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Screening the ability of several microbial isolates for production of some industrial enzymes

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Abstract microbes release more stable enzymes which can be obtained at the lowest coast. Species of fungi and bacteria were isolated from different sources including *Aspergillus niger*, *Aspergillus flavus*, *Penicillium chrysogenum*, *Fusarium solani* ;*Pseudomonas aeruginosa*, *Bacillus mycoides* and *Bacillus cereus* as well as two isolates of Rhizobia, BUS26 and RMI4 in addition to the *Ensifer meliloti* as a reference strain of rhizobia. These microbial species were used to determine their ability to produce some industrial enzymes after culturing them on Czapek dox agar by replacing the carbon source with pectin, chitin or cellulose. Then the average diameter of the colony was measured and the percentage of the enzymes production was calculated. The results showed that all the fungus utilized in this study produced the enzymes in different proportions. *A. niger* isolated from air and soil was able to grow and use orange peel as a single source of carbon to produce pectinase with the highest percentage of 80.77% and 71.72% respectively. As well as the highest yield of cellulase was produced by *A. niger* with a rate of 81.15%, while the rest of fungi differed in their ability to produce the enzymes between the medium and low amount. *F. solani* had the lowest ability to produce pectinase and chitinase by rates of 19.21% and 17.03% respectively. The ability of bacterial isolates to produce enzymes was also varied as *B.mycoides* and BUS26 gave the highest productivity of cellulase by 74.74% and 68.89% respectively. The productivity of pectinase was close as BUS26 isolate showed productivity rate of 50% following by *P.aeruginosa* with a ratio of 47.83% while *B.cereus* had a pectinase production with a rate of 34.66%.
Keywords: Pectinase, Cellulase, *A. niger*, *B. mycoide*, *E. melioti*.

فحص قدرة عدة عزلات ميكروبية على انتاج بعض الإنزيمات الصناعية

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الملخص الإنزيمات المتحصل عليها من الميكروبات تعتبر أكثر استقراراً، ويمكن الحصول عليها بأرخص ثمن، تم عزل وتشخيص أنواع فطرية معزولة من مصادر مختلفة، وشملت *Aspregillus niger*، *Aspregillus flavus*، *Penicillium chrysogenum*، *Fusarium solani* وبكتيريا *Pseudomonas aeruginosa*، كذلك نوعان من بكتيريا *Bacillus* ssp. هما *Bacillus mycoides* و *Bacillus cereus*، وعزلتان من بكتيريا الجذر العقدية Rhizobia هما BUS26 و RMI4 أضافةً إلى السلالة المرجعية *Ensifer meliloti*، حيث أُستخدمت هذه الأنواع الميكروبية لمعرفة مقدرتها على إنتاج بعض الإنزيمات الصناعية، وذلك بعد تنميها على وسط تشابك دوكس آجار المحور باستبدال المصدر الكربوني بالبكتين والكيتين والسيليلوز، حيث تم قياس متوسط قطر مستعمرة النمو وحساب النسبة المئوية لإنتاج الإنزيمات، وقد كانت جميع الفطريات منتجة لإنزيمات الدراسة وبنسب متفاوتة، حيث أثبت فطر *A. niger* قدرته على النمو واستخدام مخلفات قشور البرتقال كمصدر وحيد للكربون لإنتاج إنزيم البكتينز حيث أعطى أعلى نسبة وصلت إلى 80.77% ، 71.72% لعزلتي الفطر المعزول من الهواء الجوي والتربة على التوالي، وايضاً اعطى أعلى إنتاجية لإنزيم السليلويز بنسبة 81.15%، بينما تفاوت نسب باقي الفطريات في إنتاج الإنزيمات بين المتوسطة والقليلة، إذ كان فطر *F. solani* الأقل في الإنتاجية لإنزيمي البكتينز والكيتينز بنسب 19.21%، 17.03% على التوالي، أما بالنسبة للأنواع البكتيرية المختبرة فقد كانت انتاجيتها من الإنزيمات أيضاً متفاوتة حيث أعطت *B. mycoides* والعزلة BUS26 أعلى إنتاجية لإنزيم السيليلويز بنسب 74.74% ، 68.89% على التوالي، وقد كانت إنتاجية انزيم الكتينيز

