British Microbiology Research Journal
3(4): 478-491, 2013

SCIENCEDOMAIN *international*
www.sciencedomain.org

α-Amylase Production on Low-Cost Substrates by *Naxibacter sp*. Isolated from Mauritian Soils

Aadil Ahmad Aullybux[1] and Daneshwar Puchooa[1*]

[1]Department of Agriculture and Food Science, Faculty of Agriculture, University of Mauritius, Réduit, Mauritius.

Authors' contributions

Both authors contributed equally in the manuscript and both authors read and approved the final manuscript.

Research Article

Received 2nd May 2013
Accepted 14th July 2013
Published 25th July 2013

ABSTRACT

Alpha amylase is an important enzyme used in different industries, which degrades starch into smaller disaccharides. Extracellular α-amylase producing organisms were isolated from soil samples from Mauritius and identified by standard biochemical tests. In this study, the high yielding strain was used for amylase production. The potential of four readily available substrates, namely sugarcane bagasse, potato peel, kitchen wastes and banana peel to induce amylase production was investigated. Different parameters like temperature (30ºC, 40ºC, 50ºC, 60ºC & 70ºC), different pH (5.0, 6.0, 7.0, 8.0 & 9.0) and inoculum sizes (10%, 20%, 50%, 100% & 150% v/w) were used for the α-amylase production. It was found that α-amylase production and activity was highest for potato peel at 50ºC at pH 6.0 and inoculum size 50% (v/w). Amylase assays performed at different incubation temperatures (30ºC - 60ºC) and pH (5-9) showed that the amylase worked best at 50ºC and pH 7.Based on results of biochemical tests and 16S ribosomal RNA gene sequences, the isolate was identified to belong to the *Betaproteobacteria*, closely related to *Naxibacter haematophilus* (99% sequence similarity to the type strain).

Keywords: α-amylase; Naxibacter haematophilus; solid substrate fermentation; Mauritian soils.

**Corresponding author: Email: sudeshp@uom.ac.mu;*

1. INTRODUCTION

α-Amylase (EC3.2.1.1, 1,4-α-D-glucan-glucanohydrolase) hydrolyses starch to a range of products such as glucose and maltose or specific malto-oligosaccharide or mixed malto-oligosaccharides [1,2,3,4]. Amylases have diverse applications in a wide variety of industries such as food, fermentation, textile, paper, detergent, pharmaceutical and sugar industries [5,6]. They are employed in industries for different purposes; glucose- and maltose-forming α-amylases in alcohol fermentation and sugar syrup formulation, and malto-oligosaccharide-forming α-amylases in food processing [7,8]. Although amylases can be derived from several sources, including plants, animals and microorganisms, microbial enzymes generally meet industrial demands. Commercial production of α-amylase from micro organisms represents approximately 30% of the world enzyme market. Microorganisms like fungi and bacteria have been extensively screened for α-amylase production [9]. Because of the importance of amylases, isolation of new microorganisms suitable for amylase production could provide potential new sources of the enzyme. Isolation can be performed from soils due to their rich diversity of organisms and this approach has successfully led to the isolation of a number of starch-hydrolysing microorganisms in countries with different environments [10,11,12,13]. Similarly, soils of Mauritius could also represent a potential source of new amylase-producing bacteria but so far, no screening has been performed. Therefore, investigating microbial populations of Mauritian soil could result in the isolation of a strain suitable for producing amylase and for application in industrial processes. Also, the production of amylase is dependent on the strains, methods of cultivation, cell growth, nutrient requirement, metal ions, pH, temperature, time of incubation and thermo stability. In addition, selection of a suitable substrate is critical for fermentation processes [14] and investigating the potential of wastes for producing amylase could lead to the availability of new substrates for this purpose. In this respect, several works have reported the possible use of a number of wastes for amylase production [14,15,16].This study reports the isolation and characterisation of *Naxibacter sp*. from Mauritian soils capable of producing α-amylase. In our present study the cultural conditions were optimised for the best production of α-amylase and the effects of different low-cost substrates on α-amylase production were investigated.

2. MATERIALS AND METHODS

2.1 Isolation and Selection of Highest Amylase-Producing Strain

Soil samples from five different regions of Mauritius were collected. 1 g of sample was serially diluted and 0.1 ml was aseptically transferred to 0.1% starch agar plates. After 24 h incubation at 37ºC, isolated colonies were transferred to fresh starch agar and allowed to incubate for another 24h at the same temperature. Plates were then flooded with 1% Gram's iodine solution and the isolate producing largest zone of hydrolysis was retained for further studies. Pure culture was obtained and maintained through repeated streaking of the isolated strain on fresh starch agar medium.

2.2 Substrates Used and Conditions Optimisation

Enzyme production was investigated on bagasse, potato peel, kitchen wastes and banana peels. In 250 ml Erlenmeyer flasks, 5.0 g of solid substrate was supplemented with 20 ml of basal medium of the following composition (%) - 0.5 peptone + yeast extract, 0.25 NaCl, 0.1 K_2HPO_4 (0.1 M), 0.05 $MgSO_4$ and 0.005 $CaCl_2$. pH was adjusted to 7.0 (except for pH effect measurements) before autoclaving. Cooled flasks were inoculated with 2.0 ml (except when

determining effect of inoculum levels) of bacterial culture and incubated at 37ºC (except when effect of temperature was investigated) for 20 ± 1 h while un-inoculated flasks were used as controls. To investigate effect of different parameters on α-amylase production, incubation was performed at temperatures 30, 40, 50, 60 and 70ºC and using 10%, 20%, 50%, 100% and 150% (v/w) inoculum levels. For variation in pH (5, 6, 7, 8 and 9), the basal medium was supplemented with 2.5 ml of appropriate buffer before autoclaving. All experiments were performed in triplicates.

