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ISOLATION AND IDENTIFICATION OF KERATINASE-PRODUCING BACTERIA

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ABSTRACT

In order to isolate keratinase producing bacteria, the enrichment process using some natural sources including chicken feather, soil, compost and poultry waste digester was carried out. Screening program was conducted at two steps at 30 and 55°C using milk agar plates to detect the proteolytic potency of obtained isolates. The third step of screening programme was carried out using a liquid cultivation medium containing feather as source for nitrogen and carbon source measuring both total soluble protein and free amino acids as indicators for keratinase production at 37°C. The identification tests were also followed for the selected isolates. Different cultivation media were also used for bacterial growth and keratinase production. Obtained results showed that sporeforming bacteria represent 51.7% of the total obtained isolates and about 36.4% of the total bacterial isolates of chicken feather. Data showed also that two isolates of sporeforming bacteria which were most active in keratinase production are belonging to the genus *Bacillus*. Following the protocol of the Bergy's Manual of Systematic Bacteriology, the two isolates showed to be closely related to *B. licheniformis*. To select the most keratinase productive cultivation medium amongest five media used, the values of keratinase activity, total soluble protein, and free amino acids obtained from each medium were also considered.

Keywords : chlicken feather , biodegradation , bacterial keratinase , total soluble proteins free amino acids, , bacterial proteinase, *Bacillus spp.*

INTRODUCTION

The intensive large scale production of animal and poultry in modern slaughter houses have generated enormous wastes disposal problem. Native keratinaceous wastes viz feather, wool, hair, hoof, nails and horn are insoluble protein complexes almost completely resistant to proteolytic enzymes. The common occurrence in mature of microorganisms that readily and/or preferable grow on keratinaceous compounds supported the general believe that certain microorganisms can degrade keratin. Keratinase is one of proteinases, which hydrolyzes all protein substrates including collagen, elastin and feather keratin (Shih, 1993). Several investigators have emphasized the importance of microorganisms in keratinase production and keratin olysis. Scatt (1993) examined Coccid-shape bacteria in the pilar canal of non inflamed hair follicles. The genus *Bacillus* has examined as a keratinase producer (Williams *et al.*, 1990; Lin *et al.*, 1995; Thomas *et al.*, 1995; El-Fadaly, 1996 and El-Fadaly and Zaied, 1999).

The aim of the present investigation is to examine the ability of some bacterial isolates to produce keratin olytic enzyme and associative materials such as proteinase, total soluble protein and free amino acids as well. The identification of the most keratinase producing bacterial isolates was also performed.

MATERIALS AND METHODS

I. Materials:

I.1. Chicken feather (CF):

Chicken feather (CF) samples were collected from private shops of chicken slaughtering, local market of Mansoura City, Dakahlia Governorate, Egypt. Samples were then mixed, washed twice, milled and dried at 60°C to constant weight.

I.2. Cultivation media:

Nitrient agar (NA) was used for microbial isolation at pH 7.2. Milk agar (MA) was used for isolation, counting and screening for proteolytic bacteria at pH 7.2. The same medium was also used for detection of qualitative measurement of proteolytic activity. Sabauraud glucose agar (SGA) was used for isolation of fungi and yeast at pH 5.6. Potato Dextrose agar (PDA) was used for fungi cultivation at pH 5.6. The composition of these media was as described in Oxoid (1982). The basal medium of Hussein *et al.* (1986) was used for isolation of *Streptomyces sp.* at pH 7.0.

1.3. Media for fermentation and keratinase production:

1.3.1. Williams and Shih (1989) medium :

The ingredients (gL^{-1}) of this medium were NH_4CL, 0.5; NaCl, 0.5; K_2HPO_4, 0.3; KH_2PO_4, 0.4; $MgCl_2.6H_2O$, 0.24; yeast extract, 0.1 and ball-milled feathers, 2.0. The final pH was adjusted to 7.5.