ضئيلة جداً من قبل العزلة RMI4 والسلالة *E. melioti* وبنسب1.32%، 12.05% على التوالي، وتقاربت الإنتاجية لإنزيم البكتينيز إذ أعطت العزلة BUS26 نسبة 50% ، ومن ثم *P. aeruginosa* أعطت 47.83%، أما *B. cereus* وصلت إنتاجتها من البكتينيز إلى 34.66%.

الكلمات المفتاحية: انزيم البكتينز، انزيم السليلويز، *A. niger*، *B. mycoides*، *E. melioti*.

Introduction

The enzymes are the stimulators for the biological systems which consider to be the nerve of life for all the livings and they are the massive biochemistry active site for the metabolic processes [1]. Most of the enzymes are like glutinous solutions in water so they can be easily isolated by the same methods used to isolate the proteins but some of them are connected to the cellular organelles membranes, for instance: Cell membrane, Mitochondria, Plastids and Endoplasmic reticulum which leads to difficulties in isolating the enzymes. The enzymatic science techniques are developed to become a modern science and one of the most important sciences with the increase of its demand in many industries [2]. The enzymatic fermentation known as the utilization of the microbes to produce a useful product after growing them on a suitable nourishing environment and under specific environmental conditions as the enzymes are considered to be one of these productions. however, there are two types of fermentations that can be used in the industry: Solid-Stat fermentation SSF and Submerged fermentation SF and each type has advantages and disadvantages in the production process [3]. There are a lot of industrial wasting materials and secondary yields which were used as a Mash in the production of the enzymes like orange rind and sugar cane [4]. The microbes (Bactria, fungi) have a massive ability to produce enzymes outside the cells, which are called the extracellular enzymes, most of these enzymes are involved in the breakdown of the nutrients in the surrounding environment [5]. Pectinase, chitinase and cellulase are some of the digestive extracellular enzymes that are studied in this research. Pectinases are involved the breakdown of the glycosidic linkages of pectic substances and these extracellular enzymes can be found in both plants and microbes including fungi and bacteria [6]. Chitinase is hydrolytic extracellular enzyme and it is called chitinase because it is involved in the analysis of Poly-N-acetyl-D glucosamine as it breaks down the Beta 1-4 glycosidic bonds between the carbon atom (C1) and the carbon atom (C4) for every two opposite units of N-acetyl glucosamine in the chitin [7]. As well as cellulase is an extracellular enzyme produced by fungi and bacteria that catalyze cellulolysis, cellulose breakdown is of considerable economic importance because it makes a major constituent of plants available for consumption and it uses in chemical, food and medical industries [8]. The specific reaction of cellulase is the hydrolysis of 1,4-beta-D-glycosidic linkages in cellulose and beta-D-glucans in cereals [9].

The enzymatic research are quickly developed and became one of the research which has clear outlines and it was described as an independence science and connects strongly with the other sciences. The development of the enzymatic science lead to be useful in many usages and applications , therefore The objectives of this study were firstly to determine the efficiency of some microbial isolates (bacteria and fungi) in producing important enzymes in industrial fields secondly this study also aims to detect the microbial isolates that analyze the modified Czapeck Dox Agar medium and additive to it some enzymes such as: Chitinase, Cellulase & Pectinase.

Materials and Methods

Isolation of the Fungi

Four species of fungi were isolated from the soil, the air and orange peel, species were identified as *Aspergillus flavus*, *Aspergillus niger*, *Penicillium chrysogenum* & *Fusarium solani*. As mentioned above, all these fungi were isolated on Czapeck Dox Agar [10]. The isolated fungi were microscopically examined and identified by the comparison with identification books and the classification keys according to their appearance and the nature of their growth in the agars [11,12,13].

