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ORIGINAL ARTICLES

Effect of temperature on cellular fatty acids and proteins of three Gram positive bacterial isolates isolated from soil in Egypt

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ABSTRACT

Three gram positive bacterial isolates, isolated from soil and identified as *Bacillus licheniformis*, *Bacillus circulans* and *Geobacillus thermoglucosidasius* were grown at 20, 35, and 50°C then subjected to cellular fatty acids analysis. Although in different amounts, the fatty acids (10:0), (12:0), (14:0), (16:0) and (17:0) were detected in cells of the three bacterial isolates obtained from all the incubation temperatures. Increasing temperature from 20 to 50°C raised the proportion of the saturated fatty acids by 26.10%, 09.89% and 29.61% in *B. licheniformis*, *B. circulans* and *G. thermoglucosidasius,* respectively. Cellular protein contents and protein banding pattern on SDS-PAGE of the three isolates were estimated at 20, 25, 30, 35, 40, 45 and 50°C. The highest amount of protein concentration in all isolates was obtained at 20ºC. In contrast, the highest number of protein bands was not obtained from these treatments.

Key words: *Bacillus licheniformis*, *Bacillus circulans*, *Geobacillus thermoglucosidasius*, bacterial antagonistic activity, cellular fatty acid analysis, cellular protein analysis.

Introduction

Several bacteria are found to grow in environments in which the physical parameters such as temperature, salinity, pH or pressure, are extreme with respect to the conditions in which eukaryotic organisms live preferentially. Most extremophiles belong to the kingdom of the archaea, but also certain bacteria and even some eukarya can tolerate some of these extreme conditions.

Bacteria can adapt to these extreme environments by modifying their membranes to maintain them in a fluid state (Scandurra *et al*., 2000; Madigan, 2003; Mrozik *et al*., 2004; Satyanarayana *et al*., 2005 and Oarga, 2009). Membranes of bacteria consist of a bilayer or monolayer of lipid molecules, which form a matrix in which various membrane proteins float (Konings *et al*. ,2002). Modifications in the cytoplasmic membrane occur due to changes in the fatty acid composition and interaction between proteins and lipids (Suutari and Laakso, 1992 and Mrozik *et al*., 2004).

Torsvik and Øvreås (2007) reported that at high temperatures, the membrane fatty acids acquire longer chains, and they become more saturated and more branched. Such changes in the membrane structure and composition lead to decreased membrane fluidity and consequently better thermostability. Three changes in bacterial phospholipids with the drop in temperature have been reported by Ray (2006) : (i) increase in the level of unsaturated fatty acids, (ii) reduction in chain length, (iii) increase in the ratio of anteiso- to iso-form of branched chain fatty acids. In mesophilic *Bacillus subtilis* the membrane fluidity following cold-shock is altered both due to increased unsaturation of fatty acids by activity of desaturase and conversion of iso-branched to anteiso-branched saturated fatty acids.

The primary structure of thermophilic enzymes is one of the factors that provides thermostability. An analysis of the amino acid sequence of several enzymes revealed that mesophilic organisms have proteins containing peptides with the sequence of glycine, serine, lysine, and asparagine which promote flexibility to the back-bone of the protein (Barton, 2004).

Micro-organisms adapted to high temperatures have mechanisms for protecting their proteins and nucleic acids from irreversible denaturation. Biomolecules from such organisms are thermostable and remain active at temperatures that generally inactivate proteins, lipids, and nucleic acids in mesophilic organisms (Rothschild and Mancinelli, 2001). In some proteins, the stabilization is caused by changes in amino acid residues that make the proteins more hydrophobic and increase the stability of subunit interactions (Singleton and Amelunxen, 1973). Although proteins from thermophilic organisms and their mesophilic homologues have structures that are very similar, in case of thermophilic organisms they usually show high intrinsic thermal stability (Szilágyi and Závodszky, 2000). Metpally and Reddy (2009) reported that serine, aspartic acid, threonine and alanine are overrepresented in the coil regions of secondary structures in psychrophilic bacteria, whereas glutamic acid and

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leucine are underrepresented in the helical regions. In addition, when comparing with mesophiles, they found that psychrophiles comprise a significantly higher proportion of amino acids that contribute to higher protein flexibility in the coil regions of proteins, such as those with tiny/small or neutral side chains. Also, they found that amino acids with aliphatic, aromatic and hydrophilic side chains are underrepresented in the helical regions of proteins of psychrophiles. Further more ,the patterns of amino acid substitutions between the orthologous proteins of psychrophiles versus mesophiles were found to be significantly different for several amino acids when compared to their substitutions in orthologous proteins of the mesophiles or psychrophiles.