1.3.2. Williams *et al.* (1990) medium :

The composition (gL^{-1}) of this medium were NH_4Cl, 0.5; NaCl, 0.5; K_2HPO_4, 0.3; KH_2PO_4, 0.4; $MgCl.6H_20$, 0.1; yeast extract, 0.1. The pH value was adjusted to 7.5. Feather was added to the medium at concentration of 1%.

1.3.3. Lin *et al.* (1995) medium :

The ingredient (gL^{-1}) of this medium were NaCl, 0.5; $MgCl_2.6\ H_2O$, 0.1; $CaCl_2$, 0.06; KH_2PO_4, 0.7; K_2HPO_4, 1.4 and feather, 10. The pH was adjusted to 7.5.

1.3.4. Thomas *et al.* (1995) medium :

The ingredient (gL^{-1}) of this medium were NaCl, 0.5; K_2HPO_4, 0.3; KH_2PO_4, 0.4; $MgCl.6H_2O$, 0.1. The feather meal was replaced with chicken feather in the ratio of 1%.

1.3.5. Basal medium (BM):

The prepared basal salts-chicken feather liquid culture medium was used for fermentation and enzyme production as recommended by El-Fadaly (1996). The ingredients of this medium were (gL^{-1}) potassium chloride, 0.2; ammonium dihydrogen phosphate, 1.0; magnesium sulphate, 0.2 and dried feather, 20. The value of pH was adjusted to 7.0.

II. Methods:

II.1. Bacteriological procedures:

II.1.1. Enrichment for keratinase producing-microorganisms:

The enrichment of feather-degrading microorganisms was done using samples of some natural sources. These samples including chicken feathers (CF), soil (S), compost (C), and poultry waste digester (PWD). One gram of each sample was put in an Erlenmeyer flask containing 50 ml of basal medium supplemented with feather as a selective substance and incubated at 37°C. After 24 hr incubation under static conditions, a volume of 10 ml of the culture was transferred into 40 ml nutrient broth plus 10% skim milk. After an additional 24 hr, 5 ml was inoculated into 45 ml of fresh basal medium containing feather.

II.1.2. Isolation of keratinase producing-microorganisms:

In order to isolate the microorganisms producing keratinase and capable to degrade feather, different serial dilutions after enrichment were made with vigorous shaking. One ml sample of appropriate dilution was then taken and plated onto different specific cultivation media for actinomycetes, fungi, yeast and bacteria. After appropriate period of incubation at 37°C for actinomycetes and bacteria whereas at 28°C for fungi and yeast, single colonies were transferred to the slope agar of specific media. These cultures were repeated again on milk agar medium.

II.1.3. Purification of obtained isolates:

From the preceding step, well separated colonies which showed clear zones were transferred to slants and selected as proteinase (Keratinase) producers. Purification of the isolates was carried out by two cycles of cultivation using the same basal medium as shown in the preceding paragraph followed by single colony isolation after streaking onto milk agar plates. Slope cultures were considered pure when they showed uniform morphological feature by microscopic examination.

II.1.4. Maintenance of isolates:

All selected bacterial isolates were maintained on NA slant at 5°C till demand. Prior to use, the microbial cultures were transferred to NA slant and reincubated again at appropriate temperature (37°C) for 24 hr. This process was repeated twice.

II. 1.5. Identification of selected isolates:

Preliminary identification of two selected isolates was achieved by Gram stain, motility, spore staining and the presence of water soluble pigments. Some of the standard biochemical tests recommended for identification of bacteria in the Bergy's Manual of Systematic Bacteriology (William, 1994) were also followed. Cultivation media used in the tests listed in results and discussion were as described in Oxoid (1982).

II.1.6. Experimentation:

II.1.6.1. Preparation of standard inoculum:

Standard inoculum of each bacterial strain used was prepared by scraping the growth from the surface of nutrient agar slant in the presence of 5 ml of sterilized distilled water with the aid of platinum loop. Aliquot of

desired volume of homogenized bacterial suspension was diluted up to 6 x 10^3 cfu/ml, to be used as a standard inoculum during the experimental work.