2.3 Inoculum Preparation

A 24-h grown culture was inoculated in 100 ml peptone water and kept on a shaker at 200 rpm at 37ºC. Cultures giving an optical density of 0.50 ± 0.01 at 600 nm were used as standard inoculums.

2.4 Amylase Extraction

Amylase was extracted based on the method described by Mukherjee and others [17]. Fermented matter was mixed with 25 ml of distilled water and kept on shaker for 30 min at 200 rpm. The mixture was then filtered and centrifuged at 10 000 rpm for 10 min at 4°C. Crude supernatant was used for amylase assay.

2.5 Amylase Assay

Amylase assay was based on dextrinising activity using the method of Senthilkumar and others [18] with some modifications. To 1.0 ml of 0.15% potato starch in phosphate buffer of pH 7.0, 1.0 ml of crude enzyme was added before incubation at 60ºC for 10 min. The reaction was stopped with 1.0 ml of 0.1 M HCl, followed by 1.0 ml of 0.2% iodine/2.0% KI. A blank was set up under similar conditions with 1.0 ml water instead of enzyme. Absorbance values were then measured at 620 nm. Enzyme activity was calculated as shown below with 1 Unit (U) being defined as the amount of enzyme which hydrolyses 1 mg of starch per minute under the assay conditions.

$$Units\ of\ enzyme\ activity\ =\ (A_{620}\ control - A_{620}\ sample)\ \div\ A_{620}\ /mg\ starch\ \div\ 10\ min\ \div\ 1\ ml,$$

Where,

A_{620} sample refers to absorbance obtained for measured sample.
A_{620} control refers to absorbance obtained from control (un-inoculated flasks).
A_{620} /mg starch refers to absorbance obtained for 1 mg of starch (from standard curve).
10 min refers to incubation time.
1 ml refers to the amount of crude enzyme added.

2.6 Determining Optimum Temperature and pH of amylase

Optimum working temperature of the amylase was determined by incubating the enzyme mixture at temperatures 30, 35, 40, 50 and 60°C. To find optimum pH, the potato starch was dissolved in buffer of required pH (5, 6, 7, 8 and 9) before performing the assay.

2.7 Biochemical Tests

Identity of the isolate was determined by standard biochemical tests as per Phillips [19] and including oxidase, catalase, citrate and glucose fermentation tests.

2.8 Isolation of Genomic DNA

DNA was extracted by the method described by Wilson [20]. In brief it consisted of centrifuge of 1.5 ml of an overnight-grown culture before adding 567µl of TE buffer, 3µl 10% SDS and 3µl of 20 mg/ml proteinase K to the collected pellet. After 1h incubation at 37ºC, 100µl of 5M NaCl and 80µl of CTAB/NaCl were consecutively added before incubation for another 10 min at 65ºC. An equal amount of chloroform:isoamyl alcohol (24:1) was added and to the resulting aqueous phase transferred into a new tube, an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) was added. The aqueous phase was again transferred before adding 0.6 volume of isopropanol. The precipitated DNA was washed with 70% ethanol, dried and re-suspended in 150µl of TE buffer prior to storage.

2.9 Amplification of 16S rRNA Genes by Polymerase Chain Reaction (PCR) and Analysis of the PCR Products

The 16S rRNA genes were amplified with bacterial universal primers specific for eubacterial 16S rRNA gene (Forward, AGTTTGATCATGGCTCAG) and (Reverse, TTACCGCGGCTGGCA). The PCR (25µl) contained 0.5µl of each forward and reverse primer, 1.5mM of 10X Taq buffer (stock 20mM), 0.2mM (2.0µl) of each deoxynucleotide (ddATP, ddGTP, ddCTP and ddTTP), 1unit of *Taq* DNA polymerase (5units/µl) and 1.5µl DNA. PCR conditions were as follows: initial denaturation at 95ºC for 5 min, followed by 30 cycles, each with 1 min denaturation at 95ºC, 1 min annealing at 55ºC and extension at 72ºC for 1 min, before a final extension at 72ºC for 5 min. The PCR products obtained from DNA extracted from the samples were first analyzed by electrophoresis in 1.5% agarose gel and were stained with ethidium bromide and visualized under short-wavelength UV light.

2.10 Nucleotide Sequencing and Alignment

16S rDNA sequencing of the isolated strain was carried out by Inqaba Biotec (South Africa). Sequences obtained were compared to the non-redundant nucleotide database at the National Center for Biotechnology Information by using their World Wide Website, and the BLAST (Basic Local Alignment Search Tool) algorithm to find percentage homology with known organisms.

3. RESULTS

3.1 Identification of Isolate

Fig. 1 shows the zones of hydrolysis obtained during the isolation process prior to selection of the highest-yielding strain. Selected colonies were round, smooth, raised and had a creamy to yellow appearance. Results obtained for the biochemical tests performed are as shown in Table 1. Initially thought to be a *Pseudomonas* species, further tests performed showed that the isolate also produced fluorescence under UV light on blood agar. In addition to this, successfully amplified DNA samples (Fig. 2) were sent for sequencing and analysis of

the resulting 16S rDNA sequence revealed that the isolate was related to the genus *Naxibacter*, from the family of *Oxalobacteraceae*, with 99% homology. Pairwise alignment with different species of the genus showed a 98% sequence similarity with *N. haematophilus* followed by *N. varians* (97.5%).