This study was carried out to investigate the effect of different growth temperatures on cellular fatty acids and proteins of *Bacillus licheniformis*, *Bacillus circulans* and *Geobacillus thermoglucosidasius* isolates isolated from the Egyptian soil.

Materials and Methods

Bacterial isolates:

Three antagonistic bacterial isolates were used in this study. *Bacillus licheniformis* (Bs2), was kindly provided by Dr. K. Abuelyosr, Faculty of Agriculture, Assiut University, Egypt and it was isolated from soil. *Geobacillus thermoglucosidasius* (MA3), was detected surrounded by an inhibition zone on PDA plate during isolation of fungal organisms from a soil sample. These two isolates were identified by Biolog automated system and by consulting Logan and De Vos (2009) and Logan *et al*. (2009). *Bacillus circulans* (Sun), obtained from the culture collection of the Bacteriology Laboratory, Botany and Microbiology Department, Faculty of Science, Helwan University, Egypt, it was isolated from cultivated soil and identified during the M. Sc. thesis of Amin (2009). Bacterial isolates were maintained on nutrient agar slopes, stored at 4^{o}C and subcultured monthly.

Effect of temperature on bacterial growth:

Eight of 100 ml Erlenmeyer flasks, each containing 50 ml of LB broth were inoculated with 0.5 ml (OD_{600}~0.8) bacterial suspension obtained from an overnight shake culture of the isolate under test. Then incubated in a shaking incubator at 200 rpm for 24 h at the following temperatures 20, 25, 30, 35, 37, 40, 45, 50^{o}C. OD_{600} was measured in each treatment.

Fatty acid methyl ester (FAME) analysis:

Fatty acids were extracted by the method of Miller and Berger (1985) and analysed by gas liquid chromatography. One µl was injected into a chroompack CP900 equipment fitted with a flame ionizing detector and a 10 m x 0.53 mm capillary column CP-Wax 52 CB. Solvent blank was tested for impurities. Operating conditions included nitrogen carrier gas, flow rate 7 ml per minute, injector temperature 245ºC and temperature increase rate 4ºC per minute. Fatty acids were identified by the comparison of their retention time (RT) with the standard. The total of the areas of the chromatogram peaks of the fatty acids was considered as the total amount of fatty acids. The areas of the individual peaks were used to determine the relative percentage of each fatty acid.

Determination of protein concentration:

Protein concentration was estimated by the method of Bradford (1976).

Preparation of soluble cell protein extracts:

Cellular protein samples were prepared according to Kim *et al*. (2010).

Flasks (250 ml) each containing 50 ml LB broth were inoculated with the bacterial species and incubated overnight at 20, 25, 30, 35, 40, 45 and 50 ◦C by shaking until OD_{600}~0.4, then 1.5 ml was transferred to an eppindorff tube and centrifuged at 12,000 ×g (13.000rpm) for 5 min at 4^{o}C. The pellet was then suspended by adding 15µ of the sample buffer. For protein denaturation, samples were heated for 5 min at 95^{o}C in a water bath. The cell debris was settled by centrifugation, and the supernatant was collected for analysis.

Electrophoresis:

The method for sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was based on that of Laemmli (1970). The separating and stacking gels contained 10% and 4.55% (w:v) acrylamide respectively. Electrophoresis was performed at room temperature at a constant current of 20 mA and a

maximum of 450 V. Molecular mass markers were included in the run and the gel was stained with Coomassie Brilliant Blue R 250.

Results:

Effect of temperature on bacterial growth:

The three bacterial isolates were grown at 20, 25, 30, 35,40 , 45 and 50°C. The obtained results revealed that 35°C is the most suitable growth temperature for all the isolate, Fig. (1).