Isolation of the Bactria

In this research two isolates of Rhizobia preserved in the research laboratories- Science Faculty-university of Sebha were used, those isolated Rhizobia (BUS26, RMI4) were isolated from the *Melilotus officianalis* and *Medicago sativa* respectively and a reference strain *Ensifer meliloti* which was obtained from the biological and biomolecular research laboratory-Science Faculty-Mohamed V university in Morocco. In this research three types of bacteria were used: *Psedomonas aeruginosa* which was already identified and obtained from biological research laboratory-Science Faculty- university of Sebha [14]. Two species of *Bacillus* were isolated from the garden's soil of Science Faculty- university of Sebha which was identified as: *B. Mycoides* and *B. Cereus*. The method of collecting soil sample and isolating of bacteria were conducted as described previously by [15,16].

The ability of the isolated microbes to grow on modified media and produce enzymes

The detection of the enzymatic activities by using solid agar media is one of the most common used methods which allow to detect and give a quick evaluation of different Extracellular enzymes of bacteria and fungi [17]. To know the range of growth of isolated fungi on modified Czapeck Dox Agar [18]. A disk of fungi was taken by penetrating cork (5mm diameter) from the edge of the 5-day-old fungi colony and it was placed in the center of the Petri dish with 4 repetitives for each modified medium by addition 5 grams of

pectin, chitin and cellulose each one separately instead of the carbon source (Sucrose), in addition to the control, which was the fungal disk on the non- modified medium. The plates were incubated for a period 5 days at 28 °C. and then the results were calculated by measuring the average of colony diameters.

Regarding the enzymatic activity of the bacterial isolates was measured by immersing a desk of filter with a diameter of 5 mm in the bacterial suspension of isolates with a concentration of (10^{-5} bacterial cells / ml). The Turbidity of the bacterial suspension was measured by comparing it with the MacFarland index, then the paper disks were placed in the middle of the Petri dishes that containing the modified media, with four repetitives per modified media, In addition to the control, which was a disk of filter paper impregnated with bacterial suspension on the non-modified medium. The results were taken after 48 hours at 30 °C for *B. cereus* and *B. mycoides*, at 37 °C for *P. aeruginosa*, while for Rhizobia isolates the results were recorded after 72 hours at 28 °C. The results were recorded by calculating the mean of bacterial growth diameters, The percentage of enzyme production was calculated as mentioned by Abbott's formula Corrected%= [1-(n in T after treatment)/ (n in Co after treatment)]*100 Where: n= population, T= treated, Co= control [19].

statistical analysis

One way Analysis of variance (ANOVA) test was used by SPSS 23 Version where the different averages were significantly distinguished among them at a probability level of (0.05).

Results and discussion

The ability of fungi to produce enzymes

The results showed **Tab.1, Fig.1.** that all fungal species were producing enzymes and there was a contrast in the difference between the species of fungi. *A. niger* which was isolated from the air and soil revealed the highest percentage of pectinase on the modified Czapeck Dox Agar by adding pectin with the mean diameters of 6.250mm and 10.5 mm respectively which is statically significant comparing with the control **Tab.1**, the high efficacy of the pectinase obtained from *A. niger* was indicated by another study using solid–state fermentation method compared with submerged fermentation processes which gives a high enzyme productivity and reduces production costs [20]. *A. niger* which was isolated from soil and grown on the Czapeck Dox Agar was supplemented with chitin gave a high productivity of chitinase by 53.87%, while *A. niger* that was isolated from air gave moderate ratio of the chitinase on the same medium. There are a lot of research that demonstrate the high effectiveness of the chitinase produced from *A. niger* using the solid-state fermentation method compared to the submerged fermentation method [21]. The variation in the production of chitinase is mainly due to the genetic structure of fungal isolates which reflects the variation in many physiological and phenotypic characteristics. The ability of all the fungal isolates in this study to produce chitinase could related to its prominent role in the growth of fungi and the branching processes of fungal hyphae and the formation of barriers and the apical growth of fungi [22,23].

Table 1. Percentage of enzyme production by fungal isolates.

