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Full Length Research Article

OPTIMIZATION OF AMYLASE PRODUCED FROM BACTERIA ISOLATED FROM CASSAVA PEEL DUMPSITE USING SUBMERGED FERMENTATION

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ABSTRACT
The present investigation aimed at optimizing amylase production from amylolytic bacteria isolated from cassava peel dumpsite using submerged fermentation. Amylase production was carried out with four fermentation media designated as I, II, III and IV using all the bacteria isolated under static and non-static incubation condition for 24 h, at 37^{o}C and pH 7. Fermentation conditions were optimized by varying the following parameters: pH (5-9), inoculum size (0.5-4.0%v/v), temperature (27-75ºC), substrate concentration of maize flour (medium I) (0.5-4.0 % w/v), starch concentration of medium III (0.5-4% w/v), incubation time (18-72 h), effect of carbon source on medium III (basal medium). The highest enzyme activity was exhibited by *Enterobacter hormaechei* SR3 and *Bacillus cereus* MR1. The effect of different media and the incubation condition on the amylase production were found to be significant (P< 0.05). Amylase production was found optimum at; pH of 7 and 8, and temperature of 50^{O}C and 60^{O}C, for a period of 48 and 72 h of incubation, 2.0 and 3.0 %w/v concentration of maize flour and starch respectively. Maximum enzyme produced in maize flour by *Bacillus cereus* MR1 was 2.668 ±0.003U/mL/min and 2.718±0.001 U/mL/min by *Enterobacter hormaechei* SR3 in basal medium III. The results showed that maize flour is a good substrate for amylase production and enzymes produced by this study can be used for industrial purposes.

Keywords: Amylase, Cassava peel, Dump-site, Fermentation, Optimization

INTRODUCTION
Amylases in function, are powerful hydrolyzing enzymes and as a result causes the chemical decomposition of molecules involving the splitting of a bond and the addition of the hydrogen cation and the hydroxide anion of water. As hydrolyzing enzymes, they mediate the degradation of certain biopolymers (proteins, complex sugars) by the chemical process that results in smaller polymers or monomers like amino acids or monosaccharides (de Souza & e Magalhães, 2010; Parmar & Pandya, 2012). Singh & Kumari (2016) further explained that, amylases act by hydrolysing bonds between adjacent glucose units, yielding products characteristic of the particular enzyme involved. They constitute a class of industrial enzymes having approximately 25- 30% of the world enzymes market follow guidelines Amylases have opened new and numerous opportunities for commercial biotechnological processes among which are: renewable energy, pharmaceuticals, saccharification or liquefication of starch, detergent industries, warp sizing of textiles, fibers, paper industries, food stuffs, baking, classification of haze formed in beer or fruit juices and for pre-treatment of animal feed to improve digestibility (Abalaka & Adetunji, 2017; Fentahum & Kumari, 2017).

Starch is a significant part of the human diet and for this purpose, it is used chemically and enzymatically. They can be processed into a variety of products such as starch hydrolysates, glucose syrups, fructose maltodextrins derivatives or cyclodextrins used in food industry and are also used as microbial substrates in amylase enzyme production (Singh & Kumari, 2016; Abalaka & Adetunji, 2017).

Alpha-amylase (α-1,4 glucan-4-glucanohydrolase) can be found in microorganisms, plants and animals. They are produced by different species of microorganism. However, for commercial applications, the enzymes are derived from the genus *Bacillus* which is produced from *Bacillus licheniformis, Bacillus stearothermophiles* and *Bacillus amyloliquefaciens* and several others (de Souza & eMagalhães, 2010; Parmar & Pandya, 2012). Fungi and mold species are also good producers of amylase. Mold species producing significant levels of amylase are: *Aspergillus niger, A. oryzas, Thermomyces lanuginosu and Pencillium expansum* in addition to many species of the genus *Mucor* (Prameela *et al*., 2016; Singh & Kumari, 2016).

Two major methods are used for α-Amylase production on a commercial scale. These are: submerged fermentation, and solid state fermentation. Submerged fermentation (SmF) employs free flowing liquid substrates, such as molasses and broths. The products yielded in fermentation are secreted into the fermentation broth (Prameela *et al*., 2016; Sundarram & Murthy, 2014). The substrates are utilized quite rapidly; hence the substrates need to be constantly replenished. This fermentation technique is suitable for microorganisms such as bacteria that require high moisture content for their growth. SmF is primarily used for the extraction of secondary metabolites that need to be used in liquid form (Sundarram & Murthy, 2014).

Agro-based products and wastes have been found to be good substrates for the cost-effective production of many hydrolytic enzymes including alpha amylase, hence are attracting the attention of researchers (Shinde *et al*., 2014). In this regard, brans and flours of different grains and tubers, such as maize, rice, sorghum and wheat, and peels of cassava and potato had been used in the development of fermentation medium to increase

amylase production from bacteria and Fungi (Madika *et al*., 2017). Maize grain contains on average, about 61.50 to 77.40% starch (dry basis), and provides a good raw material for the production of starch and different sweetners, and had proven to be efficient in fermentation processes as microbial substrates for the production of enzymes, sugars, and acids (Abalaka & Adetunji, 2017; Madika *et al*., 2017).

The soil is a globally acclaimed and proven repository for screening of microorganisms with enzyme producing abilities. Therefore isolating and screening of amylolytic bacteria from soil samples are significant strides to discover novel industrial enzymes. Starch-rich soils are conducive habitats for amylolytic microorganisms since they take on starch as substrate.Improvement in the yield of amylase and consequent cost reduction depends on the selection of strains, optimization of the factors affecting biosynthesis, kinetic studies, biochemical characterization of enzyme amongst others. Hence this study: Optimization of amylase from bacteria isolated from cassava Peel dump-site using submerged fermentation.

The aim of this study is to produce amylase enzyme using suitable isolates from cassava peel dumpsite and investigate the thermal stability of the enzyme.

MATERIALS AND METHODS

Collection of Soil Samples

Soil sample was collected at different depths in polythene bags from a cassava peel dumpsite at Ojokodo in Ankpa Local Government Area of Kogi State, labelled properly and transported to Ilorin Kwara State and taken to the laboratory for the isolation of amylolytic bacteria.

Preparation *of* substrate for amylase producing bacteria

Dried maize grains was purchased from Ankpa major market in Kogi state, cleaned of debris and milled into flour with the aid of the grinding machine. Proximate analysis was carried out to know the composition of the maize flour. This made for one of the medium used in the amylase production and optimization studies.

Isolation and Screening for Amylolytic *Bacteria*

For the isolation of amylase producing bacteria from cassava peel dumpsite, 10g of soil sample was added to 100mL 0f distilled water, centrifuged and the supernatant was used. A tenfold serial dilution (10^{-1},10^{-2},10^{-3},10^{-4},10^{-5} and 10^{-6}) of the soil sample was carried out. Nine mililitre of distilled water was introduced into the test-tubes labeled 10^{-1} to 10^{-6}. One mililiter of the centrifuged supernatant was introduced into 10^{-1} dilution and from it, 1mL was taken and serially introduced into the other dilution factors to thin out the microbial population sufficient amount as described by Willey *et al*. (2008). One mililitre of the dilution factors 10^{-1} - 10^{-6} was introduced into petri dishes in triplicates. Pour plate method was used. The medium containing 1% starch and 2% agar was used as described by Sinha (2010). Plates were incubated at 370C for 72 h. Only the amylase producing bacteria were able to utilize the starch as sole carbon source and were able to grow on this medium. After the isolation of amylase producing bacteria, they were streaked subsequently on starch agar plate and Nutrient agar until pure isolated colonies were obtained. Bacteria isolates were flooded with iodine on starch agar plates and zone of clearance was determined after 60 minutes as described by Ajijolakewu *et al*. (2015).