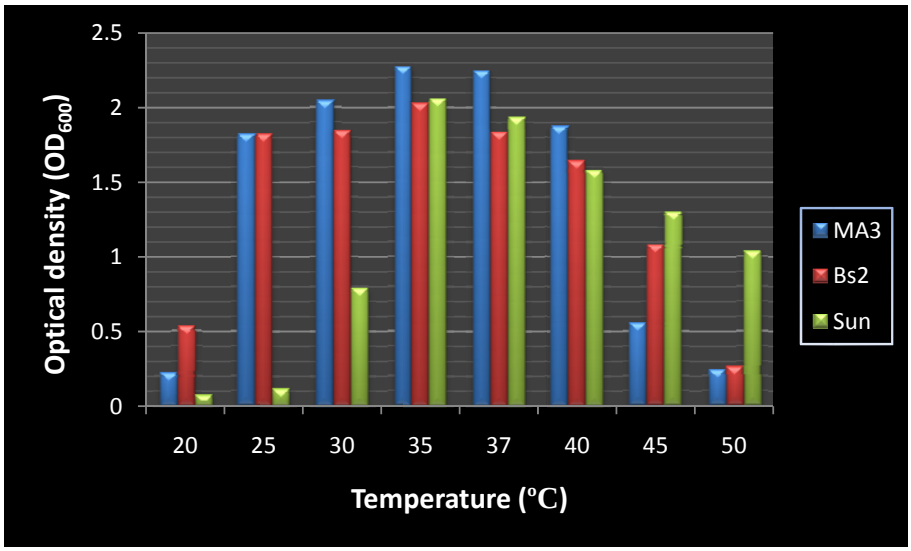

Fig. 1: Effect of different temperatures on the growth of *B. circulance* (Sun), *B. licheniformis* (Bs2) and *G. thermoglucosidasius* (MA3).

Fatty acid analysis:

Although in different amounts the two fatty acids (10:0) and (16:0) were detected in the three bacterial isolates grown at 20°C, 35°C and 50°C (Table 1). The fatty acids (12:0), (14:0) and (17:0) were recorded in *G. thermoglucosidasius* and *B. circulans* obtained from the three incubation temperatures. When *B. licheniformis* was incubated at 35°C, the fatty acid (12:0) constituted 38.7% of the total fatty acids detected in this treatment, this amount was reduced to 3.57% at 50°C and it was not detected at all at 20°C. Furthermore, the fatty acids (14:0), (16:1) and (17:0) were contained in *B. licheniformis* grown at 20°C and 35°C, but not at 50°C. However, the fatty acids (17:1), (18:0) and (18:1) were detected only in *G. thermoglucosidasius* when grown at (35°C), (20°C, 35°C and 50°C) and (20°C and 35°C), respectively (Table 1).

The amount of (16:0) was increased to 8.33 times when *B. licheniformis* incubated at 20°C and to 11.72 times when incubated at 50°C relative to that obtained when the bacterium was incubated at 35°C. Also in case of *G. thermoglucosidasius*, the amount of (16:0) was increased by 1.5 times when incubated at 50°C. In contrast, it was reduced to 0.67 that obtained at 35°C (Table 1). The highest amount of the fatty acid (16:1) was obtained when either *G. thermoglucosidasius* and *B. circulans* were incubated at 20°C. In addition to (18:3) which was only detected in *B. licheniformis* at low percentage (1.21%) when incubated at 50°C, (20:0) was recorded when the same bacterium was incubated at 20°C and 50°C (Table 1).

Cellular protein concentration:

Protein concentration of the three strains incubated at different temperatures are presented at Table (2). In case of *B. licheniformis*, high significant increase in protein concentration was detected at 20, 40 and 45°C relative to that found at 35°C. In contrast, highly significant reduction in protein concentration was obtained at 25, 30 and 50°C. However, the highest amount of protein was detected at 20°C, the increase at this incubation temperature is highly significant relative to all other treatments. In case of *G. thermoglucosidasius*, highly significant increase in protein concentration in all treatments relative to that obtained at 35°C. The highest amount of protein was observed at 20°C. When incubating *B. circulans* at 30°C, highly significant reduction in

protein concentration was obtained relative to all other incubation temperatures. At 20, 25, 40 and 45°C highly significant increase in protein concentration was observed relative to 35°C. The highest amount of protein concentration was obtained at 20°C.

Table 1: Percentage of cellular fatty acids of B. circulance, B. licheniformis and G. thermoghlucosidasius incubatd at different temperatures.