Table 1. Results for biochemical tests

Starch hydrolysis	+ve
Catalase	+ve
Citrate	-ve
Glucose fermentation	-ve
Oxidase	+ve
Growth at 42°C	+ve
Growth in the presence of 6% sodium chloride	+ve

Fig. 1. Results of iodine test prior to selection of strain producing largest zone of hydrolysis

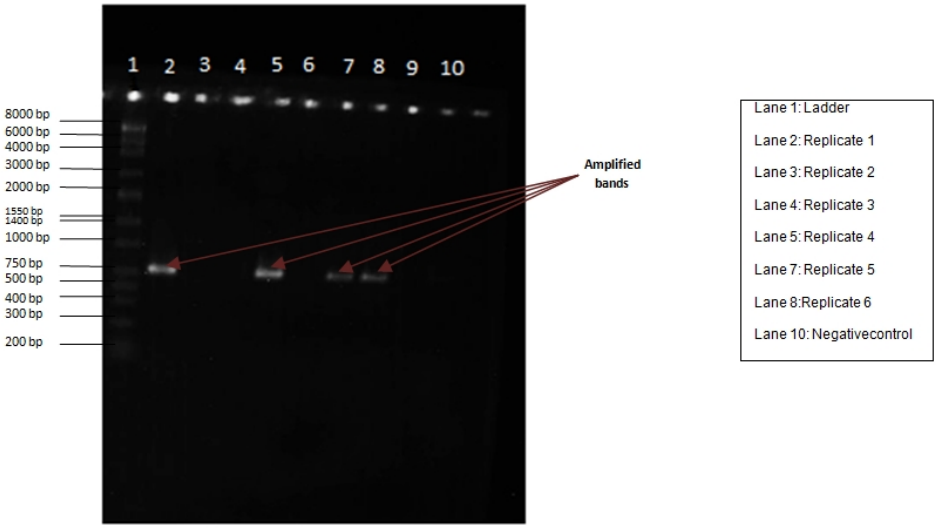

Fig. 2. Bands obtained after electrophoresis of PCR product showing bands of about 750 bp when compared to the ladder (DirectLoad™ Wide Range DNA Marker)

3.2 Optimisation of Culture Conditions

The enzyme activity for four substrates under different conditions of temperature and pH was determined to find the optimum ones for enzyme production.

3.2.1 Effect of temperature on amylase production

Inoculated substrates were incubated at temperatures 30, 40, 50, 60 and 70ºC for 20 hours at pH 7.0. Results (Fig. 3) showed that enzyme production could occur on all substrates. Highest enzyme activity was recorded for potato peel (0.14 Units), while kitchen wastes supported production over the broadest range of temperatures. As far as temperature was concerned, highest activity was recorded at 50ºC. Enzyme production on all four substrates was supported even at 70ºC, while for 30ºC, only one substrate showed enzyme activity. However, one-way ANOVA (Minitab 2010 version 16.1.1) showed that differences in enzyme production were not significant (P >.05) with respect to both substrate and temperature.

3.2.2 Effect of pH on amylase production

Fig. 4 shows variation in enzyme production with pH for the four substrates used. Enzyme activity was not detected for any substrate at pH 5 while potato peel gave the highest (8.88 Units) at pH 6. In this case, enzyme production varied with the substrates (P <.05) but changes in pH produced no significant changes in enzyme yield (P >.05).

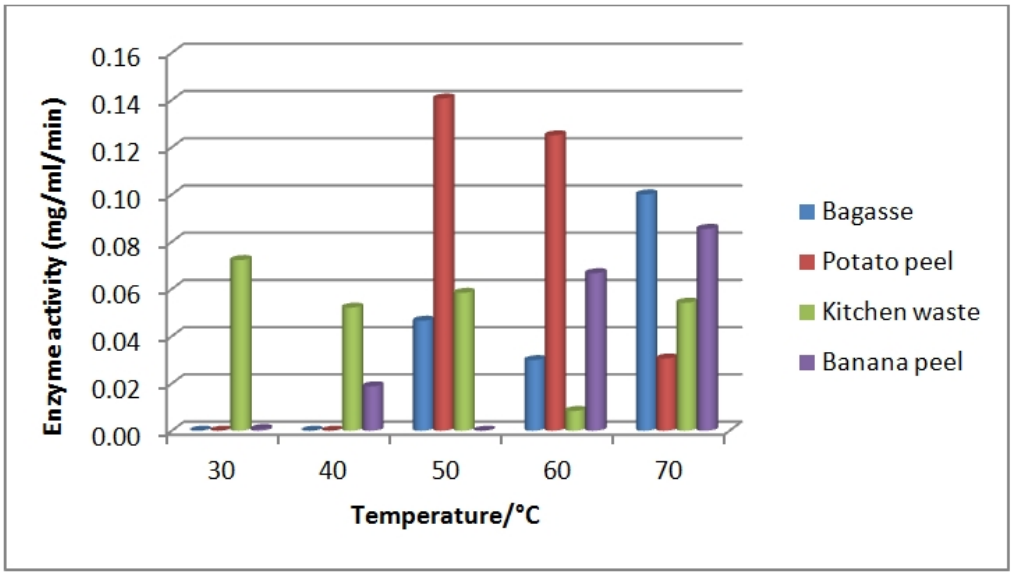

Fig. 3. Effect of temperature and substrate on amylase production by selected isolate