II.1.6.2. Working flasks preparation:

Six groups were prepared, each contains four flasks (three replicates and one as control) for each bacterial strain. Fifty ml of basal cultivation medium was dispensed in 250 ml Erlenmeyer flasks, then supplemented with 1% dried feather and autoclaved at 121°C for 20 min after adjusting the pH to 7.2.

II.1.6.3.: Fermentation procedure:

For fermentation process, the autoclaved flasks were then inoculated with appropriate inoculum size of appropriate dilutions of 24 hr old bacterial suspension (6 x 10^3 cfu/ml). The incubation was then carried out under static conditions at 37°C. During 12 days incubation period, one group of the prepared flasks was taken every 3 days as a representative sample. For sample analysis, Keratinase activity (KA, KU/ml), proteinase activity (PA, TU/ml), total soluble peptide content (TSP, ppm), and total free amino acids (FAA, ppm) were measured. Three replicates were analyzed and the mean value was recorded.

II.2. Monitoring of keratinase production:

II.2.1. Keratinase activity measurement (KA):

The activity of Keratinase was measured after Nickerson *et al.* (1963) using pure keratin (K .0253, Sigma Co., USA). A unit of Keratinase activity was defined as that amount of enzyme in one ml of cultural filtrate that produce 1.00 μg protein in 2 hr as a product of Keratin hydrolysis. Bovine serum albumin (BSA) was used as a standard.

II.2.2. Lowry protein estimation (TSP):

Total soluble protein was colorimetrically determined at 750 nm as described by Lowry *et al.* (1951) using Carl Zeiss Jena Spekol 11 colorimeter. Reference curve by using BSA (0.05 - 0.5 ml) was carried out through whole procedure.

II.2.3. Determination of proteolytic enzymes:

II.2.3.1. Qualitative enzyme activity:

The method used for qualitative proteinase assay was similar to that used in testing the antibiotics by diffusion method according to Collins and Lyne (1985). The activity of the proteinase was indicated by measuring the clear zones (mm) surrounding the microbial colonies.

II.2.3.2. Quantitation of proteinase activity (PA):

This assay was adopted using the modified casein digestion method described by Lupin *et al.* (1982). A unit of proteinase activity was defined as that quantity of enzyme, which produced TCA - soluble fragments giving blue color equivalent to 1.0 μg tyrosine under the assay conditions. A tyrosine calibration curve was set up.

II.2.4. Determination of total free amino acids (FAA):

The colorimetric method used by Lee and Takahashi (1966) was adopted. Reference curve was prepared by using glycine (10-100 μg) as a standard amino acid.

II.3. Statistical analysis:

All experimental data were subjected to the statistical analysis by the analysis of variance. The treatment means were compared at 0.05 and 0.01 probability levels using the Least Significant Difference (L.S.D.) method as mentioned by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

I.1. Isolation of keratinase-producing microorganisms:

After the process of enrichment have taken place (Materials and Methods), the isolation of keratinase-producing microorganisms was carried out which followed by the purification steps. Twenty nine isolates of bacteria, in addition to two isolates of fungi, plus one isolate of actinomycetes were obtained. As shown in Table (1), of the twenty-six bacterial isolates, spore forming bacteria producing keratinase showed to be 57.7% of the total bacterial isolates obtained from different environmental sources; chicken feather, soil, compost and poultry waste digester samples. At the same time, spore- formers represent 36.4% of the total bacterial isolates of chicken feather. Meanwhile, 28.60% spore formers was found from the total isolates of soil samples. Interestingly, the keratinase producing spore forming bacteria were 100% obtained in case of compost and poultry waste digester as well. Goktan (1984) found that most keratinase producing bacteria of the genus *Bacillus* which grow at 30-50°C.

Table (1): Shape and number of Keratinolytic microorganisms isolated from different natural sources.