Strain	*B. licheniformis*			*Geo. thermoglucosidasius*			*B. circulans*		
Temperature	20°C	35°C	50°C	20°C	35°C	50°C	20°C	35°C	50°C
Fatty acid									
8:0	0.00	0.00	0.00	1.84	2.16	1.53	1.05	0.00	0.00
9:0	0.00	3.84	0.00	0.00	0.00	0.00	0.00	0.00	0.00
10:0	5.57	36.37	3.11	21.98	28.43	29.65	43.81	52.07	61.55
12:0	0.00	38.70	3.57	23.95	26.46	22.76	32.43	30.41	27.11
13:0	0.00	1.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00
14:0	5.84	3.27	0.00	5.37	4.26	5.87	3.38	2.42	0.69
16:0	57.25	6.87	80.50	11.85	17.59	26.65	3.15	4.31	7.14
16:1	1.84	5.58	0.00	11.73	3.09	4.64	9.90	5.59	0.00
17:0	0.89	4.23	0.00	6.32	10.47	8.50	6.29	5.21	3.52
17:1	0.00	0.00	0.00	0.00	4.40	0.00	0.00	0.00	0.00
18:0	0.00	0.00	0.00	2.27	1.50	0.41	0.00	0.00	0.00
18:1	0.00	0.00	0.00	10.10	1.66	0.00	0.00	0.00	0.00
18:2	22.33	0.00	2.52	4.59	0.00	0.00	0.00	0.00	0.00
18:3	0.00	0.00	1.21	0.00	0.00	0.00	0.00	0.00	0.00
20:0	6.65	0.00	8.91	0.00	0.00	0.00	0.00	0.00	0.00
Saturated	76.2	94.41	96.09	73.58	90.87	95.37	90.11	94.42	100.0
Unsaturated	24.17	5.58	03.73	26.42	9.15	4.64	9.90	5.59	0.00

Table 2: Protein concentration of the three isolates at different incubation temperatures.

Temperature (°C)	Protein concentration (µg ml^{-1})		
	B. licheniformis	*G. thermoglucosida-sius*	*B. circulans*
20	84.47	78.75	90.53
25	72.133	77.00	90.33
30	71.60	75.00	82.613
35	78.333	71.47	86.78
40	80.90	76.78	88.07
45	82.43	74.30	90.33
50	73.90	73.40	85.06
LSD 1%	1.20	0.0891	0.71
LSD 5%	0.90	0.06	0.51

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of cellular proteins:

SDS-PAGE of cellular proteins of *B. circulans* gave 8, 7, 8, 7, 9, 8 and 8 well-resolved bands when the bacterium incubated at 20, 25, 30, 35, 40, 45 and 50°C, respectively (Fig. 2 and Table 3). Most of the bands were found in cells of this isolate over the whole incubation temperature investigated, but the relative amount as judged from staining intensities, differed for certain proteins (Table 3). Comparison of cellular protein of *B. circulans* grown at 35°C with that of the same isolate grown at temperatures under test indicated that band 1 was missing in cells incubated at 20, 30 and 45°C. Band 2 was not detected in cells grown at 45 and 50°C. Band 3 was absent in cells grown at 40 and 45°C. In contrast, band 4 was not detected in any treatment. However, band 5 was missing in cells grown at 40 and 50°C. Also band 6 was absent in cells grown at 25 and 50°C. Band 7 was not detected in cells grown at 20 and 50°C (Table 3). Additional bands which are not present in cells grown at 35°C were observed in other treatments. For example, 2 bands of M.W. of 73.36 and 130.59 kDa were detected in cells grown at 20°C. Also, 4 bands with M.W. of 34.64, 75.72, 87.29 and 135.86 kDa were observed only in cells incubated at 50°C (Table 3).

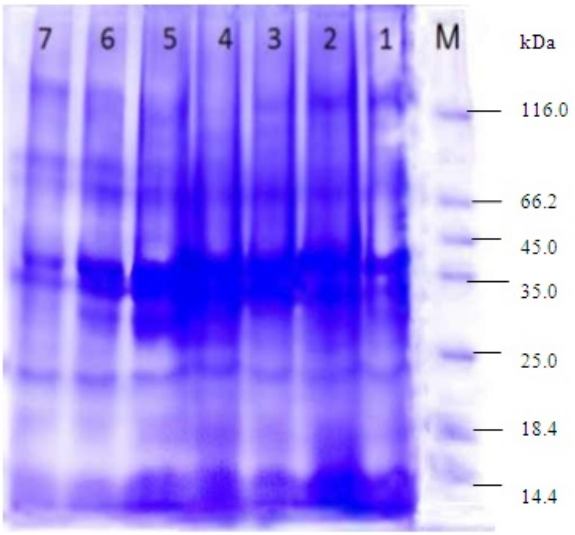

Fig. 2: SDS of cellular proteins extracted from *Bacillus circulans* (Sun) at 20, 25, 30, 35, 40, 45 and 50°C (Lanes 1, 2, 3, 4, 5, 6 and 7 respectively). M, molecular weight marker.