	Fungi (Percentage of enzymes production)						
Treatment	*A. niger "air"*	*A. niger "soil"*	*A.lavus*	*P. chrysogenum "0rang"*	*P. chrysogenum "air"*	*P. chrysogenum "soil"*	*F. solani*
Control	*32.500	37.125	41.375	26.625	23.250	23.625	57.250
Pectin	6.250	10.500	30.125	19.625	18.500	17.625	46.250
	**80.77%	71.72%	24.64%	26.29%	20.43%	25.40%	19.21%
Chitin	19.500	17.125	28.375	18.375	18.375	18.125	47.500
	40%	53.87%	24.95%	30.99%	20.97%	23.28%	17.03%
Cellulose	6.125	20.000	30.000	15.500	15.250	13.000	37.125
	81.15%	46.13%	29.14%	41.78%	34.41%	44.97%	35.15%
P-value	0.000	0.000	0.000	0.004	0.001	0.000	0.45

*The mean diameter of 4 replication (mm).
**Percentage.

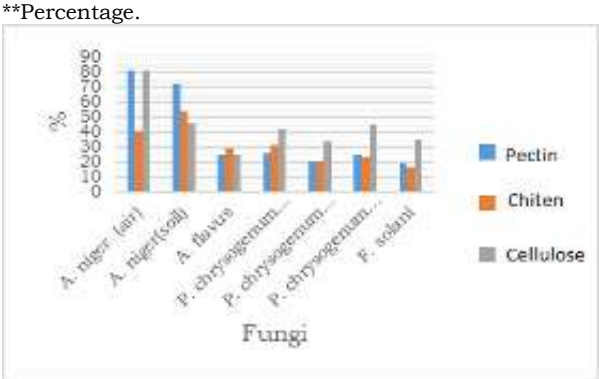

Figure 1. The percentage of enzyme production of fungi.

The production percentage of the studied enzymes by *A. flavus* was very convergent by 29.14%,24.95% and 24.64% with mean diameters 30,28.375 and 30.125mm of cellulose, chitinase and pectinase production respectively with a P-value less than 0.05 which is statically significant **Tab.1,Fig.1**.The study conducted by [24] to demonstrate the ability of 12 strains of fungi to produce cellulase, their finds were similar to the results of this study where they proved that strains of *A. flavus* and *A. niger* revealed the highest production of cellulase under the conditions of solid state fermentation processes compared to the submerged state fermentation.

Regarding *Penicillium chrysogenum* that isolated from the air, soil and orange peel, its results showed a variation in the production rates of the enzymes. Although *P. chrysogenum* is well known for its high productivity of industrial enzymes. The results showed that *P. chrysogenum* production

rates of pectinase and chitinase were convergent and did not exceed 26.29% and 30.99% respectively which is considered a small percentage while *P.chrysogenum.* production rates of cellulose did not exceed 44.97% **Tab.1, Fig1.** A study of [25] to solve the problem of the accumulation of fish waste which causes a pollution in Egypt by utilizing the solid state fermentation processes and adding fish waste as a source for chitin, their study showed that *P. chrysogenum* is the most active fungi to produce chitinase compared to *A. niger, A. flavus* and *F. solani* by using the solid state fermentation method as they were less effective in releasing chitinase which is somewhat in consist with the results of this study [21]. The ability of *Aspergillus* spp. and *Penicillium* spp to release large amounts of extracellular enzymes is the reason to consider their prominent role in food production and this feature was also harnessed for the industrial purposes [26].

Fusarium solani had the lowest ability to produce pectinase and chitinase with a percentage of 19.21% and 17.03% respectively with mean diameters of 46.250 and 47.500mm **Tab.1**, **Fig.1**. The finds of [27] showed that fungi such as *Aspergillus* sp., *Penicillium* sp. were the best isolated producing cellulase with high production capabilities while *F. solani* had the lowest ability of cellulase production.