Identification of the bacteria

The isolated bacteria were identified according to standard procedure (Fawole and Osho, 2004; Willey *et al.,*2008) and various biochemical tests necessary for identification were carried out and Bergey's Manual of Determinative Bacteriology consulted (1957). Molecular characterization of bacteria with highest yielding amylolytic activity from cassava peel dump-sites were carried out using the method described by Pitcher *et al*. (1989). Polymerase chain reaction (PCR) amplification was done to confirm the identity of the bacterial strains, the small sub-unit 16s rRNA genes were amplified from the genomic DNA with 27F: AGAGTTTGATCMTGGCTCAG and 1525R: AAGGAGGTGWTCCARCCGCA primer.

Media Selection for Fermentation

Four different media were used for fermentation. These were; media: I (maize flour – 20.0g, distilled water – 1000mL), II (Glucose - 10.0g, $FeSO_4$ -0.05g, NaCl - 0.9g, $CaCl_2$ - 0.3g, $MgSO_4$ - 0.5g, KCl - 1.0g, distilled water –1000mL), III (Starch - 10.0g, Peptone- 5.0g, yeast extract–3.0g, $ZnSO_4.7H_2O$- 0.001g, $FeSO_4$ - 0.2g, $CaCl_2$ -0.25g, NaCl - 1.0g, $MnSO_4$ - 0.002g, distilled water – 1000mL) and IV (Lactose - 10g, yeast extract - 4.0g, KH_2PO_4 -1.0g, $CaCl_2$ - 0.05g, $ZnSO_4.7H_2O$ - 0.002g,$(NH_4)_2S0_4$ - 3.2g, $FeSO_4$ -0.045g, $MgSO_4$-0.3g, distilled water – 1000mL).With 20g of agar added to the above compositions respectively, all the isolates were streaked on the four media and incubated at 37^0C for 24 h to see which best supported the growth of the organisms. The media were sterilized by autoclaving at 121^OC for 15 minutes. The media which best supported the growth of the organisms were noted.

Fermentation for Amylase production

Amylase producing ability of all the bacteria isolated were investigated using submerged fermentation. Media I, II, III and IV were used 1k respectively without agar. PH of the media was kept at 7.0 using 1M NaOH or H_2SO_4 as appropriate. 100mLs of the media were dispensed into 250mL conical flask respectively in triplicjates according to the number of the isolates and were sterilized using the autoclave at 121oC for 15 minutes. Flasks were allowed to cool after sterilization and 2mL of the overnight grown culture of the isolates were introduced into each flask containing media: I, II, III and IV respectively. Inoculated fermentation broths were incubated at 37^0C differently under two conditions: with and without agitation for 24 h. At the end of the incubation period, fermentation broths were centrifuged at 5000 rpm for 15 minutes using a cold centrifuge and the supernatant was taken as the crude enzyme. The crude enzymes were subsequently used to carry out the enzyme assay in order to know the yield of the various isolates through their enzyme activity. Two isolates with the highest enzyme activity were selected for optimization studies, and the incubation condition (with or without agitation) under which the organisms showed highest enzyme activity was also selected for further studies. Two media which showed the best growth of the organisms and also supported highest amylase yield were selected for optimization studies.

Enzyme assay
Amylase enzyme activity was determined using 1% starch dissolved in distilled water as substrate. The reaction mixture containing 1.0mL substrate and 2mL enzyme solution was incubated at 40°C for 15 minutes. And the reaction was stopped by adding 3 mL DNS (3,5-dinitrosalicylic acid) reagent. All enzyme samples were assayed in triplicates with boiled enzyme blanks for each determination. The enzyme activity was expressed as U/min/mL which corresponded to µ-mole of glucose equivalent released per minute under the assay conditions as described by Islam *et al*. (2014). Absorbance was taken at 600nm.

Optimization Studies
Optimization studies were carried out to determine the effects of pH, fermentation time, temperature, carbon source (medium III), substrate concentrations (starch as the major substrate in medium III and Maize flour for medium I), effect of metal ions (on medium III), nitrogen source optimization (media I and III) and inoculum size on amylase enzyme production. Two of the isolates: *Enterobacter hormaechei* SR3 and *Bacillus cereus* MR1, which showed the highest enzyme activity out of the nine bacteria isolated from the cassava peel dumpsites were chosen for optimization studies. Incubation without agitation which proved better for the organisms judging from the enzyme yield as shown by their various activities in U/mL/min was used all through the investigation. Overnight culture of the two isolates in broth was used all through the optimization studies. Media I (maize flour) and III (basal medium with starch as carbon source) were sterilized using the autoclave at 121^{0}C for 15 minutes. Two mililiters (2 % v/v) of inocula of *Enterobacter hormaechei* SR3 and *Bacillus cereus* MR1 were respectively introduced into 98mL of the fermentation broth. Inoculated fermentation broth of both media were incubated at 37^{0}C and pH were kept at 7 using 1N H_2SO_4 or 1N NaOH respectively where applicable.

Effect of pH on Amylase production
Fermentation media (I and III) were kept at pH of 5, 6, 7, 8 and 9 in order to study their effect on amylase enzyme production, and enzyme assay was carried out after 24 h of incubation.

Optimizing Fermentation time
The experiment was carried out individually at various incubation periods of 18, 24, 48 and 72 h. 2mL (2%v/v) of inocula of *Enterobacter hormaechei* SR3 and *Bacillus cereus* MR1 were introduced into fermentation broth (media I and III) respectively, and enzyme assay was carried out at the end of each fermentation period.

Effect of Temperature on Amylase production
The effect of temperature on amylase production was evaluated by incubating the culture media at various temperatures 27^{0}C, 37^{0}C, 45^{0}C, 50^{0}C, 65^{0}C, 75^{0}C. 2mL (2% v/v) of inocula of the two isolates were introduced into fermentation broth (media I and III).The enzyme assay was carried out after 24 h of incubation.

Carbon source Optimization for medium III
The effect of glucose, lactose, sucrose, maltose and starch as carbon sources on amylase production was investigated on medium III. Ten gram of the listed carbon sources were substituted into medium III and dissolved in 1000mL of distilled water. One hundred mililitre of the medium containing different carbon sources were dispensed into 250mL conical flasks for *Enterobacter hormaechei* SR3 and *Bacillus cereus* MR1 respectively. Amylase assay was carried out after 24 h of incubation.

Substrate Concentration Optimization
The effect of substrate concentration of media I (maize flour) and III (starch as the main substrate) on amylase enzyme production were studied using concentrations of 0.5, 0.7, 1.0, 2.0, 3.0 and 4.0 % w/v respectively. Assay was carried out after 24 h of incubation.