Table 3: Molecular weights of protein banding patterm of Bacillus circulans grown at different temperatures.

Row	Lanes													
	1		2		3		4		5		6		7	
	M.W.	Peak intensity	M.W.	Peak intensity	M.W.	Peak intensity	M.W.	Peak intensity	M.W.	Peak intensity	M.W.	Peak intensity	M.W.	Peak intensity
1	12.83	214.00									12.74	129.72		
2			13.92	231.00			13.45	206.00	13.73	173.00			13.18	107.00
3					14.01	182.28								
4			17.54	173.00										
5	18.28	141.57			18.28	117.144	18.03	147.72						
6									18.58	93.28	18.77	75.00	19.05	66.00
7									22.76	116.00	22.65	124.00	22.98	107.00
8	23.44	152.43	23.10	157.00	23.10	136.57	23.68	161.57						
9							27.30	193.43						
10	29.35	208.28							29.05	223.00	29.50	165.00		
11	33.23	221.72	32.55	231.00	32.72	231.00	32.22	229.86						
12									33.58	231.00	33.06	223.00		
13													34.64	160.43
14											35.61	223.00		
15							36.55	231.00	36.55	231.00				
16	37.84	226.43	38.17	194.57	37.51	231.00							38.50	170.72
17			69.96	180.14	69.41	204.86	69.41	169.57	69.41	179.86				
18											71.64	167.28		
19	73.36	183.86												
20													75.72	110.86
21									78.77	160.43				
22											82.59	152.43		
23													87.29	128.57
24					103.85	143.86								
25														
26									111.51	140.00				
27					122.60	140.00								
28			125.54	180.14										
29	130.59	167.28												
30													135.86	134.28
Total bands	8		7		8		7		9		8		8	

*SDS-PAGE was Coomassie Brilliant Blue R250 stained and then molecular weights of protein bands were identified using gel-documentation system " Gel Analyser V 3.0 Computer program " lanes 1-7 represents cellular proteins of *B. circulans* inoculated at 20, 25, 30, 35, 40, 45 and 50°C, respectively. M.W., molecular weight.

Analysis of protein content of *B. licheniformis* when incubated at different growth temperatures resulted in 13, 9, 5, 16,17,15 and 10 bands at 20, 25, 30, 35, 40, 45 and 50^{o}C, respectively (Fig. 3 and Table 4). Comparison of the protein banding pattern obtained at 35^{o}C, as the most suitable growth temperature, with that obtained at the other growth temperatures revealed that bands 1 & 2 are detected in all treatments, where as band 6 was detected in all treatments but cells grown at 20^{o}C. Bands 4 & 5 were detected in all treatments but 30^{o}C. Bands 9, 10, 14 and 16 were found in cells grown at 35, 40 and 45^{o}C. Band 2 was not obtained at the lower temperatures (20, 25 and 30^{o}C). Furthermore, band 7 was not detected at 20 and 30^{o}C. Each of bands 8 and 13 were detected only in one treatment which is 40 and 20^{o}C, respectively. Band 11 was not detected in cells grown at 20 and 30^{o}C. However, band 15 was not contained in all other treatments. At least 4 protein bands with M.W. of 57.54, 70.74, 90.66 and 108.50 kDa were detected only in cells grown at 20^{o}C. Also, two bands with M.W. of 33.12 and 44.29 were found in cells grown at 50^{o}C. (Table 4).

