analysis. The juice of only one fruit type, orange, was used for testing clarification ability, which limits generalization to other fruit juices with different physicochemical properties. The degree of clarification was assessed via turbidity reduction, without any accompanying comprehensive sensory or nutrient retention analyses. Moreover, this study did not evaluate enzyme reusability or stability under conditions relevant to industrial processing. Future research should focus on large-scale optimization, purification, kinetic analysis, and broader application to enhance industrial relevance.

Conclusion

A total of 22 amylase-producing *Streptomyces* spp. were isolated from soil samples. Acidity or alkalinity, C and N source, agitation, and fermentation period affected amylase production. *S. griseoflavus* ELI_1 was the best amylase producer under optimized conditions; purification of the enzyme produced increased its activity. The turbidity of orange juice was reduced by 16.8% upon treating with the amylase produced by ELI_1, as it effectively hydrolyzed starch, thereby enhancing juice clarity. Thus, this enzyme has potential for juice clarification and can be used on an industrial scale.

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