Effect of Metal ions on Medium III
Different metal ions (0.002%) were used in order to study their effects on amylase productions by *Enterobacter hormaechei* SR3 and *Bacillus cereus* MR1. The test was carried out only for medium III. To investigate their effects on amylase enzyme production, since medium III already had some of the metal ions, those metal ions already present were removed respectively (except for Na^{+1} and Ca^{+2} that were kept constant) from the composition and metal ions which were not already part of the original composition of medium III were also added respectively. The metal ions studied were; Fe^{2+}, Mn^{2+}, Mg^{2+}, Zn^{2+}, K^{+1} and Fe^{2+} + K^{+1} + Mn^{2+}+ Mg^{2+}+ Zn^{2+} + Na^{+1} + Ca^{+2}. The major components of medium III (starch, peptone and yeast extract) were weighed as specified in 100mL. pH was kept at 7. Fermentation broth was allowed to cool and inoculated with the selected isolates. Incubation was carried out at 37^{0}C for 24 h and enzyme activity was quantified.

Nitrogen Source Optimization
The effect of different nitrogen sources on amylase enzyme production was carried out for both media in the following order; yeast extract + potassium nitrate (KNO_3), yeast extract, KNO_3 (Potassium nitrate), Ammonium sulphate, peptone, yeast extract + peptone. 0.5% concentration of the various nitrogen sources were used and incorporated into every 100mL preparation of media I and III differently for *Enterobacter hormaechei* SR3 and *Bacillus cereus* MR1.Two mililitre of inocula (2% v/v) of the isolates were respectively introduced into 100mL of fermentation broth contained in 250mL conical flask. Fermentation broths were kept at pH of 7. Incubation was carried out for 24 h at 37^{0}C and enzyme assay was carried out.

Inoculum size optimization
Inoculum size optimization in both fermentation media I (maize flour) and III (basal medium with starch as carbon source) for *Enterobacter hormaechei* SR3 and *Bacillus cereus* MR1 were carried out in the following order 0.5mL (0.5% v/v), 0.7mL (0.7% v/v), 1.0mL (1.0% v/v), 2.0mL (2.0% v/v), 3.0mL (3.0% v/v) and 4.0mL (4.0% v/v) and incubated at 37^{0}C for 24 hrs. Thereafter, incubated fermentation broth was centrifuged at 5000rpm for 15 minutes. The supernant was used to determine the enzyme activity.

Thermal stability test of amylase Enzyme
The thermal stability of the enzyme was determined by incubating enzyme fractions at different temperatures ranging from 25^{0}C to 70^{0}C without the substrate for one hour at ten minutes intervals, aliquots of 0.5 mL of the incubated enzyme were assayed for

activity.

Total Amylase Activity of Optimized Amylase

The optimized amylase enzyme was produced with both *Enterobacter hormaechei* SR3 and *Bacillus cereus* MR1. Media I (maize flour) and III (starch as carbon source) were used by incorporating the most favourable outcomes of all the parameters studied in one single fermentation. These parameters for *Enterobacter hormaechei* SR3 and *Bacillus cereus* MR1 in both fermentation broth are: pH 7.0 and 8.0, 48 and 72 h of incubation, $50^{O}C$ and $60^{O}C$ temperature, 2.0 and 3.0 % w/v substrate concentration, 2 and 3% v/v inocula sizes, 0.5% peptone, Potassium nitrate and yeast extract + peptone, respectively, presence of 0.002% potassium ion and absence of zinc ion.

The method described by Islam *et al*. (2014) was used with slight modifications. Amylase activity was determined using 1% starch dissolved in distilled water as substrate. A blank tube containing 1mL of substrate and 0.2mL of 0.1 M Potassium phosphate buffer, enzyme blank tube containing 1.0mL substrate and enzyme sample test tube containing 1mL of substrate and 2mL of enzyme sample were taken. Each test tube was properly vortexed and incubated for 15 minutes at 40°C. 3mL DNS (3,5-dinitrosalicylic acid) was added to the test tubes and 2mL was added to enzyme blank test tube. The reaction mixture containing 1.0mL substrate and 2mL enzyme solution was incubated at 40°C for 15 minutes. And the reaction was stopped by adding 3 mL DNS reagent, 6mL of distilled water were added to all the test tubes. Test tubes were heated in boiling water bath for five minutes to effect colour change and then cooled with running water tap (Sinha, 2010).This procedure was carried out respectively for enzymes produced by *Enterobacter hormaechei* SR3 and *Bacillus cereus* MR1 using both fermentation medium I (maize flour) and medium III (basal medium with starch).The absorbance was observed at 600nm.

Statistical Analysis of Results

Statistical analysis of data obtained from the experiments were done using one-way analysis of variance (ANOVA).

RESULTS

Nine amylolytic bacteria were isolated from cassava peel dump-site and were identified as *Bacillus megaterium*, *Achromobacter iophagus*, *Enterobacter hormaechei* SR3, *Bacillus stearothermophilus*, *Escherichia coli*, *Bacillus licherniformis*, *Bacillus cereus* MR1, *Pseudomonas* sp. and *Bacillus subtilis*. The cultural, morphological and biochemical characteristics of these organisms are presented in Table 1. Table 2 presents the molecular characterization of the two best amylase yielding Bacteria isolated from cassava peel dump-site. Table 3 presents the result of the screening for the nine (9) isolates. Media I (maize flour) and III showed better support for the growth of virtually all the organisms. Medium IV showed no growth at all, while medium II supported the growth of few organisms. The fermentation for amylase production using the four media for all bacterial isolates under agitated and non-agitated incubation conditions are presented on Tables 4 and 5. The effects of pH on amylase production in medium I (maize flour) and medium III (basal medium) using *Enterobacter hormaechei* SR3 and *Bacillus cereus* MR1 are shown in Figure 1. Figure 2 show the effect of fermentation time on amylase production by *E. hormaechei* SR3 and *B.cereus* MR1 in media I (maize flour) and III. Effect of temperature on amylase production for media I (maize flour) and III (basal medium) are shown in Figure 3. In Figure 4, the effect of carbon source on amylase production in medium III is shown. Figure 5 shows the effects of maize flour and starch concentration on amylase production The effects of the presence and absence of metal ions on amylase production in medium III are shown in Figure 6. In Figure 7 the effect of nitrogen source on amylase production by the two organisms in media; I (maize flour) and III (basal medium).is depicted. Figure 8 show the effect of inoculum size on amylase production in fermentation media III and I (maize flour). Thermal stability test of amylase enzyme produced using media I (maize flour) and III are presented in Tables 6 and 7 respectively. In Table 8 the enzyme activity of optimized amylase produced from *Enterobacter hormaechei* SR3 and *Bacillus cereus* MR1 is presented.

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Table 1: The Cultural, Morphological and Biochemical Characteristics of Bacterial Isolates from Soil sample of banana peel dump site.