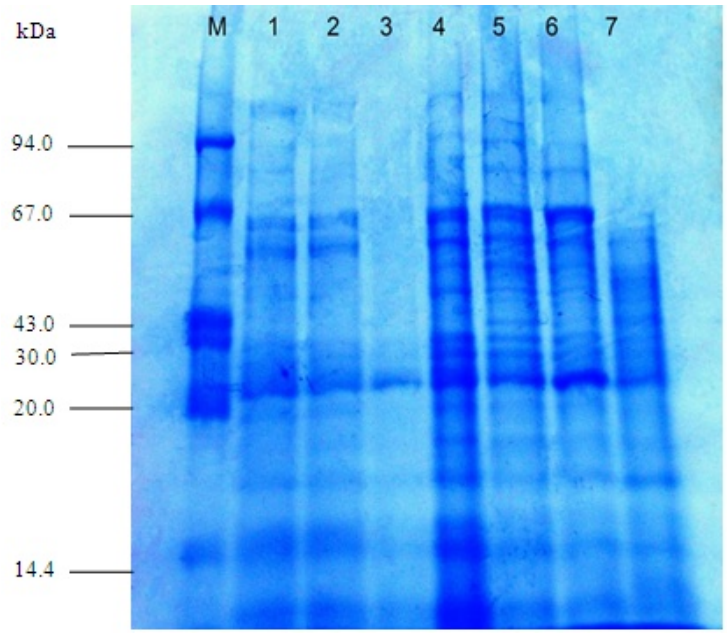

Fig. 3: SDS of cellular proteins extracted from *Bacillus licheniformis* (Bs2) at 20, 25, 30, 35, 40, 45 and 50°C (Lanes 1, 2, 3, 4, 5, 6 and 7 respectively). M, molecular weight marker.

Table 4: Molecular weights of protein banding pattern of *B. licheniformis* grown at different temperatures.

Row	Lanes													
	1		2		3		4		5		6		7	
	M.W.	Peak intensity	M.W.	Peak intensity	M.W.	Peak intensity	M.W.	Peak intensity	M.W.	Peak intensity	M.W.	Peak intensity	M.W.	Peak inten-sity
1	14.54	177.56	14.57	165.00	14.72	114.78	14.51	214.00	14.52	162.34	14.59	146.89	14.57	147.56
2							15.16	198.00						
3									15.48	125.79	15.56	116.56	15.34	119.44
4	16.89	147.78	16.84	135.67	16.91	108.34	17.05	187.00	17.00	131.11	17.04	123.78	16.90	148.78
5	18.36	143.22	18.28	127.56			18.55	189.00	18.30	131.11	18.36	119.56	18.51	129.56
6	19.47	155.22	19.67	137.67			19.74	188.00	19.86	133.00	19.91	136.67	20.02	134.00
7	21.93	195.22	22.59	179.34										
8					23.09	165.89	23.39	223.00	23.49	206.00	23.89	223.00	23.34	179.34
9	25.91	187.56			25.89	111.00								
10			26.92	141.22										
11							27.69	211.34	27.38	182.113	27.57	171.22	27.69	165.89
12	29.70	154.89	29.47	123.00	29.12	99.89								
13							31.61	204.22	32.08	168.56				
14											33.80	150.56	33.12	162.34
15														
16									43.59	153.22			44.29	165.00
17							49.05	190.78	49.68	166.67	50.44	147.78		
18							53.46	178.56						
19									54.37	190.89	54.85	169.56		
20	57.54	163.34											60.74	124.00
21			58.72	156.113			59.86	210.44	60.43	202.44	60.96	195.34		
22	64.57	144.00	64.90	127.67			65.73	205.11						
23									67.98	197.11	67.54	214.00		
24	70.74	103.67												
25							73.39	145.78						
26	82.18	110.00							83.29	135.78	82.65	133.00		
27							85.49	135.78						
28	90.66	90.11									93.46	108.11		
29									94.19	152.22				
30							98.32	107.44						
31									103.96	150.44				
	108.50	107.34												
							116.93	104.44			116.92	104.56		
Total bands	13		9		5		16		17		15		10	

*SDS-PAGE was Coomassie Brilliant Blue R250 stained and then molecular weights of protein bands were identified using gel-documentation system " Gel Analyser V 3.0 Computer program " lanes 1-7 represents cellular proteins of *B. licheniformis* inoculated at 20, 25, 30, 35, 40, 45 and 50°C, respectively. M.W., molecular weight.

