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Investigation of Microbes that Produce Hydrolytic Enzymes in their Microenvironments

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ABSTRACT

Microbial enzymes in natural environments have a role in biochemical activities and the biotechnological potential of microbes. During this study, 150 isolates from different environments, soil, water and plants were studied, and their ability to produce different enzymes (caseinase, gelatinase, amylase, carboxymethyl cellulase, and esterase) was examined. Ninety per cent of the isolates produced at least one enzyme. The isolated microbes from the three environments produced the highest percentage of caseinase enzyme. The soil microbes were also characterized by high production of cellulase and amylase enzyme. The microbes of the aquatic environment were characterized by high production of the enzyme esterase compared with the microbes of the plants that had a high efficiency in the production of the cellulase enzyme. The study showed that optimum incubation time for enzymes production was 72 h and 7.5 pH. The ability of most microbes to produce enzymes used in biotechnology exploration produced was affected by the type of microbial environment of the microorganism.

Key words : Caseinase, gelatinase, amylase, carboxymethyl cellulase, esterase, optimum conditions

INTRODUCTION

Microbes are spread in different natural environments such as soil, water, air and plants, and they are characterized by their ability to adapt according to their environment and coexist with the growth requirements present in them. Enzymes play a major role in this adaptation (Aleem *et al.*, 2018).

The microbes are largely stable, and produced by animals and plants. Microbial enzymes are used in many lives and industrial applications in particular. Microbes produce degrading enzymes, which are of great benefit, as they produce enzymes that degrade fats, proteins, cellulose and starch. Protease enzyme is one of the most important enzymes, as it is widely used in the manufacture of detergents and dairy (Ananthi *et al.*, 2014).

Amylase is a second-order enzyme produced by microbes that breaks down starch into multiple products; including dextrins and progressively smaller polymers composed of glucose units Microbial amylase is also considered a successful alternative to the industrial decomposition of starch in many industries such as food, textile and paper. Microbes produce multiple cellulose degrading enzymes : Carboxymethyl (CM) cellulose, cellobiohydrolases and β-glucosidases (Ashok *et al.*, 2019). Cellulose is of great importance as a source of carbon and energy for many microbes such as bacteria, fungi and algae and has useful applications in the production of bioenergy and bio-fuel, in addition to their uses in beverage, paper and textile industries (Ariole *et al.*, 2014).

The esterase enzyme is one of the degrading enzymes produced by microbes, which has practical applications in many chemical industries. These enzymes have applications in cosmetics, paper and pulp production, and also as food additive (Banerjee *et al.*, 2016).

Therefore, this research was an extensive survey of exogenous enzymes produced by microbes in different environments (Ananthi *et al.*, 2014).

MATERIALS AND METHODS

Ten samples of water, 15 of soils and 15 of plants were collected. The plant samples were collected from orchards in the province of Babylon without tagging them randomly. Dilutions were made for the mentioned

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samples and were grown on LB agar with incubation at 25°C for 48-72 h. The distinct colonies were grown on the same medium again to obtain pure colonies, and then the pure isolated colonies were grown in LB supplemented with 50% (v/v) fetal bovine broth serum and kept at -80°C (De Deckker, 2016). The media was inoculated with the obtained isolates and placed in an incubator at 25°C for 24 h. Then their ability to produce enzymes was examined according to the methodology of Facchin *et al.* (Table 1).

The factors affecting enzyme production such as incubation period (24-96 h) and pH (6-9) were investigated. To determine the optimal conditions for production, it was noted that the productivity of the five enzymes differed by their influence on these conditions (Sikdar *et al.*, 2015).

RESULTS AND DISCUSSION

Out of 40 samples collected from plants, soil and water, 150 isolates of bacteria and fungi were obtained. It was found that 135 isolates produced at least one of the enzymes, and 15 isolates were not productive for any type of the studied enzymes (Table 2).

