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Microbial Degradation of Native Keratin in Batch Fermentation

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Abstract

Feather fermentation was carried out using different bacterial strains of the genera *Bacillus* and *Micrococcus* and their transconjugants as well. During the time course of fermentation, keratin biodegradation was monitored by measuring both activities of keraitnase and proteinase as well as the content of total free amino acids. Results of the parental strains proved that *B. licheniformis* CF_7 was the most producing strain in keratinase and free amino acids after 15 days of fermentation being 206.7 KU/ml and 73.3 μg/ml cultural filtrate, respectively. *B. subtilis* IBF_6 was in the first in proteinase production giving 148.3 TU/ml cultural filtrate. Experimental data of transconjugants illustrated that all of them gave higher yield than their mid parents after the 3rd day of fermentation. The percent of yield reached to 274, 335, and 269 percent for keratinase, proteinase and free amino acids as well. The same trend of these results was found after calculating the relative increase of these parameters. Values of estimated the correlation coefficient (r) suggesting that the increase in free amino acids is a-good indicator for the action of both keratinase and proteinase on keratin.

Introduction

Microorganisms are capable of utilizing the organic matter of the environmental wastes as a source of their energy required for growth as well as nutrients for the synthesis of cell biomass. The involvement of microorganisms in recycling of wastes was emphasized. The microbial degradation of insoluble macro molecules like keratin depends on the secretion of extracellular enzymes with the ability to act on compact substrate surfaces (Fredrich and Antrakian, 1996).

A number of microorganisms have been reported to utilize keratin such as *Bacillus* strains (Williams *et al*., 1990), some species of saprophytic and parasitic fungi (Rajak *et al*., 1992) and a few actinomycetes (Bockle *et al*., 1995). The production of microbial proteins, peptides, amino acids and enzymes from agricultural and industrial wastes have received great attention. Keratinase is one of these enzymes which could play an important part in biotechnological applications such as enzymatic improvement of feather meal and leather industry. Keratinase could also used in the production of amino acids and peptides from high molecular weight substrates. This feature can also provide potential applications in feed technology as well as in waste management. This investigation deals with the comparison between the effectiveness of locally isolated bacterial strains and their transconjugants on the yield of the fermentation products of chicken feather. The correlation coefficient between these products and the relative increase were also calculated.

Materials and Methods

Bacterial strains: Three bacterial strains belong to the genus *Micrococcus* (M) and three strains of the genus *Bacillus* (B), and their transconjugants listed below (Table 1) were used in this investigation.

Table 1: Bacterial strains and their transconjugants used in feather degradation

Parental strains	Transconjugants	No.
I. *Micrococcus halobius* IBF_1		
Bacillus cereus CF_3	$IBF_1 \times IBF_6$	I.1
Bacillus subtilis IBF_6	$IBF_1 \times CF_7$	I.2
Bacillus licheniformis CF_7	$IBF_1 \times CF_3$	I.3
II. *Micrococcus halobius* IBF_2		
B. cereus CF_3	$IBF_2 \times CF_3$	II.1
B. subtilis IBF_6	$IBF_2 \times IBF_6$	II.2
B. licheniformis CF_7	$IBF_2 \times CF_7$	II.3
III. *Micrococcus lylae* TFF_5		
B. cereus CF_3	$TFF_5 \times CF_3$	III.1
B. subtilis IBF_6	$TFF_5 \times IBF_6$	III.2
B. licheniformis CF_7	$TFF_5 \times CF_7$	III.3

These strains were previously isolated and identified as well as their transconjugants were also obtained (El-Fadaly and Zaied, 1997).

Feather waste: Chicken feathers were collected from the local market of Mansoura City, washed twice, milled and dried at 75°C for 72 hr.

Cultivation medium and preparation: The basal salts-chicken feather medium (pH 7.5) used by El-Fadaly (1996) were employed for cultivation. Six groups of experimental flasks, each contains seven flasks (six for tested strains and one as control) for parent bacterial strains, were prepared. Ten flasks were also prepared for bacterial transconjugants of each group. Fifty ml of the fermentation medium was dispensed in 250 ml Erlenmeyer flasks and supplemented

with 2 percent (w/v) dried feather, then autoclaved at 21°C for 20 min.