Isolate	A	B	C	D	E	F	G	H	I
Colonial morphology	Medium Round, smooth, glistening white colony with yellow at the reverse, soft, convex and entire	Small white circular colonies with fading purple (like touches of iodine), colonies in chains of 2 and 3, entire, raised	Small, white, round, dot-like colonies with regular edge and flat elevation	Small, creamy, round, smooth, raised, translucent colonies with regular edge	Medium, golden yellow with whitish center and golden at the reverse, entire, moist, glistening	Large, smooth circular white colonies with cream colour at the reverse, entir, non-spreading, with hairy out-growths, convex elevation	Medium, white, non-spreading colonies with whip-like outgrowths, flat regular edge	Medium, umbonate, round, green colonies that looks slurry with irregular edge and flat elevation	Rough, off-white, spreading and opaque colonies with raised elevation
Gram reaction and	+	-	-	+	-	+	+	-	+
Microscopic description	Short single rods	Single rods	Straight Single rods	Short single rods densely arranged	Long rods in singles densely arranged	Short rods in pairs	Short rods sparsely arranged	Straight short rods	Uniformly stained medium sized rods densely arranged
Motility test	-	+	-	-	+	+	-	+	+
H_2S production using TSI	-	-	-	-	-	+	-	-	-
Citrate utilization	+	+	+	+	-	+	+	+	+
Glucose fermentation	+AG-	+AG-	-AG+	+AG-	+AG+	+AG-	+AG-	+AG+	+AG-
Sucrose fermentation	+AG-	+AG+	+AG+	-AG-	+AG+	+AG-	+AG-	+AG-	+AG-
Maltose fermentation	+AG+	+AG-	-AG+	-AG-	+AG+	+AG+	+AG-	-AG+	-AG+
Lactose fermentation	-AG-	-AG-	+AG-	+AG-	-AG+	-AG-	-AG-	-AG-	-AG-
Catalase test	+	+	+	-	+	+	+	+	+
Urease test	+	+	+	+	-	+	+	+	+
Oxidase	-	-	-	-	-	+	-	-	-
Probable Organisms	*Bacillus megaterium*	*Achromobacter iophagus*	*Enterobacter hormaechei*	*Bacillus stearothermophilus*	*Escherichia coli*	*Bacillus licheniformis*	*Bacillus cereus*	*Pseudomonas* sp	*Bacillus subtilis*

Table 2: Molecular identification of the highest yielding Amylolytic Bacteria Isolated from Cassava peel Dumpsite

Isolates	Isolate Gene bank Homolog	Number of Bases	Identity (%)	Accession Number
C	*Enterobacter hormaechei* SR3	479	95	KC634321.1
G	*Bacillus cereus* MR1	537	91	KJ685546.1

Table 3: Screening for Amylolytic Bacteria on Starch–Iodine Plates

Isolate Name	Diameter of Zone of clerance(cm)	Diameter of colony(cm)	Zone of clerance (cm)
Enterobacter hormaechei SR3	4.6	1.0	3.6
Bacillus cereus MR1	4.4	1.2	3.2
Bacillus substilis	3.7	3.4	0.3
Bacillus stearothermophilus	2.9	2.3	0.6
Bacillus megaterium	3.5	1.9	1.6
Achromobacter iophagus	-	-	-
Pseudomonas sp	4.7	1.9	2.8
Bacillus licheniformis			2.3
Escherichia coli	4.6	2.0	2.6

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Table 4: Fermentation using Bacteria for Amylase Production under agitated Incubation

Isolates	Medium I (U/mL/min)	Medium II (U/mL/min)	Medium III (U/mL/min)	Medium IV(U/mL/min)
Enterobacter hormaechei SR3	1.692 ±0.006^{i}	0.521±0.003^{i}	1.223±0.001^{i}	0.413±0.001^{g}
Bacillus cereus MR1	1.503±0.006^{h}	0.314±0.015^{f}	1.192±0.001^{h}	0.538±0.001^{h}
Bacillus stearothermophilus	1.038±0.005^{f}	0.072±0.012^{a}	0.173±0.003^{a}	0.364±0.001^{e}
Escherichia coli	0.720±0.006^{c}	0.212±0.001^{c}	0.612±0.001^{f}	0.214±0.001^{b}
Bacillus substilis	1.220±0.005^{g}	0.117±0.001^{b}	0.338±0.001^{c}	0.115±0.003^{a}
Bacillus licherniformis	0.827±0.005^{e}	0.262±0.001^{d}	0.571±0.001^{e}	0.272±0.001^{c}
Bacillus megaterium	0.481±0.006^{b}	0.315±0.001^{g}	0.314±0.002^{b}	0.113±0.001^{a}
Pseudomonas sp.	0.727±0.005^{d}	0.271±0.001^{e}	0.825±0.001^{g}	0.402±0.001^{f}
Achromobacter iophagus	0.281±0.012^{a}	0.335±0.020^{h}	0.375±0.001^{d}	0.307±0.001^{d}

Values represented are means of triplicates ± standard deviation. Means with different superscript across a row are significantly different ($p < 0.05$)

Table 5: Fermentation using bacteria for Amylase Production un-agitated Incubation

Isolates	Medium I (U/mL/min)	Medium II (U/mL/min)	Medium III (U/mL/min)	Medium IV (U/mL/min)
Enterobacter hormaechei SR3	2.313±0.003^{i}	0.483±0.001^{h}	1.693±0.001^{h}	0.515±0.003^{h}
Bacillus cereus MR1	2.112±0.002^{h}	0.352±0.001^{e}	1.856±0.002^{i}	0.345±0.001^{e}
Bacillus stearothermophilus	1.444±0.001^{e}	0.115±0.001^{a}	1.060±0.002^{f}	0.176±0.002^{a}
Escherichia coli	1.165±0.001^{d}	0.363±0.002^{f}	0.983±0.003^{e}	0.215±0.001^{b}
Bacillus substilis	1.838±0.003^{g}	0.254±0.001^{c}	0.582±0.001^{b}	0.259±0.003^{c}
Bacillus licherniformis	0.971±0.003^{c}	0.395±0.001^{g}	0.813±0.001^{c}	0.321±0.001^{d}
Bacillus megaterium	0.895±0.002^{b}	0.163±0.002^{b}	0.962±0.002^{d}	0.414±0.001^{g}
Pseudomonas sp.	1.508±0.04^{f}	0.318±0.001^{d}	1.197±0.001^{g}	0.377±0.002^{f}
Achromobacter iophagus	0.765±0.002^{a}	0.490±0.001^{i}	0.514±0.002^{a}	0.221±0.002^{b}

Values represented are means of triplicates ± standard deviation. Means with different superscript across a row are significantly different ($p < 0.05$)

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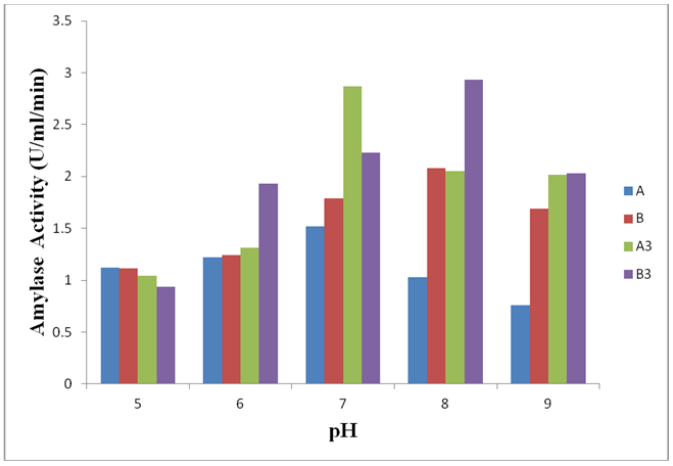


Figure 1: The effect of pH on amylase production in medium I (Maize flour) and medium III (Basal medium) using *Enterobacter hormaechei* SR3 and *Bacillus cereus* MR1

