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Quantitative Screening of Some Wastewater Actinomycetes for Production of Industrial Enzymes

Wael N. Hozzein[1,2], Mohammed A.M. Wadaan[1], Marzouka S. Abdel Tawab[3] and Mohammed B. Ahmed[4]

[1]Bioproducts Research Chair (BRC), Zoology Department, College of Science, King Saud University, Riyadh, Saudi Arabia;
[2]Department of Botany, Faculty of Science, Beni-Suef University, Beni-Suef, Egypt;
[3]Beni-Suef Wastewater Treatment Plant, Beni-Suef, Egypt.
[4]Department of Biochemistry, Faculty of Science, Beni-Suef University, Beni-Suef, Egypt.

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Enzymes play a key role in the hydrolysis and biotransformation of organics in wastewater treatment plants. The main objective of the present study was to screen some actinomycete strains isolated from the wastewater samples for production of some industrially important enzymes. In this regard, ten actinomycete strains were isolated from Beni-Suef wastewater treatment plant and screened for their amylolytic, cellulytic, lipolytic and proteolytic activities. The results showed that the wastewater actinomycetes have good capabilities for the production of the tested enzymes. It was found that all the ten actinomycete isolates gave moderate amylolytic and high proteolytic activities, while nine isolates gave weak cellulytic activities and seven isolates recorded very weak lipolytic activities. The production of proteases was very high comparing to the other tested enzymes.

Key words: Wastewater, Actinomycetes, Screening, Enzymes.

Biocatalysis is a major part of biotechnology and can be performed by living cells and/or their enzymes to catalyze chemical reactions [1]. Certain enzymes are of special interest and are utilized as organic catalysts in numerous processes on an industrial scale. Commercial applications have been found for enzymes in the food, detergent, medical, pharmaceutical and textile industries.

Several enzymes may be detected in wastewater samples, including amylases, cellulases, lipases and proteases [2, 3]. Amylase is one of the most important industrial enzymes which can be used in a number of industrial processes including brewing, baking, textile and detergent industry [4].

Cellulases constitute a family of enzymes that hydrolyze cellulose to fuel-grade alcohol which represents an important energy source. On the other hand, microbial lipases are currently receiving much attention with the rapid development of enzyme technology. Lipases constitute the most important group of biocatalysts for biotechnological applications in food industry, flavour industry, detergent manufacture, pharmaceutical industry, esters and amino acid derivatives, making of fine chemicals, agrochemicals, use as biosensor, bioremediation, cosmetics and perfumery [5].

Microorganisms account for a two-third share of commercial protease production worldwide and alkaline serine proteases are the most important group of commercial enzymes [6].

* To whom all correspondence should be addressed.
Tel.: 00966569481811; Fax: 00966114675783;
E-mail: hozzein29@yahoo.com

Within the pool of biochemical reactions that are involved in flavor development and many other industrial applications, proteolysis plays an outstanding role [7].

Despite the promising performance of newly studied enzymes in the laboratory, their application in the industry might fail due to their lack of robustness [8]. Therefore, the search for new microbial sources of enzymes with superior chances of success in biotechnological applications requires a serious.

In view of the above, it was the intention of the current research to exploit wastewater actinomycetes as producers of industrially important enzymes to further undertake a thorough investigation to isolate and characterize them.

MATERIAL AND METHODS

Sampling, isolation and characterization of the wastewater actinomycetes

Sampling, isolation of the actinomycete strains from the wastewater samples collected from Beni-Suef Wastewater Treatment Plant (WWTP) and characterization of the 10 selected wastewater actinomycetes were carried out using the media and conditions described before in details by Hozzein and others [9]. The pure isolates were preserved in 20% glycerol at -20°C.

Quantitative screening for enzymatic activities

The ten isolated actinomycete strains were screened for enzymatic activities. This was done in Modified Bennett's (MB) broth medium [10] supplemented with 1% of the substrate (starch, Carboxy Methyl Cellulose (CMC), olive oil and casein). In all cases, 25 ml of the sterile broth was inoculated and incubated at 30°C on a rotary shaker (150 rpm) for 5 days. Then, the enzyme activity was measured as described below.

Amylase activity assay

Amylase activity was determined by measuring the amount of reducing sugar (glucose) released after hydrolysis of soluble starch. The reaction mixture contained 0.5 ml of the filtrate (crude enzyme) and 0.5 ml of 100 ml phosphate buffer (pH 6.5) containing 1% (w/v) of soluble starch. The mixture was incubated at 40°C for 30 min. The amount of reducing sugar released in the mixture was determined by measuring the absorbance at 500 nm according to the Somogyi method [11]. One unit of enzyme activity is defined as the amount of enzyme releasing 1 µmol of glucose per min at 40°C.