Fermentation procedure: For fermentation process, the autoclaved flasks were then inoculated with 6 percent inoculum size of appropriate dilution of 24 hr. old bacterial culture ($4x10^3$ cells/ml). The incubation was then carried out at 30°C under static conditions. During 15 days incubation, one group of the prepared flasks was taken every 3 days as a representative sample. For sample analysis, keratinase activity (K), proteinase activity (P), and free amino acids content (FAA) were monitored. Three replicates were analyzed and the mean value was recorded.

Analysis of fermentation products:

Keratinase activity measurement (K): The activity of keratinase was assayed according to the method described by Nickerson *et al*. (1963). Pure keratin from Sigma (k. 0253) was used in this study. 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 1 hr as a product of keratin hydrolysis.

Assay of proteinase activity (P): Proteinase activity was measured by the casein digestion method as mentioned by Lupin *et al*. (1982). Tyrosine standard was used in this study. A unit of proteinase activity was defined as that quantity of enzyme which produce TCA-soluble fragments giving blue colour equivalent to 0.5 μg tyrosine under the assay conditions.

Estimation of free amino acids (FAA): FAA content of the cultural filtrate was estimated by the method of Lee and Takahashi (1966). Glycine was used as a standard.

Statistical analysis: The statistical analysis of the experimental data was performed using the analysis of variance according to Snedecor (1980). Least significant differences (LSD) was used to compare between the mean values.

Results and Discussion

Measured values of fermentation end products: *B. licheniformis* CF_7 showed to be the most efficient bacterium in feather degradation after 15 days fermentation, in respect to keratinase activity giving 206.7 KU/ml cultural filtrate as shown in Table 2. After the same time, *B. subtilis* IBF_6 comes in the second order being 193.3 KU/ml cultural filtrate followed by *M. lylae* TFF_5 while 145.0 KU/ml was produced by *B. cereus* CF_3. In contrast, both *M. halobius* IBF_2 and *M. halobius* IBF_1 were in the last after 15 days of incubation since they gave 116.0 and 108.3 KU/ml cultural filtrate, respectively.

Transconjugants obtained after mating were also examined in order to compare their efficiency in feather degradation with their parent bacterial strains as can be seen in Table 2. Transconjugant III.2 (TFF_5, x IBF_6) exhibited the most values of keratinase activity than the others being 183.3 KU/m cultural filtrate while 173.3 KU/ml was obtained by transconjugant II.2 (IBF_2 x IBF_6).

In spite of this, data exhibited that culture III.2 gave little value of enzyme activity than the parent IBF_6 by 10 KU/m but higher value than the other parent TFF_5 by 3 unit of keratinase activity. For the other culture II.2, it produced little values than the parent IBF_6 by about 20 unit of keratinase activity but more than the other parent by equal to 33 percent. Keratinase from *B. licheniformis* has been reported by Lin *et al*. (1995, 1996).

Table 3 show that the bacterium *B. subtilis* IBF_6 was in the first order regarding protein activity produced as a results of feather degradation giving 148.3 TU/ml cultural filtrate. *E. licheniformis* CF_7 comes next being produced 115.0 TU/m cultural filtrate. The culture of *M. lylae* TFF_5 comes in the last since it gives 113.3 proteinase unit per ml of the cultural filtrate. Transconjugant II.1 (IBF_2 x CF_3) exhibite highest enzyme activity, 76.0 TU/ml being more efficient feather degradation than the others. Even so, it gives low value than its parents since the different are 16.7 and 15. TU/ml related to *B. cereus* CF_3 and *M. halobius* IBF, respectively. Culture III.1 (TFF_5 x CF_3) gave little value of proteinase activity being 71.7 TU/ml. This value is less than its two parents by equal to 22.8 and 36.7 percent related to *B. cereus* CF_3 and *M. lylae* TFF_5, respectively. The combination of microbial keratinase and proteinase was also found by *Streptomyces pactum* (Bockle *et al*., 1995).

