Pak. j. sci. ind. res. Ser. B: biol. sci. 2022 **65B**(1) 62-66

In vitro Studies of the Utilization of Industrially Important Substrates by *Lactobacillus* sp. AAF-1 Isolated from Coconut Water

Ayesha Siddiqui[a*], Ayisha Aman[a], Syeda Areej Fatima[a], Aliya Riaz[b], Rashida Rahmat Zohra[a] and Suad Naheed[a]

[a]Department of Biotechnology, Jinnah University for Women, Karachi-74600, Sindh, Pakistan
[b]Department of Biochemistry, Jinnah University for Women, Karachi-74600, Sindh, Pakistan

(received August 18, 2019; revised June 24, 2020; accepted August 13, 2020)

Abstract. Coconut water (*Cocos nucifera* L.*)* possesses natural hydrating qualities, functional health properties and various nutritional benefits. *Lactobacillus* species AAF-1 has been isolated and purified from coconut water. The isolated culture was screened for the production of extracellular secreted enzymes having industrial value. Growth of the *Lactobacillus* sp. AAF-1 on MRS agar containing respective substrates showed that the *Lactobacillus* sp. AAF-1 is the potential producer of amylase, protease, cellulase and beta galactosidase. Further cell free filtrates of the *Lactobacillus* sp. AAF-1 showed large zone of hydrolysis of starch, gelatin, cellulose and lactose that reflected the extracellular production of the above enzymes in culture medium at 50 °C. It has been observed that activities after 72 h of incubation in cell free filtrates of protease, amylase, cellulase and beta galactosidase as the zone of respective substrate hydrolysis measured as 10, 8, 6 and 10 mm respectively. Thus, *Lactobacillus* sp. AAF-1 could be the potential producer of the enzymes having industrial value.

Keywords: amylase, beta galactosidase, cellulase, coconut, *Lactobacillus*, protease

Introduction

Enzymes are the biological catalysts that increase the rate of metabolic reactions. Most of the industries now emphasize on enzymatic conversion of substrates into valuable products. They are used in various sectors such as food, pharmaceuticals, detergents, paper and textile industries (Sundarran *et al*., 2014).

Amylases are the enzymes involved in the hydrolysis of starch to yield various industrial important products. It has wide applications in food, paper, pharmaceuticals and textile industries (Paul, 2016). Proteases are the enzymes that have been used in meat tenderization. Alkaline serine proteases occupied large market of enzyme when used in detergent industry (Mienda *et al*., 2014). Similarly, cellulases gain value among industrially important enzymes as they can be used to utilize lignocellulosic waste material of agriculture sectors. Biotechnological conversion of cellulosic biomass into the potential products is the main focus of industrialists (Hassan *et al*., 2016). Beta galactosidase has great potential in dairy products. It has been used for the removal of lactose from milk in the preparation of lactose free milk products (Hussain *et al*., 2010).

Enzymes from the microbial sources have been used widely in all industries. The main concern of the industries mainly food and pharmaceutical sectors is the source from where the enzymes have been harvested (Pariza and Jhonson, 2001). *Lactobacillus* is widely used as probiotic thus has been considered the safest source for the production of industrial enzymes (Naidu *et al*., 2012). Therefore, in the present study we isolate the *Lactobacillus* sp. from coconut water and screened this strain for the production of industrially important enzymes.

*Author for correspondence;
E-mail: ayesha882000@gmail.com

Materials and Methods

Collection of coconut. Coconut was purchased from the local fruit market of Karachi, Pakistan. Coconut was washed thoroughly with sterile deionized water and then surface sterilized with 70 % (v/v) ethanol. Coconut water was collected in sterile bottles under sterile conditions and stored at 4 °C till used.

Isolation of *Lactobacillus* species. Dilution of Coconut water in the ratio of 1:1 was prepared in sterile normal saline. MRS agar plates were prepared and 100 µL of diluted sample was spread on separate MRS agar plates. Inoculated plates were incubated at 37 °C for 24 h. After incubation isolated colony was picked and further purified on MRS agar.

Identification of *Lactobacillus* species. Pure culture was identified as *Lactobacillus* sp. on the basis of

Bergey's manual of Bacteriology. Colony morphology, broth appearance and gram staining of the selected culture were performed (Ando and Hoshino, 1990). The selected culture was named as *Lactobacillus* sp. AAF-1.