Isolate source	Isolate No.	Isolate shape
Chicken feather (CF)	1, 2, 13, 26 4, 5, 9 6, 11, 12, 14 7, 8	Spore-former Short-rod Coccoid shape Fungi
Soil (S)	15, 17 18, 19 16, 20 3	Spore-former Short-rod Coccoid shape Filamentous shape
Compost (C)	21, 22, 23, 24 25, 27, 10	Spore-former
Poultry waste digester (PWD)	28 - 37	Spore former

Other bacterial groups, such as short rods and coccoid-shaped bacteria which capable to produce keratinase were also obtained from tested environmental samples. In case of chicken feather, short rod-bacteria reached to 23.1%, while 30.8% was found with coccoid-shaped bacteria. For soil samples, the percent of short-rod and coccoid-shaped bacteria was 28.6% of the total isolates for each. On the other hand, two fungal isolates (isolates No. 7 & 8) were obtained from chicken feather samples. By the microscopic examination both isolates showed to be closely related to the genus *Aspergillus*. These represent 15.4% of the total isolates obtained from chicken feather sample. This percent decrease to 6.9% of the total isolates

obtained in this investigation. Moreover, only one isolate (isolate No. 3) is belonging to the group of actinomycetes obtained from soil samples. This isolate represent 14.3% of the total isolates from soil sample, while it is equal to 3.5% of the total isolates obtained in this investigation. Hussein (1989) isolated ten of thermophilic chicken feather lysing actinomycetes from a cattle manure sample. Williams *et al.* (1990) isolated different types of microorganisms which capable to hydrolyze keratin. One of these types was rod-shape bacteria, the other was a coccus, which appeared singly and in chains.

I.2. Screening program of bacterial isolates on milk agar plates:

The ability of degrading chicken feather as a result of keratinase production by obtained isolates was tested by the ability to hydrolyze casein using the milk agar plates. Three different steps of screening were performed. The philosophy of screening was to find keratinolytic enzyme-producing bacteria with growth ability on chicken feather as an environmental waste of poultry industry. The realized programme was as follows:-

Table (2): Proteolytic potency of the bacterial isolates at 30°C.

Isolate source	Isolate No.	Zone diameter (mm) of		Casinolysis		
		Growth (G)	Hydrolysis (H)	H-G	H/G	H/G%
Chicken feather (CF)	4	35	44	9	1.26	125.7
	5	16	21	5	1.31	131.2
	7	28	41	13	1.46	146.4
	9	34	45	11	1.32	132.0
	26	8	33	25	4.13	412.5
	12	12	28	16	2.33	233.3
	13	8	16	8	2	200.0
Soil (S)	3	22	38	16	1.70	172.7
	15	13	29	16	2.23	223.0
	20	10	31	21	3.10	310.0
Compost (C)	21	18	32	14	1.78	177.8
	22	22	39	17	1.77	177.2
	23	60	70	10	1.17	116.7
	24	40	48	8	1.20	120.0
	25	18	22	4	1.20	122.2
	10	18	27	9	1.50	150.0
	27	10	30	20	3.00	300.0
Poultry waste digester (PWD)	28	11	34	23	3.09	309.0
	30	34	36	2	1.05	105.8
	31	7	20	13	2.86	285.7
	32	18	19	1	1.06	105.6
	33	19	41	22	2.16	215.8
	34	10	22	12	2.20	220.0
	35	26	27	1	1.04	103.8
	36	5	19	14	3.80	380.0
	37	23	43	20	1.87	186.9

Primary screening at 30°C:

These experiments were carried out at 30°C (Table 2). The obtained isolates showed differences in their abilities towards the action against casein

digestion. This action grouped to high potentiality (Hydrolysis over growth %) above 300% of H/G%, moderate activity, which give values between 200-300 and low being between 100-200. At 30°C, chicken feather (CF) sample showed only one isolate (CF-26) gave high value of H/G being 412.5% and isolate No. 20 from soil (S) sample gave 310.0%. For compost (C), the isolate No. 27 showed to be higher being 300.0%, while isolate No. 28 and isolate No. 36 from poultry waste digester (PWD) gave higher values of H/G% being 309 and 380, respectively. Lin *et al.* (1996) used casein hydrolysis to measure keratinase activity either in the free or in immobilized form.