Table 4 shows that *B. licheniformis* CF_7 is a potent strain in producing of FAA giving 73.3 μg/ml cultural filtrated followed by *B. cereus* CF_3 which gave 72.3 μg/ml. While both *M. lylae* TFF_5 and *B. subtilis* IBF_6 come to be similar their potency giving 71.7 and 71.3 μg/ml, respective. Furthermore, the culture I.3 (IBF_1 x CF_7) and II.3 (IBF_2 x CF_7 exhibited also the same values in their capabilities in feath degradation being 68.0 and 68.7 μg/ml, respectively. The production of both proteinase and amino acids by the gene *Bacillus* showed to be attractive in the industrial field as well as in the field of environmental biotechnology that able to degrade native keratin feathers as reported Williams *et al*. (1990), Lin *et al*. (1992) and Takami *et al*. (1992).

Evaluation of the transconjugants vigour per cent of yield: On the basis of the values of mid parents, the percent the end products (yield) was calculated. Better yields keratinase activity were obtained by all test transconjugants after 3 days of fermentation Table 5. Culture I.3 (IBF_1 x CF_2) showed to be the acting transconjugant amongst the others giving 274.3 percent followed by culture II.3 ($1BF_2$ x CF_7), since it produced 265,2 percent of keratinase unit. Transconjugant I.2 (IBF_1 x IBF_6) and II.1 (IBF_2 x CF_3) seems to be similar in the efficiency while the culture II.2 ($1BF_2$ x IBF_6) comes last because it gave the lowest percent of keratinase

Table 2: Time course of keratinase (KU/ml) production during feather fermentation by tested bacterial strains and their transconjugants.

Strain No.	Fermentation period (day)					L.S.D	
	3	6	9	12	15	0.05	0.01
TBF_1	0.0	50.0	66.67	86.67	108.33**	3.44	5.01
CF_3	50.67	70.0	95.0	110.0	145.00**	7.12	10.36
I. 1 IBF_1xCF_3	40.67	54.67	64.00	81.33	85.00**	2.62	3.81
IBF_1	0.0	50.0	66.67	86.67	108.33**	3.44	5.01
IBF_6	70.0	106.67	133.33	170.0	193.33**	6.21	9.03
I. 2 IBF_1xIBF_6	77.33	86.67	123.33	146.67	150.00**	14.13	20.55
IBF_1	0.0	50.0	66.67	86.67	108.33**	3.44	5.01
CF_7	58.33	93.33	136.67	183.33	206.67**	8.95	13.01
I. 3 IBF_1xCF_7	80.00	96.00	136.00	160.00	163.33**	7.73	11.25
TBF_2	0.0	45.0	73.33	93.33	116.00**	5.99	8.72
CF_3	50.67	70.0	95.0	110.0	145.0**	7.12	10.36
II. 1 IBF_2xCF_3	56.00	75.00	106.00	138.33	143.33**	9.84	14.32
IBF_2	0.0	45.0	73.33	93.33	116.00**	5.99	8.72
IBF_6	70.0	106.67	133.33	170.0	193.33**	6.21	9.03
II. 2 IBF_2xIBF_6	73.33	84.67	121.33	168.00	173.33**	16.63	24.18
IBF_2	0.0	45.0	73.33	93.33	116.00**	5.99	8.72
CF_2	58.33	93.33	136.67	183.33	206.67**	8.95	13.01
II. 3 IBF_2xCF_7	77.33	91.67	126.67	163.33	168.33**	7.02	10.21
IFF_5	61.67	91.67	123.33	151.67	180.00**	4.87	7.08
CF_3	50.67.	70.0	95.0	1 10.0	145.00**	7.12	10.36
III. 1 TFF_5xCF_3	68.33	82.67	120.0	160.0	163.33**	10.71	15.57
TFF_5	61.67	91.67	123.33	151.67	180.0**	4.87	7.08
IBF_6	70.00	106.67	133.33	170.0	193.33**	6.21	9.03
III. 2 TFF_5xIBF_6	78.33	88.33	133.33	179.00	183.33**	16.16	24.23
TFF_5	61.67	91.67	123.33	151.67	180.0**	4.87	7.08
CF_7	58.33	93.33	136.67	183.33	206.67**	8.95	13.01
III. 3 TFF_5xCF_7	78.33	90.00	116.67	143.33	156.67**	14.93	21.71