Cellulase activity assay

Cellulase activity was determined by measuring the amount of reducing sugar (glucose) released after hydrolysis of cellulose. The reaction mixture contained 0.5 ml of the culture filtrate and 0.5 ml of 100 ml acetate buffer (pH 4.5) containing 1% (w/v) of CMC. The mixture was incubated at 50°C for 30 min. The amount of reducing sugar released in the mixture was determined by measuring the absorbance at 500 nm according to the Somogyi protocol [11]. One unit of enzyme activity is defined as the amount of enzyme releasing 1 µmol of glucose per min at 50°C.

Lipase activity assay

Lipase activity was determined by measuring the amount of fatty acids released from the hydrolysis of the emulsified solution of olive oil by titration against NaOH solution [12]. The enzyme amount with which 1 µmole of fatty acid is produced in 1 minute is assumed as 1 enzyme unit.

Protease activity assay

Anson–Hagihara's method [13] was used to measure the protease activity using casein as a substrate. The crude enzyme (1 ml of the culture filtrate) was added to 1 ml casein solution (2.5%, w/v casein in boric acid–NaOH buffer, pH 9). The reaction was incubated at 40°C for 30 min and then the enzyme reaction was terminated by the addition of 2.5 ml of TCA (5% trichloroacetic acid). The precipitates were removed by filtration through Whatman No.1 filter paper. The absorbance of the filtrate was measured at 280 nm. One unit of protease was defined as the amount of enzyme liberating 1 µg of tyrosine per min under assay conditions. Enzyme units were calculated after comparison with tyrosine standard curve (0–100 µg).

RESULTS AND DISCUSSION:

One of the main goals of the research on enzymes is industrial applications. It was reported that [14] enzyme production is a growing field of biotechnology and the world market for enzymes is over $1.5 billion and it is anticipated to be doubled within 5 years. In this regard, a large number of new enzymes have been designed with the input

Table 1. The enzymatic activities of the ten actinomycete strains isolated from Beni-Suef Wastewater Treatment Plant measured in enzyme unit/ml

Actinomycete strains	Amylase activity (u/ml)	Cellulase activity (u/ml)	Lipase activity (u/ml)	Protease activity (u/ml)
A11	12.7 ± 0.0	2± 0.0	0± 0.0	407± 5
A13	41± 0.05	3.1± 0.0	0.1± 0.0	481± 5
B21	53 ± 0.05	13.1± 0.0	0± 0.0	525.4± 5
B22	53 ± 0.05	0± 0.0	0.1± 0.0	52± 0.5
B31	54.8± 0.05	9.2± 0.0	0.05± 0.0	658.6± 5
C11	69.4± 0.05	7.5± 0.0	0.1± 0.0	854.7± 5
C12	53± 0.05	6.2± 0.0	0.05± 0.0	629± 5
C13	84.8± 0.05	7± 0.0	0.05± 0.0	518± 5
M1	7.9± 0.0	3± 0.0	0± 0.0	35.5± 0.05
M13	15.9± 0.0	4.3± 0.0	0.05± 0.0	629± 5

of protein-engineering, biochemical-reaction engineering and metagenomics. Various molecular techniques have also been applied to improve the quality and performance of microbial enzymes for their wider applications in many industries [15].

Despite the fact that large number of different enzymes has been identified and many are being used in various biotechnological applications, the available enzymatic array is still not sufficient to meet the ever increasing demands [16].

The majority of the industrial enzymes are of microbial origin [5, 17]. Therefore, programs to select new microorganisms for enzyme production are increasing around the world. Actinomycetes are one of the most investigated groups since they constitute a potential source of biotechnologically interesting substances [18].