Key:
A: *Enterobacter hormaechei* SR3 in Maize flour (Medium I)
B: *Bacillus cereus* MR1 in Maize flour (Medium I)
A3: *Enterobacter hormaechei* SR3 in Medium III (Basal medium)
B3: *Bacillus cereus* MR1 in Medium III (Basal medium)

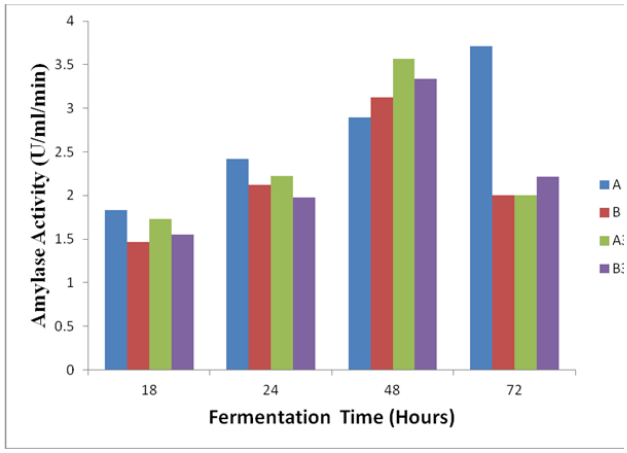


Figure 2: The effect of fermentation time on amylase production by *Enterobacter hormaechei* SR3 and *Bacillus cereus* MR1 in Media; I (maize flour) and III (Basal medium)

Key:
A: *Enterobacter hormaechei* SR3 in Maize flour (Medium I)
B: *Bacillus cereus* MR1 in Maize flour (Medium I)
A3: *Enterobacter hormaechei* SR3 in Medium III (Basal medium)
B3: *Bacillus cereus* MR1 in Medium III (Basal medium)

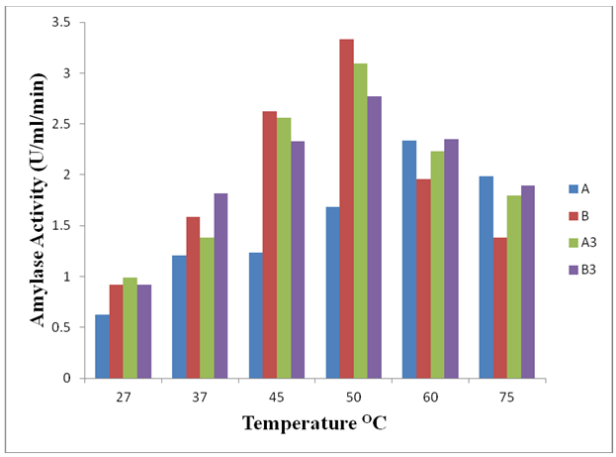


Figure 3: The effect of temperature on amylase production by *Enterobacter hormaechei* SR3 and *Bacillus cereus* MR1 in medium III and maize flour

Key:
A: *Enterobacter hormaechei* SR3 in Maize flour (Medium I)
B: *Bacillus cereus* MR1 in Maize flour (Medium I)
A3: *Enterobacter hormaechei* SR3 in Medium III (Basal medium)
B3: *Bacillus cereus* MR1 in Medium III (Basal medium)

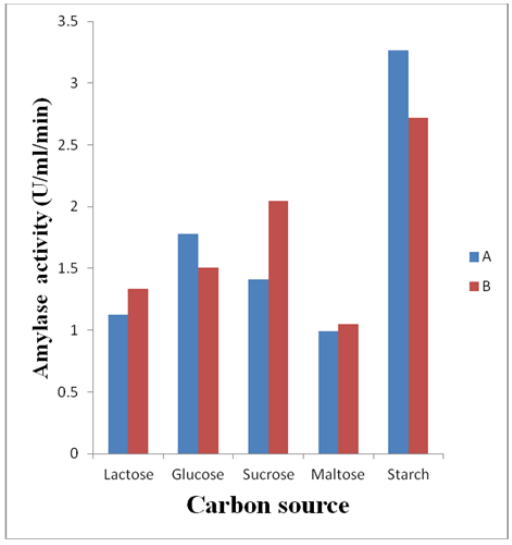


Figure 4: The effect of carbon source on amylase production in medium III (Basal medium)

Key:
A: *Enterobacter hormaechei* SR3
B: *Bacillus cereus* MR1

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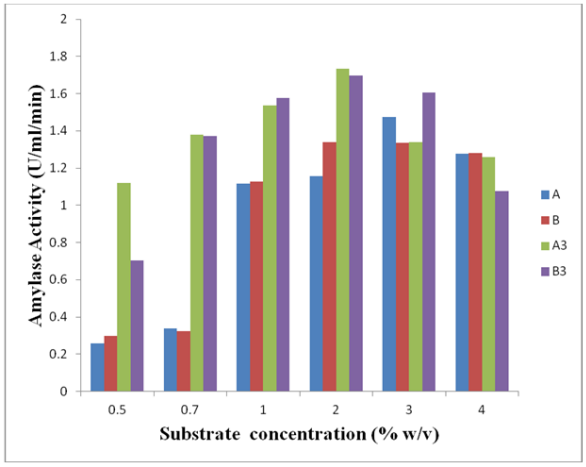


Figure 5: The effect of maize flour and starch concentration on Amylase production

Key:
A: *Enterobacter hormaechei* SR3 output with concentration of maize flour
B: *Bacillus cereus* MR1 output with concentration of maize flour
A3: *Enterobacter hormaechei* SR3 output with concentration of starch
B3: *Bacillus cereus* MR1 output with concentration of starch

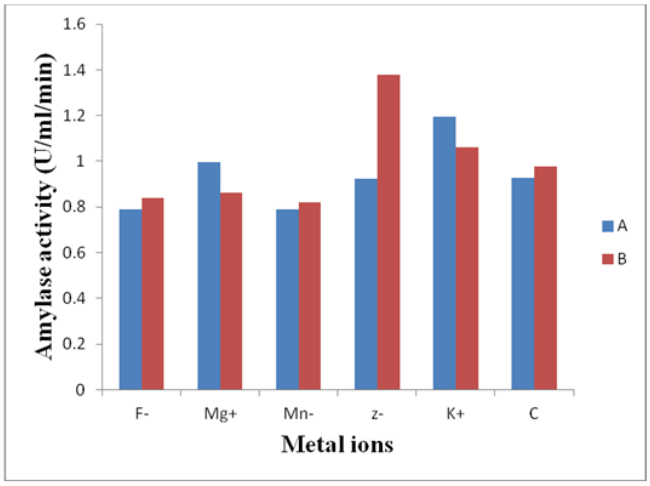


Figure 6: Effect of the presence and absence of metal ions on amylase production in medium III (Basal medium)

Key:
+: Present
- : Absent
F: Fe^{2+} (Ferrous ion)
Mg: Mg^{2+} (Magnesium ion)
Z: Zn^{2+} (Zinc ion)
K: K^{+} (Potassium ion)
C: Ca^{2+} + K^{+}+ Fe^{2+}+ Na^{+} + Mg^{2+} + Mn^{4+} + Zn^{2+}
A: *Enterobacter hormaechei* SR3,
B: *Bacillus cereus* MR1