**Significant at 0.01 probability level

being 209.5 KU/ml cultural filtrate of its mid parent. The rest of tested transconjugants have given little percent of yield than those mentioned above but, however, they are better than their parental strains. Results illustrated also that, the yield of transconjugants in other incubation period i.e., 6, 9, 12 and 15 days gave low per cent of yield than the parent strains in the case of the culture I.1 (IBF_1 x CF_3) and III.3 (TFF_5 x CF_7). On the other hand, the culture I.2 (IBF_1 x IBF_6) gave low value than its parents only after the 15th day of incubation produced 99.5 percent. The culture gave also low values after the 6th and 15th day of fermentation being 89.1 and 98.2 percent, respectively. Furthermore, the statistical analysis proved significant differences between all fermentation times which applied as well as between the different transconjuants tested as shown in the same Table. Least significant differences was also calculated to compare the mean values of all treatments.

Proteinase yield was highest in the case of transconjugant I.3 (IBF_1 x CF_7) which produced 335.4 per cent of its mid parents (Table 6). The culture II.3 (IBF_2 x CF_7) produced 277.4 per cent, while culture II.1 (IBF, x CF_3), I.2 (IBF_1 x IBF_6) and II.2 (IBF_2 x IBF_6) come next in their order. In addition, the calculated results in Table 6 exhibited obvious decrease in proteinase yield with the increase of fermentation period. The production of proteoiytic enzyme together with keratinase activity in the cultural filtrate suggesting that the microbial action on feather keratin

Table 3: Detection of feather biodegradation by bacterial strains and their transconjugants by means of proteinase production, TU/ml

Strain No.	Fermentation period (day)					L.S.D	
	3	6	9	12	15	0.05	0.01
$ITBF_1$	0.0	33.33	50.00	63.33	76.67**	7.53	10.95
CF_3	25.33	40.67	51.33	77.00	92.67**	2.82	4.10
I. 1 IBF_1xCF_3	20.67	26.67	32.67	39.33	44.67**	2.28	3.32
IBF_1	0.0	33.33	50.00	63.33	76.67**	7.53	10.95
IBF_6	24.67	46.67	71.67	120.0	148.33**	3.92	5.70
I. 2 IBF_1xIBF_6	29.33	34.00	38.67	46.67	48.67**	3.30	4.80
IBF_1	00.00	33.33	50.00	63.33	76.67**	7.53	10.95
CF_2	20.67	37.00	51.33	83.33	115.00**	6.68	9.71
I. 3 IBF_1xCF_7	34.67	40.67	49.33	58.67	60.67**	3.51	5.11
TBF_2	0.0	32.00	42.67	63.33	91.67**	3.09	4.49
CF_3	25.33	40.67	51.33	77.00	92.67**	2.82	4.10
II. 1 IBF_2xCF_3	30.67	37.33	52.00	72.67	76.00**	5.70	8.29
IBF_2	00.00	32.00	42.67	63.33	91.67**	3.09	4.49
IBF_6	24.67	46.67	71.67	120.0	148.33**	3.92	5.70
II. 2 IBF_2x1BF_6	27.33	36.67	44.00	54.67	60.67**	2.84	4.13
IBF_2	0.0	32.00	42.67	63.33	91.67**	3.09	4.49
CF_7	20.67	37.0	51.33	83.33	115.00**	6.68	9.71
II. 3 IBF_2xCF_7	28.67	36.00	45.00	63.33	63.33**	8.33	12.12
TFF_1	23.33	41.33	63.33	91.67	113.33**	7.01	10.20
CF_3	25.33	40.67	51.33	77.00	92.67**	2.82	4.10
III. 1 TFF_5xCF_3	37.33	47.33	56.00	68.67	71.67**	3.34	4.86
TFF_5	23.33	41.33	63.33	91.67	113.33**	7.01	10.20
IBF_6	24.67	46.67	71.67	120.0	148.33**	3.92	5.70
III. 2 IFF_5xIBF_6	31.00	38.67	45.33	54.67	61.67**	2.58	3.75
TFF_5	23.33	41.33	63.33	91.67	113.33**	7.01	10.20
CF_7	20.67	37.00	51.33	83.33	115.00**	6.68	9.71
III. 3 TFF_5xCF_7	27.00	38.67	48.00	60.67	66.00**	4.60	6.69

**Significant at 0.01 probability level

involved the production of adaptive enzymes (Elmayergi and Smith, 1971).