Determination of optimum temperature of *Lactobacillus* sp. AAF-1. Growth pattern of *Lactobacillus* sp. AAF-1 was determined by the incubation of *Lactobacillus* sp. AAF-1 in MRS broth at various temperatures such as 30 °C, 40 °C, 50 °C and 60 °C. Aliquots at various time intervals were collected and optical density was recorded by spectrophotometer (T80 Series, PG Instrument, UK) at 560 nm.

Screening of extracellular enzymes produced from *Lactobacillus* sp. AAF-1. MRS broth and MRS agar supplemented with 1% (w/v) each of the substrates such as starch, gelatin, cellulose, lactose and DNA were prepared separately in deionized water. Each plate was streaked by the pure culture of *Lactobacillus* sp. AAF-1 and incubated at 50 °C for 24 h. Production of extracellular enzymes was determined as described below.

Each flask of MRS broth was also inoculated by the freshly growing overnight culture of *Lactobacillus* sp. AAF-1. Inoculated flasks were incubated at 50 °C. At various time intervals i.e. 24, 48 and 72 h samples from each flask were collected and centrifuged at 10,000 rpm for 15 min at 4 °C. Cell free supernatants of each flask were collected separately and stored at -20 °C for further analysis.

Detection of amylase enzyme. Amylase screening was performed by adding 1% (w/v) soluble starch in MRS agar. Wells were made on starch-MRS agar plates by using sterile borer. Cell-free supernatant obtained at various time intervals were loaded in the wells and plate was incubated at 50 °C for 24 h. After incubation plate was flooded with Gram's iodine solution for 5 min. Zones of starch hydrolysis were measured in mm (Mazzucotelli *et al*., 2013).

Detection of protease enzyme. For screening of the production of extracellular protease, 100 µL of cell free filtrate of all aliquots (24, 48 and 72 h) were added in wells made on the MRS agar plate containing 1% (w/v) gelatin as substrate. Plate was incubated at 50 °C for 24 h and the zones of hydrolysis were observed by flooding the plate with 45% ammonium sulphate solution for 15 min (Alnahdi, 2012).

Detection of cellulase activity. Extracellular cellulase production was screened by applying 100 µL cell free filtrate of all aliquots (24, 48 and 72 h) on the MRS agar containing 1% (w/v) cellulose. Plate was incubated at 50 °C for 24 h. Zones of cellulose hydrolysis were observed by flooding the plate with gram's iodine solution for 5 min (Kasana *et al*., 2008).

Detection of beta galactosidase enzyme. Beta galactosidase activity was determined on MRS agar plates containing 1% lactose (w/v) as substrates. 100 µL of cell free filtrate of each aliquot was placed in the wells and incubated at 50 °C for 24 h. After incubation plates were flooded with gram's iodine solution and diameter of zones of hydrolysis were measured in mm (Carrim *et al*., 2006).

Results and Discussion

Pure culture of *Lactobacillus* sp. AAF-1 has been isolated from coconut water and identified according to the Bergey's manual of Bacteriology (Ando and Hoshino, 1990). The selected strain was evaluated for optimum growth temperature and the production of extracellular industrially important enzymes. It has been observed that the optimum temperature of the *Lactobacillus* sp. AAF-1 was 50 °C (Fig 1).

The present study showed that, the *Lactobacillus* sp. AAF-1 secreted number of industrially important enzymes such as amylase, protease, cellulase and beta galactosidase in culture medium at 50 °C (Fig 2).

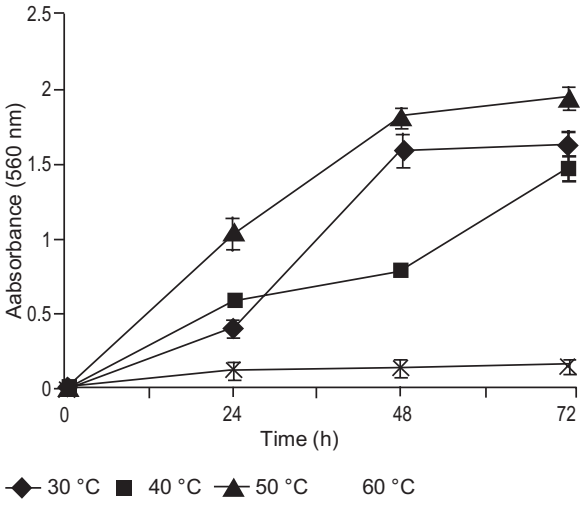

Fig. 1. Growth of *Lactobacillus* sp. AAF-1 isolated from coconut water at various temperatures.