Considering each enzyme separately, 75.3, 34.8, 50, 50 and 40% of the isolates produced caseinase, esterase, CM cellulase, gelatinase and amylase, respectively (Fig. 1). Esterase was the least enzyme produced by microorganisms that were isolated from different samples. Sample 10 produced the five studied enzymes and sample 6 produced four enzymes at high concentrations. The highest productivity of cellulase enzyme was in samples isolated from plants, while esterase enzyme was higher in water samples, especially in samples (W2-W7). Most isolates of isolated bacteria produced more than one type of enzyme. The most abundant enzyme in the three samples was caseinase (Fig. 2).

The fermentation process was carried out for 96 h with measurement of growth of P10 isolate and enzyme activity at 24 h intervals. The results showed that significant enzyme

Table 1. The media used to measure the activity of the enzyme (Ananthi *et al.,* 2014)

Enzymes	Media name	Media components	Method	Positive result
Amylase activity	Corn starchagarose	1% (w/v) agarose, 50 mM Tris-HCl pH 6.8, 1 mM CaCl2, 4-0.5% (w/v) corn starch	The isolates were grown on this medium and after the incubation period. The culture plates were flooded with 2% iodine solution to colorize the remaining starch	The amylase-producing isolates showed a clear halo
Esterase activity	Tributyrin-agarose	1% (w/v) agarose, 50 mM Tris-HCl pH 6.8, 1 mM CaCl2, 0.6% (v/v) tributyrin emulsion	The isolates were grown on this medium after the incubation period	Esterase-producing isolates showed a clear zone surrounding their colonies after incubation
Cellulase activity	Carboxymethyl cellulose-agarose	1% (w/v) agarose, 50 mM Tris-HCl pH 6.8, 1 mM CaCl2, 0.5% (w/v) carboxymethyl cellulose	After incubation, the medium was stained for 30 min with Congo red (0.25% in 0.1 M Tris-HCl, pH 8.0), followed by destaining (0.5 M NaCl, 0.1 M Tris-HCl, pH 8.0) for 5 min	Cellulase activity showed the presence of a clear zone around the strain growth
Caseinase activity	Casein-agarose	1% (w/v) agarose 1 mM CaCl2, 10% (v/v) casein in 1X PBS pH 7.4	The isolates were grown on this medium after the incubation period incubate with 1 M HCl solution to precipitate the remaining casein	Caseinase activity was detected by the presence of a clear halo
Gelatinase activity	Gelatin media	2 ml of media/assay tube: 50 mM Tris-HCl pH 6.8, 1 M CaCl2 10% (w/v) gelatin]	The isolates were grown on this medium after the incubation period	Gelatinase activity was detected by liquefaction of the media

Table 2. Number of microbial isolates from environmental samples producing or not producing (the investigated enzymes)

Samples	Soil		Samples	Plant		Samples	Water	
	P*	NP		P	NP		P	NP
S1	2	-	P1	2	2	W1	2	2
S2	3	-	P2	3	-	W2	1	-
S3	2	-	P3	2	-	W3	2	3
S4	4	-	P4	1	1	W4	2	-
S5	3	-	P6	5	-	W5	3	-
S6	9	-	P6	4	-	W6	1	-
S7	6	-	P7	1	-	W7	1	-
S8	5	-	P8	3	-2	W8	2	1
S9	9	1	P9	4	-	W9	2	-
S10	12	-	P10	10	-	W10	3	-
S11	2	-	P11	2	-			
S12	5	-	P12	4	1			
S13	3	2	P13	3	-			
S14	1	-	P14	3	-			
S15	4	-	P15	1	-			
Total	68	3		48	6		19	6

*P=producing enzymes; NP=producing no enzymes.

production (P≤0.05) started after the first period, but the maximum production was obtained after 72 h yielding 134.34/Uml caseins, 104.23/Uml esterase, 98.3/Uml cellulase, 112.3/Uml amylase and 34.7/Uml gelatinase (Fig. 3). At 96 h, there was no increase in production, but began to decrease, and this indicated that the bacteria began to produce enzymes in the stationary phase, which suggested that enzyme production and bacterial growth were correlated.