Second screening at 55°C:

These experiments were performed at 55°C. Data listed in Table (3) show that the isolates obtained from chicken feather samples gave two isolates with moderate activity against milk casein and four isolates grouped as low activity. With soil sample, only one isolate gave 300% as high potent isolate. Two isolates were moderate and one was as low in case of compost. For poultry waste digester, only one isolate was high potent giving 400% of H/G values namely PWD-28, while the rest of isolates were of low activity.

Table (3): Proteolytic activity of the bacterial isolates at 55°C.

Isolate source	Isolate No.	Zone diameter (mm) of		Casinolysis		
		Growth (G)	Hydrolysis (H)	H-G	H/G	H/G%
Chicken feather (CF)	2	13	18	5	1.38	138.5
	11	19	30	11	1.76	176.4
	26	11	28	17	2.55	254.5
	12	22	41	19	1.86	186.4
	13	15	32	17	2.13	213.3
	14	11	16	5	1.45	145.5
Soil (S)	3	7	21	14	3.00	300.0
	15	12	20	8	1.67	166.7
	19	14	17	3	1.21	121.4
	20	6	10	4	1.67	166.7
Compost (C)	21	18	32	14	1.78	177.8
	22	17	34	17	2.00	200.0
	27	12	33	21	2.75	275.0
Poultry waste digester (PWD)	28	7	28	21	4.00	400.0
	29	16	20	4	1.25	125.0
	31	25	43	18	1.72	172.0
	32	13	19	6	1.46	146.2
	34	34	50	16	1.47	147.1
	36	36	62	26	1.72	172.2
	37	7	11	4	1.57	157.1

Third screening:

In order to assure the efficiency of bacterial isolates obtained in Tables (2 and 3), Some isolates of high and moderate potentialities were taken further to be examined using liquid medium used by Lin *et al.* (1995) at

37°C and obtained results are listed in Table (4). During the course of fermentation, both total soluble proteins (TSP) and free amino acids (FAA) were measured. It could be seen that isolate No. CF-26 and PWD-28 are the most active in keratinase production. The obtained values of TSP were 0.170 and 0.145 ppm after the third day of fermentation for two isolates, respectively. The FAA values were 1.258 and 1.144 ppm at the same time for the two isolates, respectively. So both isolate No. CF-26 and No. PWD-28 were selected for further studies.

I.3. Identification of the two selected isolates:

The microscopic examination of obtained pure cultures showed that these two isolates could be placed into one group of sporeforming cells. Recorded results of morphological and biochemical examinations are shown in Table (5). According to the Bergy's Manual of Systematic Bacteriology (William, 1994), the position of these two isolates is belonging to the genus *Bacillus*. In addition, upon this standard identification key, it could be mentioned that both isolates; No. 2 (CF-26) and No. 5 (PWD-28) are falling within *Bacillus licheniformis* group. Furthermore, throughout the identification steps, *B. licheniformis*, 1320, provided by Fermentation Service Unit, GBF, Braunshweig, Germany, was used as a reference strain. El-Fadaly and Zaied (1997) isolated and identified three bacteria to be *Bacillus cereus, Micrococcus luteus* and *Bacillus licheniformis* from chicken feather. Williams *et al.* (1990) succeeded to isolate a feather-degrading bacterium from a poultry waste digester after adaptation to grow with feather. They identified this isolate as *Bacillus licheniformis* PWD-1. Lin *et al.* (1995) used identified strain of *Bacillus licheniformis* PWD-1 (ATCC53757) in producing keratinase as a result of growth on whole feather. El-Fadaly (1996) produced keratinolytic enzymes from imported bacterial strains namely, *Bacillus licheniformis*, 1316; *Bacillus licheniformis*, 1320; *Bacillus mesentericus*, 1320 and *Bacillus megaterium*, 1310.

I.4. Selection of the most efficient cultivation medium:

The two tested bacterial strains, *Bacillus licheniformis,* CF-26 and *Bacillus licheniformis,* PWD-28 were examined for keratinase production. After 10 days, growth on different five cultivation basal media, M_1, M_2, M_3, M_4 and M_5, keratinase activity, KU/ml of cultural filtrate and associative products such as total soluble proteins (TSP), ppm and free amino acids (FAA), ppm were determined.