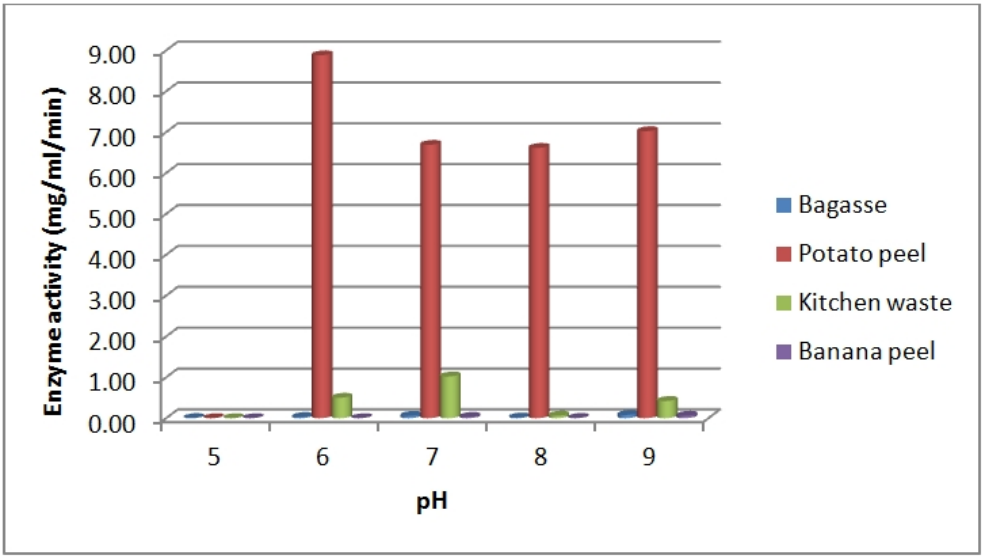

Fig. 4. Effect of pH and substrate on amylase production by selected isolate

3.2.3 Effect of inoculum level on amylase production

Varying responses were obtained when different inoculum sizes were used for enzyme production with the four substrates. Highest activity was again obtained for potato peel as the value increased from zero for an inoculum level of 10% to reach 0.67 Units at 50% before decreasing again (Fig. 5). No activity could be measured for the banana peel. Inoculum level did not affect enzyme production ($P > 0.05$) while the substrates had a significant impact on yield ($P < 0.05$).

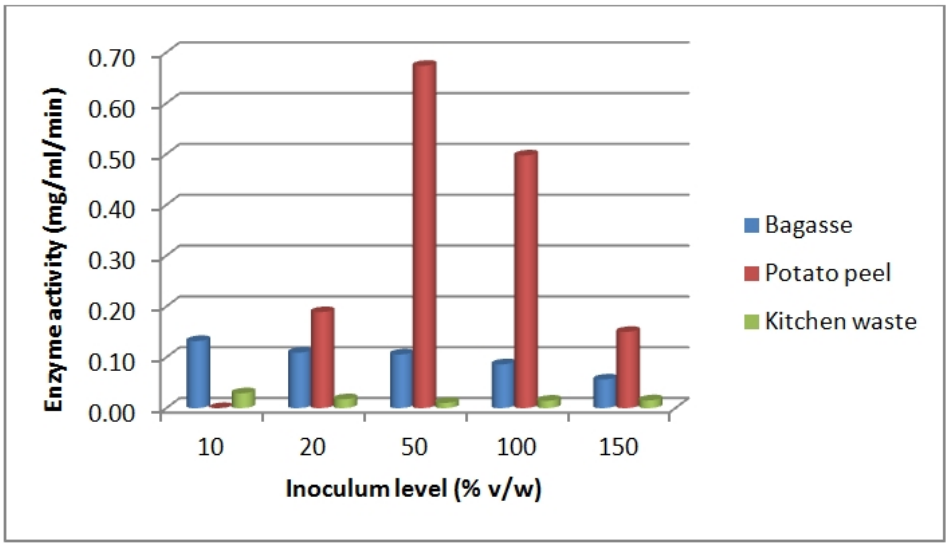

Fig. 5. Effect of inoculum level and substrate on amylase production by selected isolate

3.3 Effect of Temperature and pH on Enzyme Activity

The absorbance values for the control could not be determined in this case and consequently, the reciprocal of absorbance values at 620 nm were taken as a measure of activity for determining optimum temperature and pH for amylase activity. Fig. 6 shows that maximum activity was recorded at 50ºC, followed by 30ºC. For pH, enzyme activity increased from its lowest value at pH 5 to reach its maximum at pH 7 before subsequently decreasing as shown in Fig. 7.