Proteases are the most important enzymes used in detergent, leather and food industries. It comprises of the 60% of all enzymes required in industries (Sundus *et al*., 2016). Proteases are the best candidates for the breakdown of collagen and elastin in the process of meat tenderization and the preparation of various processed meat products (Arshad *et al*., 2016). In this study, highest production of proteases has been observed at 72 h in cell free broth and showed 10 mm wide zone of gelatin hydrolysis, while 7 and 8 mm zone of hydrolysis were observed at 24 and 48 h respectively (Fig 3). Basically metallo-endopeptidases or metalloproteinases are the enzymes that utilize gelatin as substrate and hydrolyze it into polypeptides, peptides and amino acids. These enzymes have wide applications in food and medical industries (Balan *et al*., 2012).

Amylases are the enzymes that have great significance in food, textile, detergent and biofuel industries. This enzyme is able to produce diverse products such as oligosaccharides, maltose and glucose from starch (Kumari *et al*., 2019; Naidu and Saranraj, 2013). Present results showed that significant production of amylase from *Lactobacillus* sp. AAF-1 as 5, 7 and 8 mm zone of starch hydrolysis was measured after 24, 48 and 72 h of incubation respectively at 50 °C (Fig. 4).

Beside amylase and protease, other tested enzyme such as cellulase has been produced on the MRS agar plates containing cellulose as a substrate. Cellulase is the main enzyme used in paper and pulp industries. It is the major component used to enhance the animal feed digestibility. It acts on cellulose and degrades it into different products including oligosaccharides and glucose (Imran *et al*., 2016). Figure 5 shows the production yield of cellulase

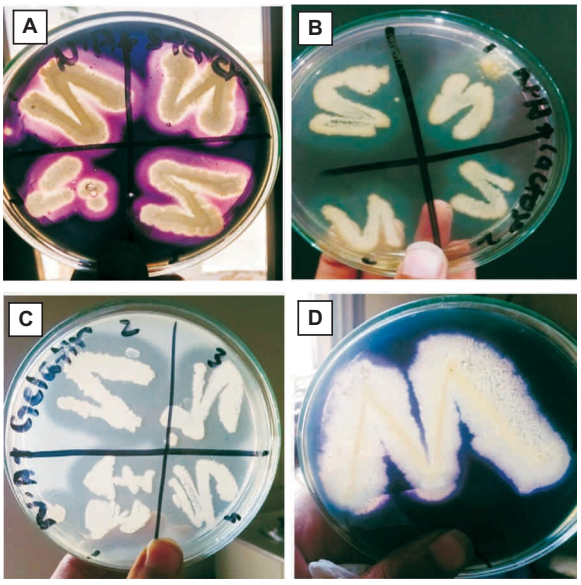

Fig. 2. Growth of *Lactobacillus* sp. AAF-1 on MRS agar **(A)**. *Lactobacillus* sp. AAF-1 showed Amylase production on MRS-Starch agar **(B)**. *Lactobacillus* sp. AAF-1 showed Protease production on MRS-Gelatin agar **(C)**. *Lactobacillus* sp. AAF-1 showed Cellulase production on MRS-Cellulose agar **(D)**. *Lactobacillus* sp. AAF-1 showed Beta galactosidase production on MRS-Lactose agar.

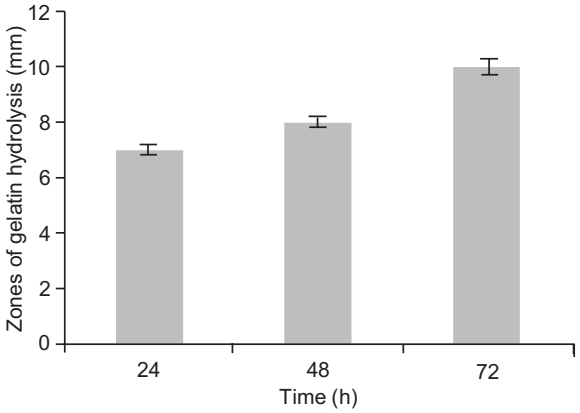

Fig. 3. Production of protease from *Lactobacillus* sp. AAF-1 isolated from coconut water with respect to incubation time in hours.