The ability of bacteria to release enzymes

There was a variation in the ability of bacterial species to produce the studied enzymes **Tab. 2, Fig. 2** whereby the BUS26 isolate, *P .aeruginosa* and *B .mycoides* produced medium convertage proportions of the studied enzymes with p-value less than 0.05, in contrast *B. cereus* produced small quantities of the studied enzymes with P-value greater than 0.05 which has no satirically significance. These results are in agreement with the study of [27] who showed that *P. fluorescens* was more releasing of pectinase and cellulase than *B. subtilis*. All the bacterial isolates was able to produce pectinase in moderate amounts **Tab.2 ,Fig 2**. The study of [28] showed the ability of some microorganisms to release pectinase as they found that bacteria were the most produced of pectinase followed by Actinomycetes and fungi by 65.26%,21.06% and 13.68% respectively. Chitinase was produced in very small proportions except RMI4 isolate which was the most producing of chitinase followed by *P. aeruginosa* and *B. cereus* by a percentage of 34.44%, 29.7% and 25.24% respectively **Tab. 2** . The reference strain *E. meliloti* produced the smallest quantities of pectinase and chitinase at rates of 28.92% and 1.32% respectively **Tab.2, Fig.2**, this shows a similarity to the study of [29].

Table 2. Percentage of enzymes production from bacteria.

Bacteria (Percentage of enzymes production)						
Treatment	*P. aeruginosa*	*B. cereus*	*B. mycoides*	BUS26	RMI4	*E. meliloti*
Control	17.250*	12.625	11.750	11.250	9.500	10.375
Pectin	9.000 **47.83%	8.250 34.66%	6.500 44.68%	5.625 50%	6.000 36.84%	7.375 28.92%
Chitin	12.125 29.71%	14.375 25.14%	9.750 17.02%	9.750 17.02%	7.375 34.44%	9.375 1.32%
Cellulose	12.378 34.06%	10.500 16.83%	3.000 74.47%	3.000 74.47%	3.500 68.89%	6.875 27.63%
P-value	0.000	0.195	0.000	0.000	0.025	0.018

*The mean diameter of 4 replication (mm).
**Percentage.

In addition the results revealed that *B. mycoides,* BUS26 *and* RMI4 were the most releasing isolates of cellulase compared to the other bacterial species which is in agreement with the study of [30,31]. which demonstrated that *Pseudomonas* sp. has the ability to analyse cellulase while *Bacillus* was unable to analyse it. On the top of that Rhizobia is known to produce cellulase because it is the main enzyme that is utilized to analyse the root hairs of plant during the symbiosis processes [32].

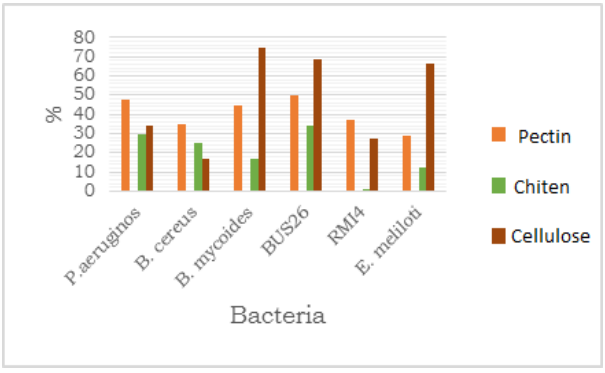

Figure 2. The percentage of enzyme production from bacteria.

Conclusion

The use and exploitation of microorganisms in industry has spread widely, they have utilized in the pharmaceutical, food industries and manufacturing of enzymes and vitamins. The presence of enzymes is an essential characteristics of the organisms living system because enzymes activate and accelerate the physiological reactions in living cells. Microorganisms produce many enzymes that play an important role in breaking down the organic materials and many of these enzymes are made commercially. Recently, there has been an increased reliance on enzymes produced by microorganisms rather than those that are chemically manufactured or extracted from plants; Therefore special attention should be given to the method of purification and extraction development of enzymes from microorganisms. Through the results obtained from this study we confirm that there are some factors that play a

fundamental role in the process of enzymes production from microbes, these factors are concentrated in the selection of the productive strains which must be not pathogenic or toxic. Thus, we recommend that attention has to be given to the selection of the optimal conditions for enzyme production from the appropriate microorganism.

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