4. DISCUSSION

In this work, Mauritian soil was screened for an amylase-producing organism and the ability of this organism to produce amylase on different wastes under different conditions was investigated. The isolate was identified as a member of the genus *Naxibacter*, of the *Oxalobacteraceae* family. The isolation of this organism might not be surprising since members of this family are commonly found in soils [21], though they may also be found in other environments [22,23,24]. In addition to high similarity of this isolate with the species *N. haematophilus* based on 16S rRNA gene sequence, morphological observations and results of biochemical tests for the isolate also seemed to agree with a description of this species given in a study [25]. The description of *N. varians*, given in the same study, was very similar to the one for *N. haematophilus* and could thus explain why the isolate also showed high homology to the former species. However, phenotypic properties tend to vary between *Naxibacter* species [22]. Also a number of novel organisms have been introduced in the *Oxalobacteraceae* family, with some as new genera [24,25] and others as species within new genera [26]. Therefore, with the absence of 100% homology, the possibility that a new species or a variant of an existing one has been isolated cannot be excluded and further characterisation of the isolate may be needed.

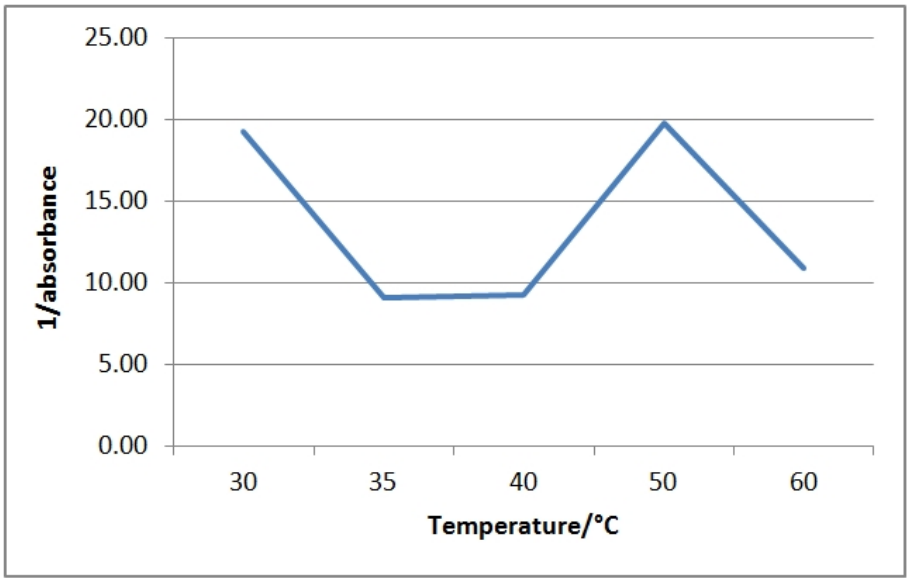

Fig. 6. Effect of temperature on amylase activity

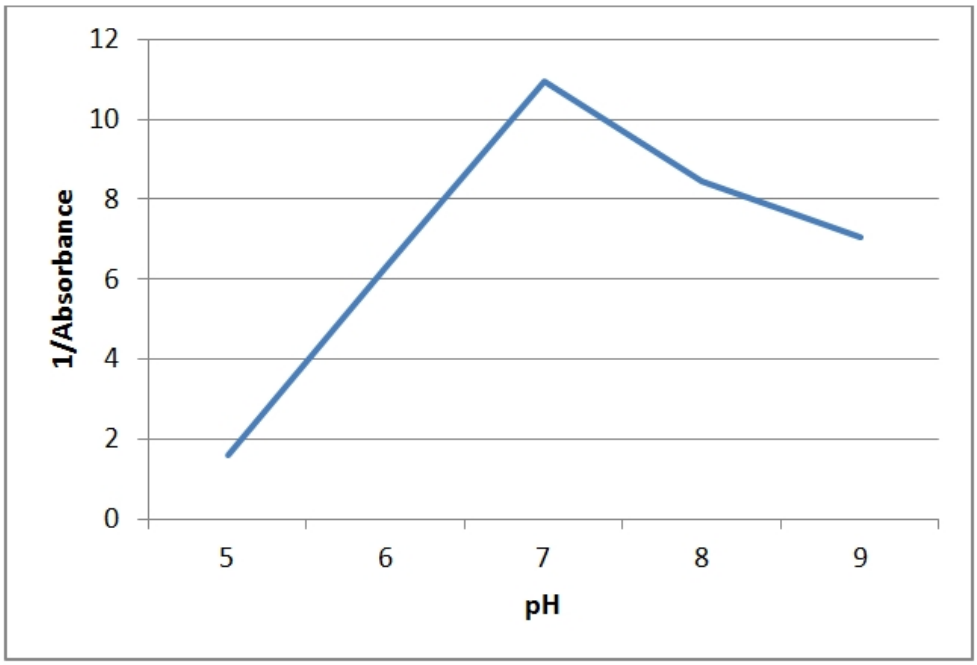

Fig. 7. Effect of pH on amylase activity

One of the important factors for developing a successful Solid-State Fermentation (SSF) is the use of an appropriate substrate [27]. Selection of a suitable substrate is usually related to its cost and availability [28], thereby favouring the use of a number of agro-industrial wastes. In this study, substrates were selected based on their availability as well as reports of their use in previous studies. The results showed that enzyme production varied with different substrates. This is mainly because an organism reacts differently to different substrates due to varying rates at which nutrients are used from each [29]. Potato peel produced highest enzyme activities compared to the other substrates and similar results were reported in other studies involving *Bacillus* species where the efficiency of this substrate was compared with other possible ones for amylase production [15,17]. Amylases are inducible enzymes whose production can be induced by starch or maltose [5] and hence the higher level of production on potato peel could be related to its starch content which can be as high as 51%, depending on the peeling process [30]. Bagasse, on the other hand, has as main fermentable matter, reducing sugars such as glucose derived from cellulose [31] while banana peel has a high proportion of carbohydrates [32], including sucrose and glucose [16]. The smaller starch content and the fact that glucose can repress amylase production [5] could explain the smaller enzyme yield for these substrates. Nevertheless, their use as possible substrates for amylase production has been reported elsewhere [33,34,35,36]. Kitchen wastes were also shown to be quite suitable for inducing enzyme production under various conditions. These wastes consisted mainly of a mixture of various vegetable and fruit peels and their heterogeneous nature could have promoted enzyme production due to the availability of a wider range of nutrients. However, this can prove to be impractical for industrial processes since this heterogeneity would make it more difficult to reproduce the same substrate composition every time to ensure consistency in enzyme yield.

Since different organisms react differently to different substrate, fermentation techniques need to be optimised for each one [29]. Therefore, for each substrate used in this work, the effect of different parameters on amylase production was determined. A number of studies describe the optimisation of conditions for amylase production using *Bacillus* sp. [33,37,38]. But in comparison, there are less works describing the use of other bacterial species for the same purpose.