Table (4): Screening of obtained microbial isolates for keratinase production from feather wastes*.

Examined	Total soluble protein (ppm) at incubation period (day) of				F-	L.S.D.		Free amino acids (ppm) at incubation period (day) of				F-	L.S.D.	
Isolate	3	6	9	12	test	0.05	0.01	3	6	9	12	test	0.05	0.01
S-3	0.067	0.124	0.066	0.059	**	0.009	0.013	1.079	0.848	0.847	0.225	**	0.020	0.030
CF-12	0.201	0.067	0.084	0.069	**	0.008	0.013	1.072	1.141	0.868	0.248	**	0.020	0.020
CF-26	0.170	0.076	0.074	0.069	**	0.018	0.001	1.258	0.862	0.859	0.227	**	0.016	0.024
S-15	0.107	0.087	0.057	0.034	**	0.02	0.030	1.128	0.880	0.969	0.233	**	0.040	0.050
C-27	0.185	0.086	0.066	0.053	**	0.018	0.030	1.123	1.078	0.653	0.172	**	0.020	0.030
Pwd-28	0.145	0.081	0.095	0.090	**	0.003	0.005	1.144	1.163	1.690	0.250	**	0.400	0.600
Pwd-31	0.019	0.053	0.057	0.059	**	0.008	0.013	1.082	0.880	0.714	0.199	**	0.015	0.020
Pwd-36	0.156	0.086	0.102	0.094	NS	0.00	0.00	1.164	1.153	0.935	0.316	**	0.030	0.050
F test	**	**	**	**				**	**	**	**			
L.S.D.														
0.05	0.011	0.007	0.005	1.883				1.04	0.070	0.030	0.0009			
0.01	0.016	0.011	0.007	2.739				1.52	0.011	0.044	0.0010			

* The medium of Lin *et al.* (1995) was used.

Table (5): Morphological and biochemical characteristics of the two selected bacterial isolates.

Test	CF-26	PWD-28
Motility	+	+
Spore formation	+	+
Capsule formation	-	-
Gram reaction	+	+
Cell dimensions (μm)	0.7 x 2.2	0.5 x 1.4
Tolerance of NaCl (%)		
2.0	+	+
5.0	+	+
7.0	+	+
10.0	+	+
Temperature (°C)		
10	+	+
30	+	+
37	+	+
50	+	+
55	+	+
65	+	+
Indole production	-	-
VP	+	+
MR	-	+
Gelatin liquefction	+	+
Casein hydrolysis	+	+
Starch hydrolysis	+	-
Citrate utilization	+	+
Propionate utilization	-	+
Catalase production	+	+
Urease	-	-
DNA ase	-	-
Lipase	+	+
Gas from glucose	-	-
Water soluble pigment	-	-
Growth in N. broth at pH		
5.7	+	+
6.8	+	+
Acid from		
Glucose	+	+
Arabinose	+	+
Xylose	+	+
Mannitol	+	+
Sucrose	+	+
Lactose	-	+
$NO_3 \rightarrow NO_2$	+	+

Table (6): Screening for productive cultivation medium supplemented with chicken feather by the two selected bacterial strains after 10 days of keratinase fermentation.

Basal media supplemented with chicken feather	Tested bacterial strains					
	Bacillus licheniformis CF-26			*Bacillus licheniformis* PWD-28		
	KA (KU/ml)	TSP (ppm)	FAA (ppm)	KA (KU/ml)	TSP (ppm)	FAA (ppm)
M_1	0.64	0.84	0.65	0.820	0.45	0.83
M_2	0.47	0.64	0.62	0.580	0.42	0.46
M_3	0.49	0.68	0.53	0.590	0.63	0.63
M_4	0.45	0.82	0.47	1.050	0.42	0.73
M_5	0.83	1.17	0.83	1.303	0.87	1.03
F test	**	**	**	**	**	**
LSD 0.01	0.3	0.74	0.26	1.50	0.19	0.20
LSD 0.05	0.21	0.51	0.18	1.01	0.13	0.14