Analysis of cellular protein contents of *G. thermoglucosidasius* grown at 20, 25, 30, 35, 40, 45 and 50°C, resulted in 8, 8, 5, 3, 8, 3 and 5 bands, respectively (Fig. 4 and Table 5). Comparison of the cellular protein of this strain when grown at 35°C with that obtained in cells grown at the other temperatures revealed that band 1was detected only in cells grown at 45°C, whereas band 3 was detected in cells incubated at 45 and 50°C. However, band 2 was detected in cells grown at 20, 30, 40 and 45°C but not 50°C (Table 5). Four bands with M.W. of 24.90, 29.74, 34.66 and 40.42 kDa were detected only in cells grown at 50°C and 2 bands with M.W. of 101.54 and 127.00 kDa were detected only in cells grown at 20°C. Also, 3 bands with M.W. of 48.27, 97.24

and 123.38 were found only in cells grown in cells grown at 25°C (Table 5). Furthermore, 3 bands with M.W. of 89.41, 118.36 and 138.21 kDa appeared only in cells grown at 40°C (Table 5).

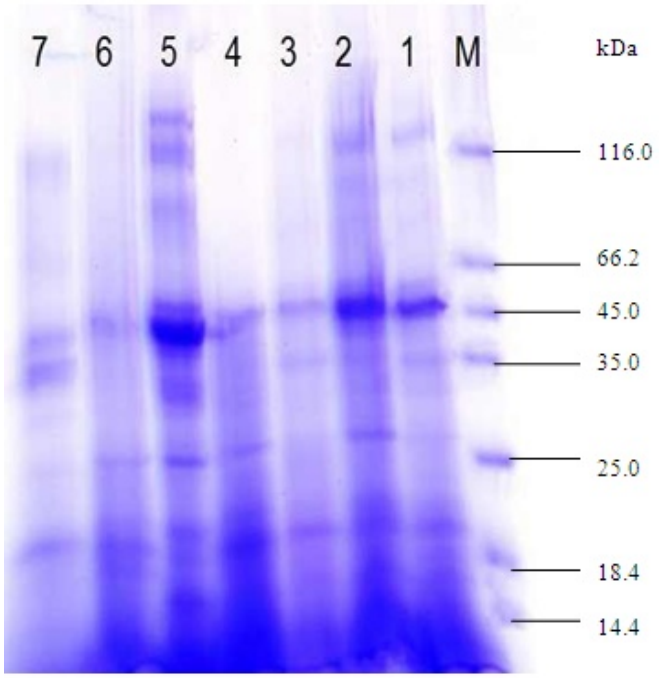

Fig. 4: SDS of cellular proteins extracted from *Geobacillus thermoglucosidasius* (MA3) at 20, 25, 30, 35, 40, 45 and 50°C (Lanes 1, 2, 3, 4, 5, 6 and 7 respectively). M, molecular weight marker.

Table 5: Molecular weights of protein banding pattern of Geobacillus thermoglucosidasius grown at different temperatures.

Row	Lanes													
	1		2		3		4		5		6		7	
	M.W.	Peak intensity	M.W.	Peak intensity	M.W.	Peak intensity	M.W.	Peak intensity	M.W.	Peak intensity	M.W.	Peak intensity	M.W.	Peak intensity
1									160.7	189.72				
2					169.6	140.00								
3	173.2	165.00					195.0	199.14			195.6	157.00	198.1	114.72
4			181.2	190.00										
5	203.6	149.00	208.2	171.86	204.2	140.00			203.8	153.57				
6													249.0	58.00
7	266.4	83.00			269.5	75.00	262.8	132.00	256.8	149.00	253.0	99.00		
8													297.4	50.00
9			275.4	137.72										
10	322.3	75.00	321.3	116.00					325.8	149.00				
11													346.6	96.72
12	351.4	91.00			354.3	75.00								
13			361.5	124.00									404.2	79.57
14									412.5	231.00				
15							429.8	115.72			432.8	101.28		
16														
17	474.6	176.43			479.3	91.00								
18			482.7	206.00										
19									894.1	92.14				
20														
21			972.4	88.72										
22	1015.4	42.00												
23									1183.6	108.28				
24			1233.8	103.57										
25	1270.0	60.28												
26									1382.1	104.72				
Total bands	8		8		5		3		8		3		5	

*SDS-PAGE was Coomassie Brilliant Blue R250 stained and then molecular weights of protein bands were identified using gel-documentation system " Gel Analyser V 3.0 Computer program " lanes 1-7 represents cellular proteins of *Geobacillus thermoglucosidasius* inoculated at 20, 25, 30, 35, 40, 45 and 50°C, respectively. M.W., molecular weight.