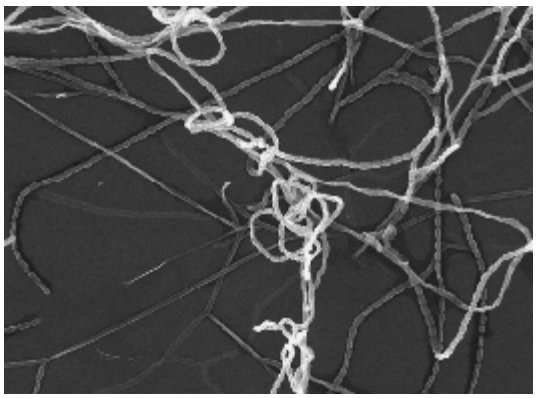

Fig. 1. Scanning electron micrograph of strain C13 showing the very long spore chains with smooth surface

In this study, ten morphologically different actinomycete strains were isolated from wastewater samples collected from Beni-Suef WWTP and characterized using standard procedures. The full description of the strains was reported in a previous study [9]. The observations revealed that strains A11, B21 and B31 showed characteristics of genus *Nocardia*, where strains A13 and B22 were characterized as members of the genus *Rhodococcus*. On the other hand, the strains C11, C12 and C13 (Fig 1) had typical characteristics for members of genus *Streptomyces*, where strain M1 had characteristics similar to those of genus *Gordonia*. Finally, strain M13 showed characteristics typical to members of genus *Nocardiopsis.*

The main objective of the present study was to screen the ten selected actinomycete strains isolated from the wastewater samples for production of industrially important enzymes. In this regard, they were screened for their amylolytic, cellulytic, lipolytic and proteolytic activities.

The results showed that the wastewater actinomycetes have good capabilities for the production of the tested enzymes. It was found that all the ten actinomycete isolates gave moderate amylolytic and high proteolytic activities; nine isolates gave weak cellulytic activities, whereas only seven isolates recorded very weak lipolytic activities.

The data recorded in Table 1 revealed that the two *Streptomyces* strains C13 followed by C11 were the most active strains in amylase production. Though, amylase is mainly a fungal and/or

eubacterial product, the possibility of using *Streptomyces* strains in amylase production was recently investigated and proved [19, 20, 21].

The two *Nocardia* strains B21 and B31 followed by the *Streptomyces* strain C11 were the most active strains in cellulase production, whereas the *Streptomyces* strain C11 followed by the two *Rhodococcus* strains B22 and A13 were the most active in lipase production. Recently, the interest raised by the enzymes produced by actinomycetes and the lack of information concerning the lipases of the genus *Rhodococcus* leaded to the discovery of LipR, the first member of a new bacterial lipase family (Family X) displaying an unusual Y-type oxyanion hole from *Rhodococcus* sp. strain CR-53 [22].

It is obvious from Table 1 that protease production was very high comparing to the other tested enzymes and that the *Streptomyces* strain C11 was the most active organism in protease production. It was reported that proteases from *Streptomyces* origin offer an advantage as the mycelium can be easily removed by filtration [23]. Therefore, production and characterization of proteases from different *Streptomyces* strains for different biotechnological applications were of interest [24, 25].

Enzymes play a key role in the hydrolysis and biotransformation of organics in wastewater treatment plants. However, no significant application has been proposed yet for the enzymes from the wastewater actinomycetes. Therefore, the aim of the current study was screening the wastewater actinomycetes for production of four enzymes of commercial interest. The results are encouraging and the purification and characterization of the most interesting produced enzymes will be reported in other studies.