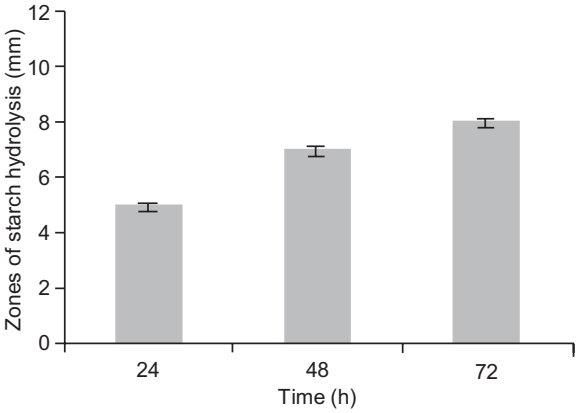

Fig. 4. Production of amylase from *Lactobacillus* sp. AAF-1 isolated from coconut water with respect to incubation time in hours.

enzyme. 5 mm zone of hydrolysis was observed after 24 h while the enzyme activity was about 6 mm after 48 as well as 72 h of incubation.

Beta galactosidase is the enzyme that is involved in the breakdown of lactose into glucose and galactose. As this enzyme is widely used in dairy products so the host organism must be generally regarded as safe (GRAS) (Ali *et al*., 2016). In our analysis, *Lactobacillus* sp. AAF-1 produced Beta galactosidase by using lactose as a substrate. The enzyme production was sequentially increased as 4, 6 and 10 mm zone of hydrolysis after 24, 48 and 72 h of incubation respectively at 50 °C as shown in Fig. 6.

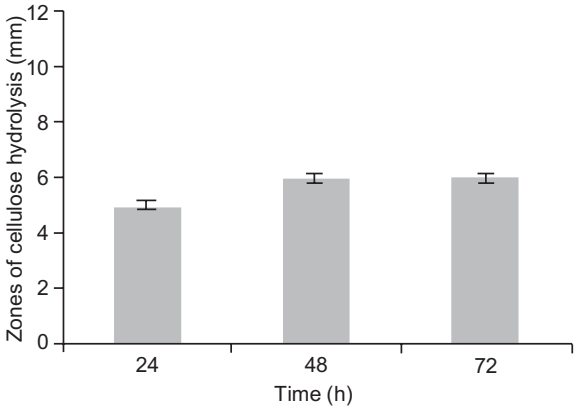

Fig. 5. Production of cellulase from *Lactobacillus* sp. AAF-1 isolated from coconut water with respect to incubation time in hours.

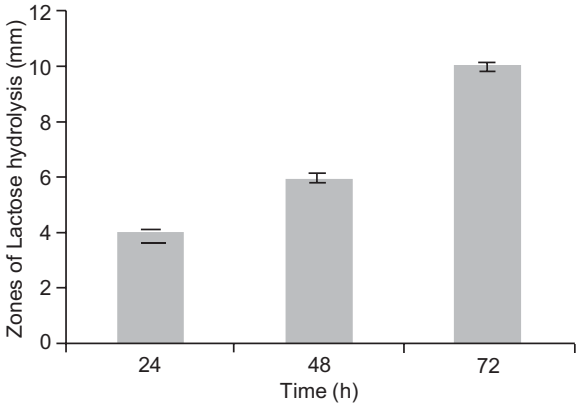

Fig. 6. Production of beta galactosidase from *Lactobacillus* sp. AAF-1 isolated from coconut water with respect to incubation time in hours.

Conclusion

It has been concluded that the *Lactobacillus* sp. AAF-1 has produced enzymes such as amylase, protease, cellulase and beta galactosidase having commercial importance in various industries. To the extent that the strains that produce industrial enzymes must be nonpathogenic, our studies revealed that the *Lactobacillus* sp. AAF-1 was obtained from coconut water which is natural healthy food source so it can be the safe alternative for the production of industrially important enzymes. Future studies will focus on the optimization of this enzyme production with respect to optimum temperature as well as purification and characterization of the above secreted enzymes and the evaluation of their characteristics according to industrial needs.