The same trend of results was found for the yield of free amino acids as illustrated in Table 7. The culture I.3 (IBF_1 x CF_7) and 11.3 (IBF_2 x CF_7) showed to be the most potent cultures because they gave 269.5 and 247.8 percent, respectively. Again both cultures I.2 (IBF_1 x IBF_6) and II.2 (IBF_1 x IBF_6) are identical in their percent of the yield of free amino acid of their mid parents followed by culture II.1 (IBF_1 x CF_3) which gave 206.3 percent. Williams and Shih (1989) noted that there is a possibility to produce free amino acids from feather by microorganisms isolated from waste digester grown on chicken feather containing medium.

Relative increase: Data proved that all transconjugants presented their high relative increase at the third day of fermentation. The superiority of the transconjugant I.3 (IBF_1 x CF_3) was found again over the others giving +174.3 percent. Transconjugant II.3 (IBF_2 x CF_7) comes next since it gave +165.2 per cent over its mid parents followed by culture II.1 (IBF_2 x CF_3) which showed to be similar to that of culture I.2 (IBF_2 x IBF_6) being about 120 percent after the third day of fermentation. The order of relative increase is transconjugant II.2, I.1, III.3, III.1 while III.2 comes in the last being +18.98 percent (Table 8). In addition, F-test and LSD analysis were applied proving high significant differences between all the tested cultures and all intervals of fermentation as well.

Table 4: Production of free amino acids (μg ml^{-1}) during the course of feather fermentation using bacterial strains and their transconjugants

Strain No.	Fermentation period (day)					L.S.D	
	3	6	9	12	15	0.05	0.01
IBF_1	0.0	13.33	22.33	29.33	30.67**	4.05	5.89
CF_3	21.33	28.67	38.67	53.33	72.33**	3.10	4.51
I. 1 IBF_1xCF_3	16.00	20.67	27.00	31.33	32.67**	2.94	4.28
IBF_1	0.0	13.33	22.33	29.33	30.67**	4.05	5.89
IBF_5	26.67	39.33	50.67	62.33	71.33**	3.84	5.59
I. 2 IBF_1xIBF_6	30.67	38.67	50.67	50.67	59.33**	2.12	3.09
IBF_1	0.0	13.33	22.33	29.33	30.67**	4.05	5.89
CF_7	30.67	37.33	45.33	68.00	3.33**	4.12	5.99
I. 3 IBF_1xCF_7	41.33	50.67	58.67	67.0	68.00**	1.92	2.79
IBF_2	0.0	17.33	25.33	34.67	37.33**	2.28	3.32
CF_3	21.33	28.67	38.67	53.33	72.33**	3.10	4.51
II. 1 IBF_2xCF_3	22.0	27.33	32.67	38.00	40.67**	2.01	2.92
IBF_2	0.0	17.33	25.33	34.67	37.33**	2.28	3.32
IBF_6	26.67	39.33	50.67	62.33	71.33**	3.84	5.59
II. 2 IBF_2xIBF_6	30.67	36.00	41.33	51.33	54.00**	3.00	4.37
IBF_2	0.0	17.33	25.33	34.67	37.33**	2.28	3.32
CF_7	30.67	37.33	45.33	68.00	73.33**	4.12	5.99
II. 3 IBF_2xCF_7	38.00	50.33	60.00	67.00	68.67**	3.54	5.14
TFF_5	14.67	30.67	40.00	50.67	71.67**	4.49	6.53
CF_3	21.33	28.67	38.67	53.33	72.33**	3.10	4.51
III. 1 TFF_5xCF_3	26.00	30.67	37.33	41.33	43.33**	4.12	5.99
TFF_5	14.67	30.67	40.00	50.67	71.67**	4.49	6.53
IBF_6	26.67	39.33	50.67	62.33	71.33**	3.84	5.59
III. 2 TFF_5xIBF_6	34.00	41.33	49.33	58.00	61.67**	3.39	4.93
TFF_5	14.67	30.67	40.00	50.67	71.67**	4.49	6.53
CF_7	30.67	37.33	45.33	68.00	73.33**	4.12	5.99
III. 3 TFF_5xCF_7	19.33	23.33	29.33	36.00	37.33**	3.08	4.48