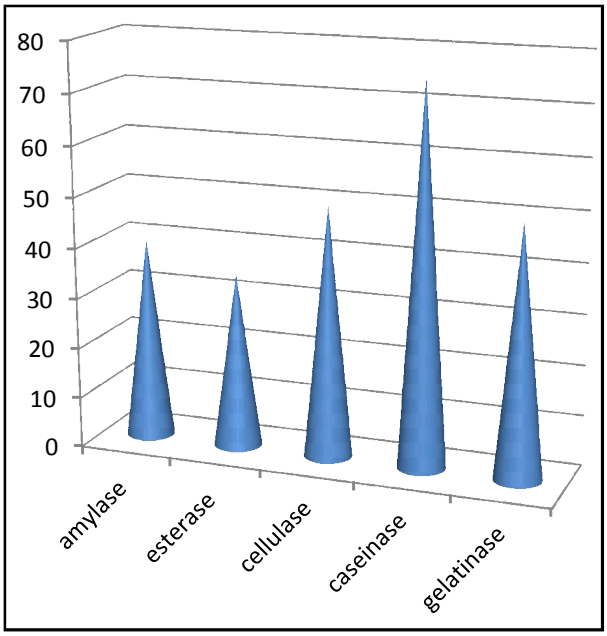

Fig. 1. Percentage of microbial isolates from environmental samples (from soil, water and plant) producing each of five different extracellular hydrolytic enzymes studied.

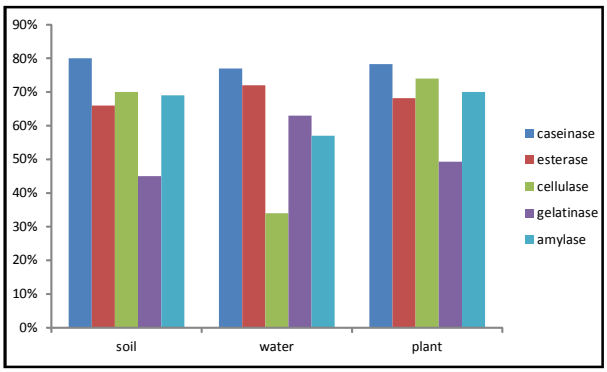

Fig. 2. Percentage of microbial isolates from soil, plant and water samples with activity of each of the five investigated enzymes.

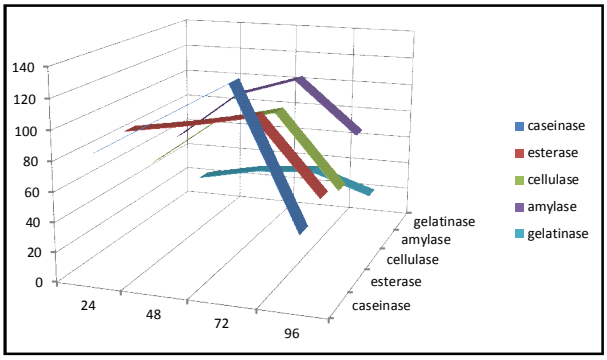

Fig. 3. Effect of incubation time in isolate P10 on growth and enzymes production.

It was found that the productivity increased up to pH 7.5. The highest productivity reached at 7.5 pH ($P \leq 0.05$), after which it began to decline (Fig. 4).

Prokaryotic microorganisms exist in a variety of environments such as water, air, soil, plants and biological environments such as human intestines and ruminant rumen (Egerton *et al.*, 2018). These organisms have different relationships with the living things of the environment in which they reside, whether plants or other diverse microorganisms (Finore *et al.*, 2014). These relationships keep them in balance within their environment. Microorganisms, present in the environment, are of great importance, as they have been used in various fields of waste treatment, water supply and regulation, and to provide healthy soil for agriculture, and this is due to its ability to produce many degrading enzymes (He *et al.*, 2018).