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Discussion:

In this investigation, eight fatty acids were detected in *B. licheniformis* incubated at 37^{o}C, pelargonic (9:0), capric (10:0), lauric (12:0), tridecylic (13:0), myristic (14:0), palmitic (16:0), palmitoleic (16:1) and margaric (17:0) acids. In contrast to this result, Martin and Swenson (1976) found that vegetative cells of *B. licheniformis* grown at 37^{o}C contained lauric (12:0), myristic (14:0), palmitic (16:0), palmitoleic (16:1), and linoleic (18:2) acids. Qualitative and quantitative changes were detected when the incubation temperature was changed. At the lower temperature (20^{o}C) linoleic acid (18:2) appeared in concentration of 22.33% of the total fatty acids of this treatment. This fatty acid was not detected at 35^{o}C and its concentration at 50^{o}C was only 2.52% of this treatment. Sikorski *et al*. (2008) found that when *Bacillus simplex* incubated at 20^{o}C, the unsaturated fatty acids increased by 2.5-3.7 fold compared with that obtained at 28^{o}C. The proportion of saturated/unsaturated fatty acids was increased by increasing the incubation temperature. These results agree with that reported about saturated/unsaturated fatty acid contents in response to growth temperature (Kaneda, 1977; Russell, 1989 and Sikorski *et al*., 2008). The obtained results revealed that unsaturated fatty acids disappeared when *B. circulans* was grown at 50^{o}C. However, De Rosa *et al*. (1974) reported that unsaturated fatty acids were completely absent in *Bacillus acidocaldarius* grown at temperature of 50 to 70^{o}C The percentage of palmetic acid (16:0) produced by *B. licheniformis*, *G. thermoglucosidasius* and *B. circulans* was increased between 20 and 50^{o}C to 1.4, 2.25 and 2.27 times, respectively. Also the percentage of arachidic acid (20:0) increased at 50^{o}C to 1.34 times relative to that found at 20^{o}C. Working with *B. megaterium*, Rilfors *et al.* (1978) found that the relative amount of long chain fatty acids (C_{16} to C_{18}) increased fivefold over that of short chain fatty acids (C_{14} and C_{15}).

High percentage of capric acid (10:0) was found in *B. circulans* in all treatments, the highest amount was 61.55% of the total fatty acids and obtained at 50^{o}C. Also palmitic acid (16:0) constituted 80.5% of the total cellular fatty acids of *B. licheniformis* incubated at 50^{o}C. These fatty acids have pharmaceutical and industrial applications (Akoh & Moussata, 1998; Macri *et al.,* 2003 and Nakatsuji *et al.,* 2009) and the obtained results indicate that our bacterial strains could be a promising source of them.

Differences in protein concentration, not only between isolates but also within the same isolate, were obtained by incubating the bacteria under test at different temperatures.

It is observed that the highest protein concentration was obtained at 20^{o}C by the three bacterial isolates**.** Thieringer *et al*. (1998) observed that when bacteria was subjected to heat or cold shock, they produce the low molecular weight stress proteins. In cold shocked bacteria some of the *de novo* production is attributed to increased desaturase levels, which act to modify membrane lipids (Russel, 1990). It has been reported that under heat stress, bacteria modify the fluidity of the membrane through interactions of heat shock proteins (Torok *et al*., 1997).

Although it has been reported that exposure of cells to increased temperature triggers the induction of a phenomenon known as the heat shock response which is characterized by the enhanced synthesis of a set of proteins, called heat shock proteins (Sabri and Hasnain, 1996) and a temperature shift from 65°C to a temperature below the normal temperature range of growth (40 to 43°C) resulted in a marked suppression in the synthesis of all cellular proteins (Wu and Welker, 1991), protein concentration was significantly reduced in *B. licheniformis* at 50ºC in comparison with that found at 35ºC. This could be due to the destruction of protein by the action of this high temperature and the failure of this bacterium to synthesize heat stable protein. Furthermore, this bacterium when incubated at 30^{o}C, it produced the lowest protein concentration as well as the smallest number of bands relative to that produced at different temperatures by the same bacterium. This might indicate that the reduction of protein concentration could be due to disappearance of certain proteins.

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