**Significant at 0.01 probability level

Table 5: Yield (%) of keratinase (KU/ml) obtained from feather biohydrolysis by different transconiugants of tested bacterial

Transconjugant	Fermentation period (day)					L.S.D	
	3	6	9	12	15	0.05	0.01
I. 1. IBF_1xCF_3	160.51	91.11	79.17	82.71	67.11**	5.98	8.70
I. 2. IBF_1xIBF_6	220.95	110.63	123.33	114.28	99.45**	15.16	22.05
I. 3. IBF_1xCF_7	274.3	133.96	132.78	118.52	103.70**	7.88	11.47
II. 1. IBF_2xCF_3	221.04	130.43	125.94	136.07	109.83**	16.44	23.91
II. 2. IBF_2xIBF_6	209.52	111.64	117.42	127.85	112.07**	18.0	26.18
II. 3. IBF_2xCF_7	265.16	132.53	120.63	118.07	104.34**	11,95	17.38
III. 1. TFF_5xCF_3	121.65	102.26	109.93	122.29	100.51**	11.94	17.37
III. 2. TFF_5xIBF_6	118.98	89.07	103.90	111.29	98.22**	13.73	19.97
III. 3. TFF_5xCF_7	130.56	97.30	89.74	85.57	80.73**	13.17	19.15
F-test	**	**	**	* *	**		
L.S.D. 0.05	10.44	8.46	10.60	4.39	4.21		
0.01	14.06	11.39	14.27	5.90	5.67		

**Significant at 0.01 probability level

Table 6: Yield (%) of proteinase obtained from feather hydrolysis by different tr unsi;oniugants of tested bacteria

Transconjugant	Fermentation period (day)					L.S.D	
	3	6	9	12	15	0.05	0.01
I. 1. IBF_1xCF_7	163.18	72.07	64.48	56.06	52.75**	7.32	10.64
I. 2. IBF_1xIBF_6	237.80	93.33	63.56	50.91	43.26**	13.99	20.35
I. 3. IBF_1xCF_7	335.43	115.64	97.37	80.00	63.30**	19.57	28.46
II. 1. IBF_2xCF_3	242.13	102.74	110.64	103.56	82.46**	17.33	25.21
II. 2. IBF_2xIBF_6	221.59	93.22	76.96	59.64	50.56**	14.57	21.20
II. 3. IBF_2xCF_2	277.37	104.35	95.75	86.37	61.29**	26.47	38.50
III. 1. TFF_5xIBF_6	153.44	115.44	97.68	81.42	69.58**	7.38	10.73
III. 2. TFF_5xCF_3	129.17	87.88	67.16	51.65	47.13**	11.36	16.52
III. 3. TFF_5xCF_7	122.63	98.73	83.72	69.33	57.81**	8.79	12.79
F-test	**	**	**	**	**		
L.S.D. 0.05	17.59	11.56	10.39	3.59	2.53		
0.01	23.68	15.57	13.99	4.83	3.41		

**Significant at 0.01 probability level

Table 7: Yield of free amino acids obtained during feather fermentation by bacterial transconiugants