Various microorganisms are of great importance in the cycle of elements in nature such as (carbon, nitrogen, phosphorus and

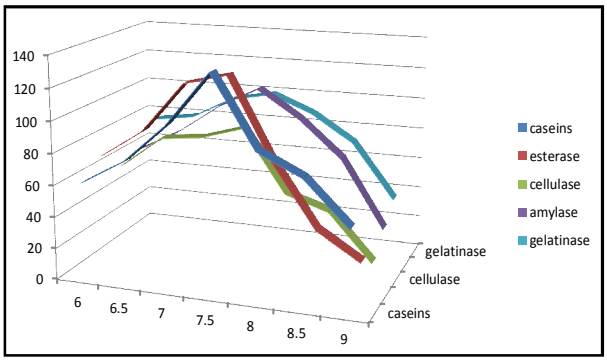

Fig. 4. Effect of initial pH on enzymes production.

sulfur) and this is due to their possession of many decomposing enzymes that help in the decomposition of organic matter and the remains of plants and animals (Jinendiran *et al.*, 2019). Microorganisms that produce amylase, caseinase and esterase enzymes were isolated from a plant in tropical forests (Liu and Kokare, 2017). Enzymes producing many enzymes have also been isolated repeatedly from environments with different conditions, including harsh and extreme conditions, including natural conditions (Sayyed *et al.*, 2015).

Several studies were conducted to investigate the bacteria that produce extracellular enzymes. One study showed that the production of amylase enzyme was more in the soil, and other studies showed that the production of protease was more in the soil. The percentage of enzymes produced in the soil varied, because the soil is a diverse environment that includes different types of microbes that differ in their physical and chemical properties (Ruzhen Wang *et al.*, 2014). In the presented study, one of our soil isolations, as shown in Table 2, produced all the studied enzymes, because soil is an environment that contains various microorganisms, differing in their habitats and requirements, which are integrated with each other and linked to each other with different and complementary relationships (Ananthi *et al.*, 2014).

Microbes are associated with plants in different relationships that may be pathogenic, parasitic, or dependent, and since plants represent a nutrient-rich environment because they contain different organic compounds, some of them are fast decomposing, and some of them are complex polymers such as cellulose, half-cellulose and lignin, which need external enzymatic activity to break down. This enzymatic activity provides it various microorganisms that live on plants (Hu, 2016). Our study showed that plant microbes had a high production of cellulase enzyme and amylase as shown in Fig. 2 and this is consistent with studies of Luo *et al.* (2017) and Li *et al.* (2018) which showed that plant microbes produced cellulase enzyme at high rates.

Most aquatic environment microbes produced different hydrolytic enzymes depending on the type of aquatic environment in which they were present (Lashin *et al.*, 2015) and obtaining microbial isolates with high productivity of the enzyme caseinase and esterase in the aquatic environment (Fig. 2). Thus, indicating the interaction between these microbes and the organic matter present in them, and that the substrate for the enzyme esterase was present in large quantities in the water (Hehemann *et al.*, 2019). Interactions between microorganisms and organic matter were of paramount importance in the functioning of aquatic environments (Egerton *et al.*, 2018). In this context, composition of organic matter, with others, the physical and chemical conditions of the environment, and the activity and structure of microbial communities.

Maximum enzyme production was obtained at the start of the stationary phase, 72 h after culture initiation, which suggested that enzyme production and bacterial growth were correlated (Mladenoska and Dimitrovski, 2015). The reduction in enzyme production after an optimum incubation period can be explained by cell autolysis, nutrient exhaustion and accumulation of enzyme repressors (Rietl *et al.*, 2016).

The initial pH of the medium and incubation temperature both influence enzymatic production by affecting transport across the cell membrane and control of enzyme gene expression (Orsi *et al.*, 2018). Findings are in accordance with those of Khalil (2015), who reported that enzymatic production varied at pH 7.5 to 8.5 and temperatures between 55 and 60°C.

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