The isolate showed the ability to produce amylase at all the temperatures used in the investigation (30ºC to 70ºC), though differences in enzyme yield were not significant. This could be attributed to the ability of the organism to survive a range of temperatures, a feature yet to be investigated since the effect of temperature on amylase production is related to the growth of the microorganism [5]. Amylase production occurred at a temperature of 70ºC on all the substrates. Furthermore, the extracted enzyme showed maximum activity at a temperature of 50ºC. However, it has been reported that *N. haematophilus* and *N. varians*, to which the isolate share highest similarity, do not show growth at 45ºC [39]. Both results suggest possible thermostability of the enzyme and the production of such enzymes from non-thermophilic bacterium have also been reported elsewhere [40]. The stability of this amylase at different temperatures remains to be investigated, as possible thermostability property of the enzyme would open a number of possible applications for the isolated strain especially since the use of thermostable enzymes is constantly increasing in industrial applications [41].

Enzyme production also occurred over a pH range of 6 to 9, with highest production occurring at pH 7 and pH 9 for most substrates but differences in enzyme production at different pH were not significant and were more likely to be due to the substrates used rather than to the pH of the medium. Nevertheless, the pH of the medium is one of the main parameters which affect enzyme yields because pH changes can affect the stability of amylases in the medium [42]. Maximum activities of amylases are between 4.5 and 7.0, slightly in the acidic region, although differences might occur depending on the source of the enzyme [43]. This coincides with the observed result obtained for the optimum activity of the amylase at pH 7. Enzymatic activities observed at pH 9 could not only be due to the ability of the isolate to grow at a slightly alkaline pH but could also possibly suggest stability of the amylase at this pH, a fact which is yet to be determined.

As far as the effect of the inoculum level on amylase yield was concerned, as a general trend, except for potato peel which showed maximum production at an inoculum level of 50% (v/w), the other substrates showed decreasing enzyme activities as the inoculum level increased above 10% (v/w). An inoculum level of 10% has been reported to be most suitable for amylase production in many studies [44,45,46]. However, despite the fact that under SSF the concentration of the inoculum affects the production of metabolites [47], differences in the measured activities, in this case, were not significant at the 5% level of confidence. In fact, high inoculum levels significantly increase the moisture content such that the unabsorbed free water creates a diffusion barrier in addition to that created by the solid nature of the substrate, thereby causing decreased growth and enzyme production [14]. On the other hand, low inoculum levels contain lower number of cells, hence causing them to take longer time to grow to an appropriate number for using the substrate and producing the required product [48]. But in this particular case, rapid growth of the isolate could have caused the inoculum to increase rapidly, thereby causing the availability of substrate to become the limiting factor in enzyme production instead of the varying inoculum level

5. CONCLUSION

In this work, a microbial strain, isolated from the Mauritian soil was identified as a member of *Oxalobacteraceae* family but due to lack of complete sequence homology further characterisation of the isolate might be required before confidently stating the identity of the isolate. The isolated strain also had the ability to produce amylase on different wastes, with temperature, pH and inoculum level having no significant effect on enzyme yield. Potato peel was found to be the best substrate with the maximum production occurring at a temperature of 50ºC, a pH of 6 and with an inoculum level of 50% (v/w). Optimisation of other parameters needs to be performed before deciding on the suitability of this isolate for fermentation processes. Furthermore, with an optimum working temperature of 50ºC, the possibility that the enzyme could display thermostability cannot be neglected though this remains to be confirmed. Nevertheless, this work can be considered to be a contribution to the studies available on the potential and possible applications of newly-isolated species to the field of industrial biotechnology.