Transconjugant	Fermentation period (day)					L.S.D	
	3	6	9	12	15	0.05	0.01
I. 1. IBF_1xCF_3	150.02	98.41	88.52	75.81	63.43**	10.79	15.69
I. 2. IBF_1xIBF_6	229.97	146.85	191.20	128.01	116.34**	8.78	12.78
I. 3. IBF_1xCF_7	269.53	200.03	173.41	137.67	130.77**	7.68	11.17
II. 1. IBF_7xCF_3	206.28	118.84	102.08	86.36	74.17**	6.38	9.28
II. 2. IBF_2xIBF_6	229.97	127.07	108.77	105.84	99.39**	8.09	11.77
II. 3. IBF_7xCF_7	247.80	184.17	169.83	130.51	124.10**	18.96	27.58
III. 1. TFF_5xCF_3	144.44	103.36	94.91	79.49	60.18**	16.24	23.62
III. 2. TFF_5xIBF_6	164.49	118.09	108.82	102.65	86.25**	13.37	19.45
III. 3. TFF_5xCF_7	85.28	68.62	68.75	60.67	51.49**	8.96	13.03
F-test	**	**	**	**	**		
L.S.D. 0.05	14.74	8.88	5.89	2.78	4.35		
0.01	19.84	11.96	7.93	3.75	5.85		

Significant at 0.01 probability level

Table 8: Relative increase (%) of keratinase (KU/nil) obtained by different trans-conjugants of bacteria during fea bioconversion

Transconjugant	Fermentation period (day)					L.S.D	
	3	6	9	12	15	0.05	0.01
I. 1. IBF_1xCF_3	+60.51	-8.89	-20.83	-17.29	-32.89**	3.54	5.14
I. 2. IBF_1xIBF_6	+120.95	+10.63	+23.33	+14.28	-0.55**	14.14	20.56
I. 3. IBF_1xCF_7	+174.30	+33.96	+32.78	+ 18.52	+3.70**	7.88	11.47
II. 1. IBF_2xC_3	+121.04	+30.43	+25.94	4 36.07	+9.83**	16.44	23.91
I. 1. $IBF_2\ xCF_6$	+109.52	+11.64	+19.04	+27.85	+ 12.07**	15.17	22.06
I. 2. $IBF_2\ xIBF_7$	+165.16	+32.53	+ 17.46	+18.07	+4.34**	12.23	17.79
III.1. TFF_5xCF_3	+21.65	+2.26	+9.93	+22.29	+0.51**	10.26	14.93
III. 2. TFF_5xIBF_6	+ 18.98	-10.93	-13.42	+11.29	-1.78*	8.02	11.67
III. 3. TFF_5xCF_7	+30.56	-2.70	-10.25	-14.43	-18.96*	11.57	16.83
F-test	**	**	**	**	**		
L.S.D. 0.05	10.44	9.44	8.91	4.43	3.10		
0.01	14.06	12.71	11.99	5. 96	4.18		

*,**Significant at 0.05 and 0.01 probability level, respectively

Table 9: Relative increase (%) of proteolytic enzyme produced = -by- different transconjugants of bacteria during feather bioconversion

Transconjugant	Fermentation period (day)					L.S.D	
	3	6	9	12	15	0.05	0.01
I. 1. IBF_1xCF_3	63.18	-27.92	-28.94	-43.94	-47.24**	12.31	17.96
I. 2. IBF_1xIBF_6	137.8	-15.0	-36.44	-48.79	-56.74**	12.61	18.34
I. 3, IBF_1xCF_7	235.43	15.64	-2.63	-19.99	-64.14**	21.75	31.64
II. 1. IBF_2xCF_3	+131.61	+15.59	+10.64	3.57	-17.54**	15.56	22.63
II. 2. IBF_2xIBF_6	+121.59	-6.78	-23.04	-40.36	-49.44**	18.79	27.33
II. 3. IBF_2xCF_2	+177.37	+4.35	-4.25	-13.63	-39.30**	25.06	36.45
III. 1. TFP_5xIBF_6	53.44	15.44	-2.32	-18.28	-30.42**	9.46	13.76
III. 2. TFF_5xIBF_6	4 29.17	-12.12	-32.84	-48.35	-52.87**	14.25	20.73
III. 3. TFF_5xCF_7	22.73	-1.27	-16.27	-30.67	-42.19**	12.40	18.04
F-test	**	**	**	**	**		
L.S.D. 0.05	20.57	8.78	9.22	3.47	2.54		
0.01	27.69	11.82	12.41	4.67	3.42		

**Significant at 0.01 probability level

Table 10: Relative increase of free amino acids obtained during feather fermentation by bacterial transconjugants