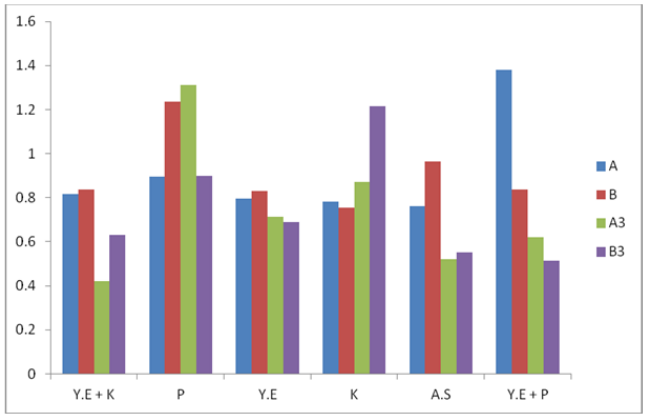


Figure 7: The effect of nitrogen source on amylase production of *Enterobacter hormaechei* SR3 and *Bacillus cereus* MR1 in media; I (maize flour) and III (Basal medium)

Key
Y.E + K: Yeast extract plus Potassium nitrate
P: Peptone, K: Potassium nitrate (KNO_3)
A.S: Ammonium sulphate ($(NH_4)_2SO_4$), Y.E +P: Yeast extract plus Peptone
A: *Enterobacter hormaechei* SR3 in medium III, B: *Bacillus cereus* MR1 in medium III
A3: *Enterobacter hormaechei* SR3 in maize flour (medium I),
B3: *Bacillus cereus* MR1 in maize flour (medium I)

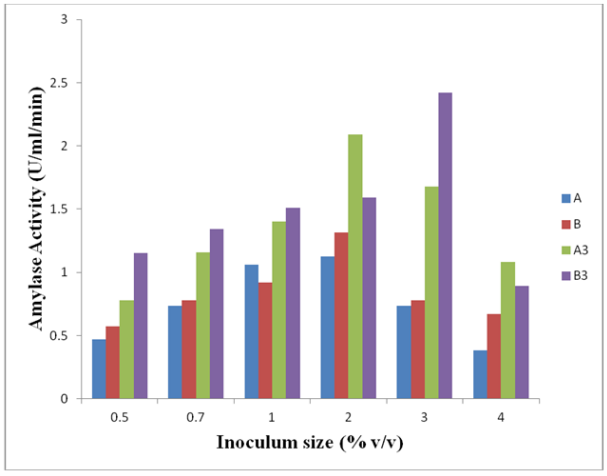


Figure 8: The effect of inoculum size on amylase production in maize flour and medium III (Basal medium)

Key:
A: *Enterobacter hormaechei* SR3 in maize flour (medium I)
B: *Bacillus cereus* MR1 in maize flour (medium I)
A3: *Enterobacter hormaechei* SR3 in medium III (Basal medium)
B3: *Bacillus cereus* MR1 in medium III (Basal medium)

Table 6: Thermal stability test of amylase enzyme produced using medium I (maize flour)

Temperature ^{O}C	*Enterobacter hormaechei* SR3 (U/mL/min)	*Bacillus cereus* MR1 (U/mL/min)
25	1.343 ± 0.003^{h}	1.209 ± 0.009^{d}
30	1.621 ± 0.003^{i}	1.652 ± 0.005^{i}
35	2.000 ± 0.009^{j}	2.543 ± 0.003^{j}
40	1.211 ± 0.003^{g}	1.600 ± 0.006^{h}
45	1.118 ± 0.011^{f}	1.524 ± 0.004^{g}
50	1.091 ± 0.003^{e}	1.421 ± 0.006^{f}
55	1.008 ± 0.005^{d}	1.273 ± 0.003^{e}
60	0.812 ± 0.007^{c}	1.091 ± 0.003^{c}
65	0.780 ± 0.003^{b}	0.850 ± 0.005^{b}
70	0.753 ± 0.007^{a}	0.598 ± 0.007^{a}

Values represented are means of triplicates ± standard deviation. Means with the same superscript across a row are significantly different ($p < 0.05$)

Table 7: Thermal stability test of amylase enzyme produced using medium III (Basal medium)

Temperature ^{O}C	*Enterobacter hormaechei* SR3 (U/mL/min)	*Bacillus cereus* MR1 (U/mL/min)
25	1.621 ± 0.005^{h}	1.752 ± 0.002^{h}
30	2.310 ± 0.006^{j}	2.110 ± 0.003^{i}
35	2.116 ± 0.002^{i}	2.406 ± 0.006^{j}
40	1.531 ± 0.004^{g}	1.690 ± 0.005^{g}
45	1.324 ± 0.003^{f}	1.459 ± 0.003^{f}
50	1.300 ± 0.008^{e}	1.423 ± 0.003^{f}
55	1.160 ± 0.003^{d}	1.290 ± 0.005^{d}
60	0.899 ± 0.007^{c}	1.206 ± 0.006^{c}
65	0.750 ± 0.003^{b}	0.895 ± 0.003^{b}
70	0.710 ± 0.003^{a}	0.760 ± 0.006^{a}

Table 8: Enzyme Activity of Optimized Amylase produced from *Enterobacter hormaechei* SR3 and *Bacillus cereus* MR1 in Maize flour (medium I) and Basal medium (medium III)

Enterobacter hormaechei SR3 **Enzyme from medium I (U/mL/min)**	*Bacillus cereus* MR1 **Enzyme from medium I (U/mL/min)**	*Enterobacter hormaechei* SR3 **Enzyme from medium III (U/mL/min)**	*Bacillus cereus* MR **Enzyme from med III (U/mL/min)**
2.512 ± 0.001	2.668 ± 0.003	2.718 ± 0.001	1.963 ± 0.001

Values represented in table are means of triplicates ± standard deviation

DISCUSSION

Conventionally, amylase, producing microorganisms like fungi and bacteria are isolated from soil (Singh & Kumari, 2016).The present investigation deals with the optimization of amylase from bacteria isolated from soil of cassava peel dumpsite using submerged fermentation. Altogether, nine bacteria were isolated, these are: *Bacillus cereus* MR1, *Enterobacter hormaechei* SR3, *Bacillus stearothermophilus*, *Escherichia coli*, *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus megaterium*, *Pseudomonas* sp. and *Achromobacter iophagus*. The amylolytic bacteria isolated in this investigation is to a great extent in agreement with the work of Parmar & Pandya (2012) they reported that *Bacillus subtilis*, *Bacillus cereus, Bacillus polmyxa, Bacillus amyloliquefaciens, Bacillus coagulants, Bacillus subtilis, Lactobacillus, Escherichia, Proteus, Bacillus lincheniformis, Bacillus stearothermophilus, Bacillus megaterium, Strepotmyces* sp. *and Pseudomonas* sp.., were involved in α-amylases and β-amylases production. Also the isolation and characterization of an *Enterobacter* sp. NACASA2 which showed maximum α-Amylase production of 56.13U/mg protein/ min) at 24 h of incubation was reported by Jadhav *et al*. (2016). These bacteria are major decomposers of carbonaceous matter, therefore, it is not strange that they were isolated from a cassava peel dumpsite.

Media II and IV showed poor support for the growth of the organisms, while on other plates, there were no growth of the organisms at all. However, medium I (maize flour) and medium III showed better results as they supported maximally the growth of the microorganisms. This finding is in agreement with the assertion of Okafor (2007) who stated that all microbiological media, whether for industrial or laboratory purposes must satisfy the needs of the organism in terms of carbon, nitrogen, minerals, growth factors, and water and in addition must not contain materials which are inhibitory to growth.