ACKNOWLEDGEMENTS

The authors wish to thank the Faculty of Agriculture, University of Mauritius for supporting this study. The support of the technical staff of the Botany laboratory of the Department of Agriculture & Food Science, in particular Mrs. A. Issany and Mrs D. Bhookhun-Seeruttun, is gratefully acknowledged.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Hashim SO, Delgado OD, Martı´nez MA, Kaul RH, Mulaa FJ, Mattiasson B. Alkaline active maltohexaose-forming a-amylase from *Bacillus halodurans* LBK 34. Enzyme Microb. Technol. 2005;36:139–146.
2. Messaoud EB, Ali MB, Elleuch N, Masmoudi NF, BejarS.Purification and properties of a maltoheptaose- and maltohexaose forming amylase produced by *Bacillus subtilis* US116. Enzyme Microb. Technol. 2004;34:662–666
3. Takasaki Y. An amylase producing maltotetraose and maltopentaose from *Bacillus circulans*. Agr. Biol. Chem. 1983;47:2193–2199
4. Dey G, Palit S, Banerjee R, Maiti BR. Purification and characterization of maltooligosaccharide-forming amylase from *Bacillus circulans* GRS313. J. Ind. Microbiol. Biotechnol. 2002;28:193–200
5. Gupta R, Gigras P, Mohapatra H, Goswami VK, Chauhan B. Microbial α-amylases: a biotechnological perspective. Process Biochem. 2003;38:1599–616.
6. Pandey A, Nigam P, Soccol CR, Soccol VT, Singh D, Mohan R. Advancesin microbial amylases. Biotechnol. Appl. Biochem. 2000;3:135
7. Kirk O, Borchert TV, Fugslang CC. Industrial enzyme application. Curr. Opin. Biotechnol. 2002;13:345–351.
8. Palacios HR, Schwarz PB, D'Appolonia LD. Effect of α-amylases from different sources on the retrogradation and recyclization of concentrated wheat starch gels: relationship to bread staling. J. Agr. Food. Chem. 2004;52:5978–5986.

9. Ivanova V, YankovD, Kabaivanova L, Pashkoulov D. Simultaneous biosynthesis and purification of two extracellular *Bacillus* hydrolases in aqueous two alpha–amylase. Biochem. Eng. J. 2011;8:61–81.
10. Raharjo S, Ardiansyah, Endang P, Tien. Isolation of thermostable α-amylase from local thermophilic bacteria for liquefaction. In: ICMNS (International Conference on Mathematics and Natural Sciences), Science for Sustainable Development. Bandung, Indonesia 23-25 November 2010.
11. Alkando AA, Ibrahim HM. A potential new isolate for the production of a thermostable extracellular α-amylase. J. Bacteriol. Res. 2011;3(8):129-137.
12. Wang S, Liang Y, Liang T. Purification and characterization of a novel alkali-stable α-amylase from *Chryseobacteriumtaeanense*TKU001, and application in antioxidant and prebiotic. Process Biochem. 2011;46:745-750.
13. Kumara SM, Rohit KC, Pradeep SK, Sandip P, Parthasarathi B, Monika P, et al. Identification and characterization of a novel isolate *Comamonaskerstersii*KSM7 for extracellular α-amylase production. J. Microbiol. Biotechnol. Res. 2012;2(5):703-711.
14. Balkan B, Ertan F. Production of α-Amylase from *Penicillium chrysogenum* under Solid-State Fermentation by Using Some Agricultural By-Products. Food Technol. Biotechnol. 2007;45(4):439-442.
15. Shukla J, Kar R. Potato peel as a solid state substrate for thermostable α-amylase production by thermophilic *Bacillus* isolates. World J. Microbiol. Biotechnol. 2006;22:417-422.
16. Unakal C, Kallur RI, Kaliwal BB. Production of α-amylase using banana waste by *Bacillus subtilis* under solid state fermentation. Euro. J. Exp. Biol. 2012;2(4):1044-1052.
17. Mukherjee AK, Borah M, Rai SK. To study the influence of different components of fermentable substrates on induction of extracellular α-amylase synthesis by *Bacillus subtilis*DM-03 in solid-state fermentation and exploration of feasibility for inclusion of α-amylase in laundry detergent formulations. Biochem. Eng. J. 2009;43:149-156.
18. Senthilkumar PK, Uma C, Saranraj P. Amylase Production by *Bacillus sp*. Using Cassava as Substrate. Intl. J. Pharm. Biol. Arch. 2012;3(2):300-306
19. Phillips I. Identification of *Pseudomonas aeruginosa* in the clinical laboratory. Med. Microbiol. 1969;2:9-16.
20. Wilson K. Miniprep of bacterial genomic DNA. In: Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Struhl K, editors. Current Protocols in Molecular Biology. New York: John Wiley &Sons. 2.4.1-2.4.5;2003
21. Bruijn FJ. Molecular Microbial Ecology of the Rhizosphere, Two Volume Set. Hoboken (NJ): John Wiley & Sons. Inc.; 2013.
22. Weon HY, Yoo SH, Kim SJ, Kim YS, Anandham R, Kwon SW. *Massiliajejuensis*sp. nov and *Naxibacter suwonensis*sp. nov., isolated from air samples. Int. J. Sys. Evol.Microbiol. 2010;60:1938-1943
23. Kämpfer P, Lodders N, Martin K, Falsen E. Revision of the genus *Massilia* La Scolaet al. 2000, with an emended description of the genus and inclusion of all species of the genus *Naxibacter as* new combinations, and proposal of *Massilia consociate*sp. nov.Intl. J. Syst. Evol. Microbiol. 2011;61:1528-1533.
24. Kim EH, Jeong HJ, Lee YK, Moon EY, Cho JC, Lee HK, et al. *Actimicrobiumantarticumgen*. nov., sp. nov., of the Family *Oxalobacteraceae,* Isolated from Antartic Coastal Seawater. Curr. Microbiol. 2011;63:213-217.
25. Zhang DC, Redzic M, Schinner F, Margesin R. *Glaciimonasimmobilisgen.*nov., sp. nov., a member of the family *Oxalobacteraceae* isolated from alpine glacier cryoconite. Intl. J. Syst. Evol. Microbiol. 2011;61(9):2186-2190.