Transconjugant	Fermentation period (day)					L.S.D	
	3	6	9	12	15	0.05	0.01
I. 1. IBF_1xCF_3	+50.02	-1.59	-11.48	-24.18	-36.57**	7.47	10.86
I. 2. IBF_1xIBF_6	+129.97	+46.85	91.25	28.01	+16.34**	8.72	12.68
I. 3. IBF_1xCF_7	+169.53	+100.03	+73.41	37.67	+30.77**	7.68	11.17
II. 1. IBF_2xCF_3	+106.28	18.84	+2.08	-13.64	-25.83**	4.79	6.97
II. 2. IBF_2xIBF_6	+129.97	+27.07	+8.77	+5.84	-0.61**	8.61	12.53
II. 3. IBF_2xCF_7	147.8	+84.17	+69.83	+30.51	+24.10**	18.96	27.58
III. 1. TFF_5xCF_3	44.44	+3.36	-5.09	-20.51	-39.81**	17.40	25.32
III. 2. TFF_5xIBF_6	64.49	18.09	+8.82	2.65	+7.69**	15.38	22.37
III. 3. TFF_5xCF_7	-14.72	-31.37	-30.95	-39.33	-48.50**	9.14	13.29
F-test	**	**	**	**			
L.S.D. 0.05	14.16	9.52	6.63	3.32	3.70		
0.01	19.06	12.82	8.92	4.47	4.99		

*Significant at 0.01 probability level

Additionally, the same trend of effectiveness of tested transconjugants were observed in the case of proteinase yield as well as the yield of free amino acids (Table 9, 10), Again the culture I.3 and II.3 were the potent. In these experiments amino acids and proteinase were released as a result of native feather degradation as cited by Fredrich and Antrakian (1996). The importance of amino acids containing poultry feed was established. So, many authors have made attempt to produce of a methionine - excreting mutant of Streptomyces fradiae (Elmayergi and Smith, 1971). Further, the statistical analysis proved the significant differences between all treatments and fermentation period as well.

Estimated values of the correlation coefficient of the fermentation products: Data summarized in Table 11 show that significant correlations between keratinase activity and the content of free amino acids of the cultural filtrate were observed along the fermentation course. In addition, proteinase activity was also significantly correlated with free amino acids after the third day of fermentation. On the other hand, the positive insignificant correlations were found after other times of incubation such as 6, 9, 12 and 15 days. Furthermore, data showed that keratinase activity was significantly correlated at most fermentation times with proteinase activity. This means that the increase in the content of free amino acids is probably good indicator of the action of both keratinase and proteinase on native keratin. So, the potent strain in keratinase production may be the highest strain in proteinase production, too.

The same finding was obviously observed by bacterial transconjugants regarding the significant correlation between keratinolytic activity and total free amino acids in the cultural filtrate at all intervals of fermentation. Significant correlations were also observed between keratinolytic activity and proteinase activity after the 3rd and 6th day of the fermentation. Insignificant positive

Table 11: Correlation coefficient (r) between some parameters obtained during feather degradation in batch fermentation by bacterial strains and their transconjugants

Fermentation period (day)	Examined parameters		Keratinase Keratinase	Free amino acids
	Keratinase	P		0.91*
3		T		0.67**
	Proteinase	P	0.97**	0.88**
		T	0.63*	0.59*
	Keratinase	P		0.94**
		T		0.68*
	Proteinase	P	0.78*	0.67
		T	0.68*	0.27
	Keratinase	P		0.93**
9		T		0.75**
	Proteinase	P	0.72	0.66
		T	0.51	0.06
	Keratinase	P		0.92**
12				0.70*
	Proteinase	P	0.77**	0.72
		T	0.50	0.08
	Keratinase	P		0.87*
15				0.69*
	Proteinase	P	0.83*	0.65
		T	0.54	0.03

P = Parental strains, T = Transconjugant, *'**Significant at 0.05 and 0.01 probability level, respectively

correlations, on the other hand, were found at other intervals. Data proved that keratinase activity is closely related to free amino acids at all intervals of the fermentation. This, however, indicated that measuring of free amino acids is a good indicator to keratinase activity. Owing to the activity of keratinase is correlated rather well with proteinase activity, the correlation between these two measured end-products is probably a good indicator for each other.

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