The fermentation for amylase production carried out for all the bacteria isolates showed that medium I (maize flour) and medium III favoured amylase production compared to medium II and IV (Tables 4 and 5), hence, the reason they were selected as fermentation medium for optimization studies. *Enterobacter hormaechei* SR3 and *Bacillus cereus* MR1 showed the highest enzyme activity compared to the other bacteria isolated in both fermentation media under agitated and non-agitated incubation conditions, hence, the reason they were picked for optimization studies. The selection of *Enterobacter hormaechei* SR3 and *Bacillus cereus* MR1 is also in consonance with their display of highest zones of clearance on starch-iodine plates (Table 3). The essence of agitation was to create aeration especially for obligate aerobes. Though production under non-agitated incubation condition was highest for all isolates in both fermentation broth, for them to still produce considerably with agitation suggests they are facultative anaerobes. The role of several factors, including agitation have been studied for amylase production. Similar study has been carried out by Silva *et al*. (2009), they produced high levels of α-amylase and glucoamylase using *Aspergillus niveus* under submerged fermentation from agricultural residue; cassava peel as carbon source. Their finding showed that in the static conditions, the amylase production was substantially greater than in the agitated condition. Contrary to the report of this investigation, Singh & Kumari (2016) and Fentahun & Kumari (2017) in separate studies produced and optimized high yields of amylase from *Bacillus* sp. under agitated incubation conditions. The production using all the bacteria isolated from the cassava

peel dump-site is equally necessary because, the microorganisms with the highest zone of clearance on starch-iodine plates might not be the best producers. From this investigation, *Bacillus stearothermophilus, Bacillus subtilis*, *Bacillus megaterium* and *Escherichia coli* had 0.6cm, 0.3cm, 1.6cm and 2.6cm as zones of hydrolysis (Table 3). However, judging by their level of production or activity (Tables 4 and 5), *Bacillus stearothermophilus* had better activity in medium I (maize flour) (under agitated and non-agitated condition;1.038±0.005 U/mL/min and 1.444±0.001 U/mL/min respectively) and medium III (basal medium with starch) (1.060±0.002U/mL/min under non-agitated incubation) compared to *Escherichia coli* which had a higher zone of clearance (hydrolysis) but lower enzyme titre in both fermentation broth under both incubation conditions. The findings of this investigation has proven that the use of zone of clearance alone might not conclusively decide the fate of high enzyme producing isolates. Concerning the choice of submerged fermentation, thermostable-amylases from various bacterial strains have been produced through this method as well as solid state fermentation. In agreement with the use of submerged fermentation for the production of bioactive substances, Subramaniyam and Vimala (2012) pointed out that the recovery of products is relatively simple using the technique. The effect of fermentation medium, incubation condition and differences in amylase production by all the bacteria isolated from the cassava peel dumpsite was found to be significant ($p < 0.05$).

Optimization studies on amylase enzyme production by *Enterobacter hormaechei* SR3 and *Bacillus cereus* MR1 in fermentation media I and III, the various factors tested had profound effects on amylase production. This concurs with the findings of Fentahun and Kumari (2017) They asserted that in the production of microbial amylase optimization of culture conditions, chemical and physical parameters like pH, temperature, salt concentration and incubation time of microbial fermentation process are highly essential.

Studies on the effect of pH (Figure 1) showed that *Enterobacter hormaechei* SR3 and *Bacillus cereus* MR1 in fermentation medium I (maize flour) displayed maximum enzyme activity of 1.520±0.005U/mL/min and 2.082±0.002U/mL/min at pH values of 7.0 and 8.0 respectively. Also, in medium III (basal medium with starch), had highest enzyme activity for *Enterobacter hormaechei* SR3 and *Bacillus cereus* MR1 (Figure 1) which were 2.869±0.005U/mL/min and 2.936 ± 0.002U/mL/min at pH values of 7.0 and 8.0 respectively. Amylase activity kept rising until it reached the optimum values of 7 and 8 respectively for both organisms, after which activity began to drop. This pH study also showed that enzyme produced by both organisms can effectively function under a wide range of pH from slightly acidic to neutral and slightly alkaline conditions. Similarly, Jadhav *et al*. (2016) found highest amylase production of 56.13 U/mg protein/min at pH of 7 using bacteria isolated from soil and identified as *Enterobacter* sp. NACASA2. Changes in pH, like that of temperature, might change the three dimensional structure of active sites. Also the enzymes and substrates binding speed to produce maximum product will be highly reduced due to pH change (Fentahum & Kimari, 2017). The effect of pH on amylase production was found to be significant at $p<0.05$.

Fermentation time studies of the isolates in maize flour (Figure 2) showed that, *Enterobacter hormaechei* SR3, had maximum activity of 3.711±0.003U/mL/min after 72 h, while activity of 3.124±0.004U/mL/min was highest for *Bacillus cereus* MR1 after 48 h. For fermentation medium III (basal medium with starch as carbon source) (Figure 2), both *Enterobacter hormaechei* SR3 and *Bacillus cereus* MR1 had highest activities of 3.567±0.008U/mL/min and 3.340±0.005U/mL/min after 48 h respectively. For both isolates in both fermentation media, increasing enzyme titres were noticed until it reached the optimum duration after which activity dropped. The findings of this work however negates the results of Singh and Kumari (2016) who reported that amylase activity decreased from 0.981 to 0.215 U/mL as the incubation time increased from 24 to 72 h at 35 ±2°C. The enzyme activity increased with increase in incubation time until it reached the optimum duration. In most cases, the production of enzyme began to decline if the incubation time was further increased. This might be due to the depletion of nutrients in the medium (Raul *et al*., 2014) or release of toxic metabolites.

The influence of temperature on amylase activity (Figure 3) showed that *Enterobacter hormaechei* SR3 had maximum enzyme activity at $50^{O}C$ and $60^{O}C$ in both fermentation media, while for *Bacillus cereus* MR1, maximum amylase titres was recorded at $50^{o}C$ in both media. In all there was progressive increase in amylase enzyme activity produced by both isolates from $27^{O}C$ to $50^{O}C$ and $27^{O}C$ to $60^{O}C$ respectively after which the enzyme activity began to decrease. For both organisms to produce at such high temperature implies that they are thermophilic. The findings of this work is in agreement with the work of Fentahun & Kumari (2017) they reported optimum temperature of $60^{O}C$ for maximum amylase production by thermophilic spore forming Bacilli isolated from starch-rich soil. The optimum temperature found in this investigation however negates the findings of Jadhav *et al*. (2016) who reported optimum temperature of $35^{O}C$ for maximum amylase production using *Enterobacter* sp.NACASA2 isolated from soil. The increase in amylase activity with increasing temperature up to the optimum temperature might be as a result of increasing kinetic energy, which favoured the rate of collisions between substrate and enzyme during hydrolysis process up to the optimum temperatures of $50^{O}C$ and $60^{O}C$ after which the kinetic energy began to decrease. The decrease after the optimum temperatures of $50^{O}C$ and $60^{O}C$ might also be a resultant effect of the breakage of secondary, tertiary and quaternary bonds that maintain the three dimensional structure of enzymes at high temperature , which might have led to conformational changes of the enzyme active sites. The effect of temperature on amylase production was found to be significant ($p < 0.05$).