26. Zul D, Wanner G, Overmann J. *Massiliabrevitalea*sp. nov., a novel beta-proteobacterium isolated from lysimeter soil. Intl. J. Syst. Evol. Microbiol. 2008;58:1245-1251.
27. Pandey A, Soccol CR, Larroche C, Editors. Current Developments in Solid-state Fermentation. Delhi: Asiatech Publishers Inc.; 2008.
28. Couto SR, Sanroman MA. Application of solid-state fermentation to food industry – A review. J. Food Eng. 2006;76(3):291-302.
29. Subramaniyam R, Vimala R. Solid state and submerged fermentation for the production of bioactive substances: A comparative study. Intl. J. Sci. Nature. 2012;3(3):480-486.
30. Schieber A, Saldana MDA. Potato Peels: A Source of Nutritionally and Pharmacologically Interesting Compounds – A Review. Food. 2009;3(2):23-29
31. Pessoa AJR, Mancilhal M, Sato S. Acid hydrolysis of hemicellulose from sugarcane bagasse. Braz. J. Chem. Eng. 1997;14(3).
32. Anhwange BA. Chemical Composition of *Musa sapientum* (Banana) Peels. J. Food Technol. 2008;6(6):263-266.
33. Kokab S, Asghar M, Rehman K, Asad MJ, Adedyo O. Bio-Processing of Banana Peel for α-Amylase Production by *Bacillus subtilis*. Intl. J. Agric. Biol. 2003;5(1):36-39
34. Bhattacharya S, Bhardwaj S, Das A, Anand S. Utilisation of Sugarcane Bagasse For Solid State Fermentation and Characterization of α-Amylase From *Aspergillus flavus* Isolated From Muthupettai Mangrove, Tamil Nadu, India. Australian J. Basic. Appl. Sci. 2011;5(12):1012-1022.
35. Krishna PR, Srivastava AK, Ramaswamy NK, Suprasanna P, D'Souza SF. Banana peel as substrate for α-amylase production using *Aspergillus niger* NCIM 616 and process optimization. Indian J. Biotechnol. 2012;11:314-319.
36. Mamatha J, Suresh V, Vedamurthy AB, Shilpi B, Shruthi SD. Production of α-amylase from *Aspergillus flavus* under solid state fermentation with optimum conditions. Intl. Res. J. Pharm. 2012;3(8):135-140.
37. Asgher M, Asad MJ, Rahman SU, Legge RL. A thermostable α-amylase from a moderately thermophilic *Bacillus subtilis*strain for starch processing. J. of Food Eng. 2007;79,:950-955.
38. [38] Tanyildizi MS, Özer D, ElibolM. Production of bacterial α-amylase by *B. amyloliquefaciens* under solid substrate fermentation. Biochem. Eng. J. 2007;37:247-297.
39. Kämpfer P, FalsenE,Busse HJ. *Naxibacter varians*sp. nov. and *Naxibacter haematophilus*sp. nov., and emended description of the genus *Naxibacter*. Int. J. Sys. Evol. Microbiol. 2008;58:1680-1684.
40. Declerk N, Machius M, Joyet P, Wiegand G, Huber R, Gaillardin C. Engineering the thermostability of *Bacillus licheniformis* α-amylase. Biol. Bratislava. 2002;57(11):203-211.
41. Zamost BL, Nielsen HK, Starnes RL. Thermostable enzymes for industrial applications.J. Indus. Microbiol. 1991;8:71-82.
42. El-Fallal A, Dobara MA, El-Sayed A, Omar N. Starch and Microbial α-Amylases: From Concepts to Biotechnological Applications. In: Chang CF, editors. Carbohydrates – Comprehensive Studies on Glycobiology and Glycotechnology. In Tech. 2012;459-488.
43. Prescott SC, Dunn CG. Industrial Microbiology, 4th ed. Delhi: CBS Publishers and Distributers; 1982.
44. Anto H, Trivedi U, Patel K. Alpha Amylase Production by *Bacillus cereus* MTCC 1305 Using Solid-State Fermentation. Food Technol. Biotechnol. 2006;44(2):241-245.

45. Singh RK, Mishra SK, Kumar N. Optimization of α-amylase production on agriculture byproduct by *Bacillus cereus* MTCC 1305 using solid state fermentation. Res. J. Pharm. Biol. Chem. Sci. 2010;1(4):867-876.
46. Kharadi S, Desai PV, Patel BV. Studies on Optimization of Amylase Production by Solid State Fermentation. Advanced Biotech. 2011;11(3):34-36.
47. Sivakumar T, Shankar T, Vijayabaskar P, Muthukumar J, Nagendrakannan E. Amylase Production Using *Bacillus cereus* Isolated from a Vermicompost Site. Intl. J. Microbiol. Res. 2012;3(2):117-123.
48. Bhardwaj S, Vedamurthy AB, Bhattacharya S, Das A. Effect of Inorganic Salts and Surfactants on the Production of α-Amylase by a Mangrove Isolate of *Aspergillus flavus* using Solid-State Fermentation. J. Chem. Biol. Phys. Sci. 2012;2(3):1390-1397.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=246&id=8&aid=1745