References

Ali, N.M., Andleeb, S., Mazhar B., Khan, A.U.A. 2016. Optimization of β-galactosidase production from yogurt and dairy soil associated bacteria using different fermentation media. *British Microbiology Research Journal*, **11:** 1-15.

Alnahdi, H.S. 2012. Isolation and screening of extracellular proteases produced by new isolated *Bacillus* sp. *Journal of Applied Pharmaceutical Sciences*, **2:** 71-74.

Ando, N., Hoshino, E. 1990. Predominant obligate anaerobes invading the deep layers of root canal dentine. *International Endodontic Journal*, **23:** 20-27.

Arshad, M.S., Kwon, J.H., Imran, M., Aslam, A., Nawaz, I., Amjad, Z., Khan, U., Javed, M. 2016. Plant and bacterial proteases: a key towards improving meat tenderization, a mini review. *Cogent Food Agriculture*, **2:** 1261780.

Balan, S.S., Nethaji, R., Sankar, S., Jayalakshmi, S. 2012. Production of gelatinase enzyme from *Bacillus* spp. isolated from the sediment sample of Porto Novo Coastal sites. *Asian Pacific Journal of Tropical Biomedicine*, **2:** S1811-S1866.

Carrim, A.J.I., Barbosa, E.C., Jose, D.G.V. 2006. Enzymatic activity of endophytic bacterial isolates of Jacardanadecurrence cham. *Brazilian Archives of Biology and Technology*, **49:** 353-359.

Hassan, H.M., Keera, A.A., Fadel, M. 2016. High yield cellulases and xylanase production from sugar-cane bagasse pith by *Aspergillus oryzae* FK-923 cultivated under solid state fermentation.

International Journal of Current Research and Academic Review, **4:** 1-13.

Hussain, Q. 2010. β galactosidases and their potential applications: a review. *Critical Reviews in Biotechnology*, **30:** 41-62.

Imran, M., Anwar, Z., Irshad, M., Asad, M.J., Ashfaq, H. 2016. Cellulase production from species of fungi and bacteria from agricultural wastes and its utilization in industry: a review. *Advances in Enzyme Research*, **4:** 44-55.

Kasana, R.C., Salwan, R., Dhar, H., Dutt, S., Gulati, A. 2008. A rapid and easy method for the detection of microbial cellulases on agar plates using Gram's iodine. *Current Microbiology*, **57:** 503-507.

Kumari, N., Rani, B., Malik, K., Avtar, R. 2019. Microbial amylases: an overview on recent advancement. *Journal of Entamology and Zoology Studies*, **7:** 198-205.

Mazzucotelli, C.A., Ponce, A.G., Kotlar, C.E., Moreira, M.R. 2013. Isolation and characterization of bacterial strains with a hydrolytic profile with potential use in bio-conversion of agroindustial by-products and waste. *Food Science and Technology Campinas*, **33:** 295-303.

Mienda, B.S., Yahya, A., Galadima, I.A., Shamsir, M.S. 2014. An overview of microbial proteases for industrial applications. *Research Journal of Pharmaceutical Biological and Chemical Sciences*, **5:** 388-396.

Naidu, K.S.B., Adam, J.K., Govender, P. 2012. The use of probiotics and safety concerns: a review. *African Journal of Microbiological Research*, **6:** 6871-6877.

Naidu, M.A., Saranraj, P. 2013. Bacterial amylase: a review. *International Journal of Pharmaceutical and Biological Archives*, **4:** 274-287.

Pariza, M.W., Johnson, E.A. 2001. Evaluating the safety of microbial enzyme preparations used in food processing: update for a new century. *Regulatory Toxicology and Pharmacology*, **33:** 173-186.

Paul, D. 2016. Microorganisms and α-Amylase: a concise review. *Innovare Journal of Sciences*, **4:** 1-5.

Sundarram, A., Pandurangappa, T., Murthy, K. 2014. α-amylase production and applications: a review. *Journal of Applied and Environmental Microbiology*, **2:** 165-175.

Sundus, H., Mukhtar, H., Nawaz, A. 2016. Industrial applications and production sources of serine alkaline proteases: a review. *Journal of Bacteriology and Mycology*, **3:** 191-194.