The influence of carbon source on amylase enzyme yield of *Enterobacter hormaechei* SR3 and *Bacillus cereus* MR1 in medium III (basal medium) (Figure 4) revealed highest enzyme titres when starch was used as carbon source. Next in activity for *Enterobacter hormaechei* SR3 is glucose; which had 1.779±0.006U/mL/min, while for *Bacillus cereus* MR1, it was sucrose with 2.049±0.004U/mL/min level of enzyme activity. Jadhav *et al*. (2016) found the highest amylase yield with starch when compared to xylose, lactose, maltose and sucrose from *Enterobacter* sp.NACASA2 isolated from garden soil. Contrary to this findings, Sundarram and Murthy (2014) reported that, *Bacillus cereus* MTCC 1305 showed enhanced enzyme production of 122±5 U/g with glucose than other carbon sources studied. The effect of carbon source on amylase production was found to be

significant ($p < 0.05$).

The effect of substrate concentration on amylase production was studied for both medium I (maize flour) and medium III (starch as the carbon source) using *Enterobacter hormaechei* SR3 and *Bacillus cereus* MR1. Amylase production increased from concentrations of 0.5 to 4.0% w/v and maximum yield was found at concentrations of 2.0 and 3.0% w/v for maize flour (medium I) and medium III (starch in basal medium) using both organisms (Figure 5).Similarly, Kumar & Duhan (2011) reported maximum amylase activity at optimum concentration of 3 % starch. The gradual increase in amylase titre got with increased substrate concentration might be that, at low substrate concentration the active sites of enzyme were not saturated and thus the enzyme activity was increased with the increase in substrate concentration until it reached the optimum. The effect of substrate concentration on amylase production was found to be significant ($p < 0.05$).

The impact of the presence and absence of metal ions on medium III on amylase production was also investigated (Figure 6). *Enterobacter hormaechei* SR3 had lowest enzyme activity of 0.788±0.002U/mL/min when manganese was absent from the fermentation medium, and highest enzyme activity of 1.196±0.002U/mL/min when potassium was present in the medium. *Bacillus cereus* MR1, had lowest amylase activity of 0.821±0.004U/mL/min when manganese was absent and highest activity of 1.377±0.003U/mL/min when Zinc was absent from the medium. When calcium, potassium, sodium, magnesium, iron, manganese and Zinc were combined in medium III (basal medium with starch), appreciably high activity was also found. In all, there was no inhibitory effect of Ca^{2+} on amylase production. Sinha (2010) pointed out that α- Amylase is known to be a calcium metallo-enzyme having at least one calcium ion associated with its molecule. Enhanced enzyme production by the two bacteria in all parameters tested might be the result of increased availability of calcium ions. The effect of metal ions can also be found from the works of Olajide & Fagade (2007) and Mahmood & Rahman (2008) in trying to optimise the production of amylase, stated that the various metal ions used had significant impact on the activity of amylase enzyme and its production respectively.

Nitrogen source optimization was carried out for media I (maize flour) and III (basal medium with starch) (Figure 7). *Enterobacter hormaechei* SR3 and *Bacillus cereus* MR1 showed greatest preference for KNO_3 (Potassium nitrate), Yeast extract + peptone, and peptone as maximum enzyme titres were recorded using these nitrogen sources. The study revealed that nitrogen source has significant ($p < 0.05$) impact on amylase enzyme yield and activity. Also different organisms have different nitrogen source requirement, for optimum growth and enzyme production. The study however negates the findings of Hassan *et al*. (2018) who reported highest amylase yield with *Escherichia coli* when Ammonium sulphate was used as nitrogen source. Hence, for maximum yield of amylase enzyme in industrial settings, it is important to know the organism's best nitrogen requirement or source.

Inocula sizes in the range 0.5 to 4.0% v/v was also optimized for *Enterobacter hormaechei* SR3 and *Bacillus cereus* MR1 in both fermentation media (Figure 8). Highest amylase yields were found at 2 and 3% v/v. For *Enterobacter hormaechei* SR3 and *Bacillus cereus* MR1 amylase activity kept increasing with increase in inoculum size until it reached the optimum of 2 and 3%v/v after which amylase production began to decline with increase in inoculum size. Vishnu *et al*. (2014) reported maximum amylase production using 2% inoculum size with *Bacillus* sp isolated from soil receiving kitchen waste. Lower enzyme biosynthesis at lower inoculum might be due to less cell suspensions which might be insufficient to use the fermentation medium in a better way, hence, a longer time was required for the organisms to grow to an optimum number to utilize the substrate and form the desired product.

Thermal stability study of the enzymes (Tables 6 and 7) produced by the bacteria *Enterobacter hormaechei* SR3 and *Bacillus cereus* MR1 using fermentation media I (maize flour) and III (basal medium with starch) indicated a general decrease in enzyme activity as temperature increased from 25-70^{0}C. Thermal stability of the enzymes produced were found to be maximum at 30^{o}C and 35^{o}C.Taking an appraisal, enzyme produced using maize flour (medium I) was highest in terms of thermal stability, also enzymes produced from *Bacillus cereus* MR1 displayed the maximum thermal stability of 2.543±0.003U/mL/min and 2.406±0.006U/mL/min in both media; I and III. The least thermal stability for enzymes produced was observed at 70^{o}C for 1 hour. The enzymes retained over 50% of their activity even at 55^{0}C for 1 h, indicating thermal stability of the enzyme. Contrary to the findings of this work, Singh (2016) reported the thermal activity of alpha-amylase produced by *Bacillus amyloliquefaciens* (MTCC 610) by incubating the enzyme at various temperatures such as 55°C, 60°C, 65°C, 70°C and 75°C for 30 minutes and then assay of enzyme was performed, maximum stability (219.44 IU/mL) was found at 65°C. The inactivation of the enzymes at high temperature might be due to incorrect conformation as a result of hydrolysis of the peptide chain, destruction of amino acid or aggregation.

In order to establish an efficient fermentation process, Ray *et al*. (2007) emphasized the importance of making the environmental and nutritional conditions favourable for the microorganism for enhanced production of the desired metabolite. Therefore, the derived optimal conditions were combined in a single fermentation to obtain higher amylase enzyme production. The result presented total amylase production of *Enterobacter hormaechei* SR3 and *Bacillus cereus* MR1 using media I (maize flour) and III (basal medium with starch) higher (Table 8) than in the initial fermentation (Tables 4 and 5). The optimized amylase yields by *Enterobacter hormaechei* SR3 and *Bacillus cereus* MR1 in maize flour (medium I) as fermentation substrate were; 2.512±0.001U/mL/min and 2.668±0.003U/mL/min respectively. Also, activities of 2.718±0.001U/mL/min and 1.963±0.001U/mL/min were found for enzymes produced from *Enterobacter hormaechei* SR3 and *Bacillus cereus* MR1 respectively using medium III (basal medium with starch). Amylase yield has always varied from one study to another. Contrary to the present study, Singh *et al*. (2017) recorded maximum amylase yield of 2200U/mL from *Bacillus licherniformis* using a complex medium after 24 h of incubation.

Conclusion

Bacillus cereus MR1 and *Enterobacter hormaechei* SR3 were able to grow and produce appreciable amounts of amylase using maize flour (a low-cost and readily available agricultural substrate) and a basal medium (composed of low-cost starch and chemicals).The results show that the bacterial strains studied are worthy of further investigation with biotechnological applications very feasible.

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