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REFERENCES

1. Carvalho, C.C.C.R. Enzymatic and whole cell catalysis: finding new strategies for old processes. *Biotechnol. Adv.*, 2011; **29**: 75–83.
2. Boczar, B.A., Begley, W.M., Larson, R.J. Characterization of enzyme activity in activated sludge using rapid analyses for specific hydrolases. *Water Environ. Res.*, 1992; **64**: 792–797.
3. Hosetti, B.B., Frost, S. Catalase activity in wastewater. *Water Res.*, 1994; **28**: 497–500.
4. Panedy, A., Benjamin. S., Soccol, C., Nigam, P. Solid state fermentation for the production of industrial enzymes. *Curr. Sci.*, 1999; **29**: 119-131.
5. Sharma, R., Chisti, Y., Banerjee, U.C. Production, purification, characterization, and applications of lipases. *Biotechnol. Adv.*, 2001; **19**: 627-662.
6. Tremacoldi, C.R., Carmoa, E.C. Production of extracellular alkaline protease by *Aspergillus clavatus*. *World J. Microb. Biotech.*, 2005; **21**: 169-172.
7. Takeuchi, S., Kinoshita, T., Kaidoh, T., Hashizume, N. Purification and characterization of protease produced by *Staphylococcus aureus* isolated from a diseased chicken. *Vet. Microbiol.*, 1999; **67**: 192-202.
8. Vermelho, A.B., Noronha, E.F., Filho, E.X.F., Ferrara, M.A., Bon, E.P.S: Diversity and biotechnological applications of prokaryotic enzymes. In: *The Prokaryotes* (Rosenberg, E, DeLong, EF, Lory, S, Stackebrandt, E, Thompson, F, eds). Berlin Heidelberg: Springer, 2013; pp 213-240.
9. Hozzein, W.N., Ahmed, M.B., Abdel Tawab, M.S. Efficiency of some actinomycete isolates in biological treatment and removal of heavy metals from wastewater. *Afr. J. Biotech.*, 2012; **11**(5): 1163- 1168.
10. Jones, K.L. Fresh isolates of actinomycetes in which the presence of sporogenus aerial mycelia is a fluctuating characteristic. *J. Bacteriol.*, 1949; **57**: 141-145.
11. Somogyi, M.J. Micromethods for the estimation of diastase. J. Biol. Chem., 1938; **125**: 299 -414.
12. Kumar, D., Kumar, L., Nagar, S., Raina, C., Parshad, R., Gupta, V.K. Screening, isolation and production of lipase/esterase producing *Bacillus* sp. strain DVL2 and its potential evaluation in esterification and resolution reactions. *Arch. Appl. Sci. Res.*, 2012; **4**(4): 1763-1770.
13. Hagihara, B. (ed): The Enzymes. New York: Academic Press Inc., 1958.
14. Lowe, D.A.: Production of enzymes. In: *Basic biotechnology* (Ralledge, C, Kristiansen, B, eds.). Cambridge, 2002; pp 391-408.

15. Nigam, P.S. Microbial enzymes with special characteristics for biotechnological applications. *Biomol.*, 2013; **3**: 597-611.
16. Burg, V., Eijsink, V.G.H. Selection of mutations for increased protein stability. *Curr. Opin. Biotech.*, 2002; **13**: 333-337.
17. Anbu, P. Screening of extracellular protease (gelatinase) at different pH by keratinophilic fungi. *J. Pure Appl. Microbiol.*, 2013; **7**(1): 39-44.
18. Lealem, F., Gashe, B.A. Amylase production by a Gram positive bacterium isolated from fermenting tef (Eraglostis tef). *J. Appl. Bacteriol.*, 1994; **77**: 348-352.
19. Hidaka, H., Koaze, Y., Yoshida, K., Niwa, T., Shomura, T., Niida, T. Isolation and some properties of amylase from *Streptomyces hygroscopicus* SF-1084. *Starch*, 2006; **26**(12): 413–416.
20. Kar, S., Datta, T.K., Ray, R.C. Optimization of thermostable ±- amylase production by *Streptomyces erumpens* MTCC 7317 in solid-state fermentation using Cassava fibrous residue. *Braz. Arch. Biol. Technol.*, 2010; **53**(2): 301-309.
21. dos Santos, É.R., Teles, Z.N.S., Campos, N.M., de Souza, D.A.J., Bispo, A.S.D., do Nascimento, R.P. Production of a-amylase from *Streptomyces* sp. SLBA-08 strain using agro-industrial by-products. *Braz. Arch. Biol. Technol.*, 2012; **55**(5): 793-800.
22. Bassegoda, A., Pastor, F.I.J., Diaz, P. *Rhodococcus* sp. strain CR-53 LipR, the first member of a new bacterial lipase family (Family X) displaying an unusual Y-Type oxyanion hole, similar to the *Candida antarctica* lipase clan. *Appl. Environ. Microb.*, 2012; **78**(6): 1724–1732.
23. Phadatare, S.U., Deshpande, V.V., Srinivasan, M.C. High activity alkaline protease from *Conidiobolus coronatus* (NCL 86.8.20): Enzyme production and compatibility with commercial detergents. *Enzyme Microb. Technol.*, 1993; **15**: 72-76.
24. Moreira, K.A., Cavalcanti, M.T.H., Duarte, H.S., Tambourgi, E.B., de Melo, E.H.M., Silva, V.L., Porto, A.L.F., Filho, J.L.D. Partial characterization of proteases from *Streptomyces clavuligerus* using an inexpensive medim. *Braz. J. Microb.*, 2001; **32**: 215-220.
25. Manivasagan, P., Venkatesan, J., Sivakumar, K., Kim, S-K. Production, characterization and antioxidant potential of protease from *Streptomyces* sp. MAB18 using poultry wastes. *Bio. Med. Res. Inter.*, 2013; **2013**: 1-12.