M_1: Williams & Shih (1989), M_2: Williams *et al.* (1990 M_3: Lin *et al.* (1995)
M_4: Thomas *et al.* (1995) M_5: El-Fadaly (1996). KU: Keratinase unit
TSP: Total soluble protein FAA: Free amino acids

Recorded data in Table (6) exhibited the superiority of the medium No. 5 (M_5) over the other media regarding the three parameters considered, KU, TSP and FFA. These results also indicate that the order of the productivity of these media was M_5, M_1 followed by M_3, M_2 and M_4 regarding keratinase production in case of *B. licheniformis*, CF-26. In case of *B. licheniformis*, PWD-28, the order of productive media was M_5, M_4, M_1 followed by either M_3 or M_2 for keratinase production. El-Fadaly *et al.* (1996) found that cultivation medium No. 5 (M_5) was better than M_1 when using poultry feather as carbon and nitrogen sources. They obtained 78.7 and 85.2 ppm TSP from feather in M_5 by *Fusarium semitectum* and *Alternaria solani*. They also obtained 65.5 and 85.2 ppm TSP from M_5 by *B. licheniformis*, CF-7 and *B. cereus*, CF-3, respectively. Hussein *et al.* (1986) found that keratinases were detected in broth after 24 hr of incubation. They added that these enzymes varied with the variants of the basal salts feather medium used. They recorded the highest content of keratinases with a maximum level on the 11th day of fermentation.

Moreover, the statistical analysis applied proved the significance between the cultivation media as seen in the same Table (6). These results also proved that these bacterial strains can use the feather as a sole source of energy as well as carbon and nitrogen. The results obtained by Elmayergi and Smith (1971) suggested that during the first 5 days of incubation reflect the consumption and depletion of glucose that probably little degradation of keratin occurs after the 5th day. The selection of mineral media was based on the economic factor as well as to avoid the late of enzyme production. From the previous data, the medium M_5, which used by El-Fadaly (1996) proved to the best for the two tested strains.

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عزل وتعريف البكتريا المنتجة لإنزيم الكيراتنيز

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لعزل البكتريا المنتجة لإنزيم الكيراتنيز قد تم عمل وسط إنماء بإستخدام بعض المصادر الطبيعية مثل ريش الدجاج ، تربة زراعية ، سماد صناعى والسائل المتخلف عن تنظيف الدجاج. تم أيضا عمل برنامج تصفية على ثلاث مراحل . المرحلة الأولى كانت على ٣٠ °م والثانية على ٥٥ °م وكلاهما تم بإستخدام بيئة أجار اللبن وذلك لتحديد قدرة العزلات على إنتاج الإنزيم المحلل للبروتين. أما الخطوة الثالثة فقد تمت على ٣٧ °م بإستخدام بيئة سائلة محتوية على ريش دجاج (مصدر كيراتين) كمادة تفاعل ومصدر للكربون والنتروجين. وفى الخطوة الثالثة من برنامج التصفية تم قياس البروتينات الذائبة الكلية والأحماض الأمينية الحرة كدليل على إنتاج الكيراتنيز على ٣٧ °م.

بينت النتائج أن نسبة العصويات الطويلة المتجرثمة كانت ٥١,٧% من إجمالى العزلات الناتجة وحوالى ٣٦,٤% من إجمالى العزلات الناتجة من ريش الدجاج.وأوضحت النتائج أيضا أن عزلتين من العصويات المتجرثمة والتى بينت كفاءة عالية فى إنتاج الكيراتنيز كانت تابعة لجنس *Bacillus*. بإتباع بروتوكول برجى لتقسيم البكتريا أوضحت النتائج أن العزلتين قريبتا الشبه بميكروب *B. licheniformis*. كذلك تم إستخدام خمس بيئات غذائية بغرض تحديد أفضلها لإنتاج الإنزيم بواسطة العزلتين المختارتين وذلك عن طريق قياس النشاط الإنزيمى للكيراتنيز والبروتينات الذائبة الكلية والأحماض الأمينية الحرة والناتجة فى كل بيئة غذائية.