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Impact of Temperatures and pH on Soluble Protein Content and Protein Profile of PY79 (Wild Type) and Sporulation Defective Mutant Strains of *Bacillus*

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Abstract: Effects of temperatures (37 and 45°C) in combination with varying pH (6, 7, 8 and 9) after 24 and 96 h of incubation were studied on soluble protein content and protein profile of PY79 (wild type) and sporulation defective mutant strains (5, 19, 96 and 99) of *Bacillus* strains. These strains were isolated from local polluted environments around Lahore, Pakistan. Soluble protein content is more in PY79 as compared to the defective sporulating mutant strains (5, 19, 96 and 99). In strain 5, it appeared that polypeptides of 55-59 kDa (pH 6, 7 and 8), 50-54 kDa (pH 6, 7 and 8), 45-49 kDa (pH 8), 20-24 kDa (pH 7) and 10-14 kDa (pH 8) were the heat stress proteins. While in strain 99, at high temperature different stress proteins appeared especially at pH 6 (30-34, 25-29 kDa), pH 7 (25-29 kDa), pH 8 (50-54, 30-34 kDa) and at pH 9 (45-49, 30-34, 20-24 kDa). At temperature 37°C, the polypeptides of 55-59 kDa at pH 6, 7 and 9 were observed which disappeared at high temperature.

Key words: *Bacillus*, soluble protein, polyacrylamide gel electrophoresis, temperature, pH

INTRODUCTION

For substantial period of time bacteria can survive under stress condition by synthesizing stress as well as general proteins (Holtmann *et al*., 2004; Schaik *et al*., 2004). With different temperatures, pH and different chemical reagent the protein and model peptides are modified as time scale of seconds to minutes (Yohannes *et al*., 2004). In *B. subtilis* stress proteins are induced in response to different environmental conditions such as changes in pH, heat shock, salt stress, glucose limitation or oxidative stress (Errington *et al*., 2003). These stress proteins have been previously grouped into general proteins (Gsps) and heat specific stress proteins (Hsps). DnaK and GroEL are Hsps in *B. subtilis*. Proteins GsiB, Ctc and RsbW belong to a class of Gsps that are induced by various stresses including heat (Volker *et al*., 1994).

Bacterial culture respond to pH changes by selective expression of numerous stress proteins, redox modulators and envelop proteins (Tucker *et al*., 2003) External acids, whose uptake is amplified by the pH gradient, induce heat shock and oxidative stress proteins as well as RpoS regulon (Arnold *et al*., 2001). The acid chaperons HdeA and HdeB enhance survival in extreme acid conditions (Gajiwala and Burley, 2000). The aim of present research is to study the effect of temperature and pH on soluble protein and protein profile of different spore defective strains of *Bacillus* strains.

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Table 1: Bacterial strains used in the study

Strains	Source
PY 79	Youngman *et al.* (1983)
5, 19, 96, 99	Microbial and Molecular Genetics Lab.

MATERIALS AND METHODS

Bacterial Strains Isolation and Characterization

Ten different samples were collected from saline, industrial and polluted area around (Muridkey, Kala Shah Kaku) and from Lahore (Muslim Town) (Table 1). Suspensions soil samples were made by adding 10 g of sieved soil in 100 mL of glass distilled water. Soil suspensions kept for 6-8 h, dilution (1 g mL^{-1} of water) plated on plates containing Schaeffer's media were incubated at 37°C for 96 h. From bacterial growth, different colonies were picked up and purified for further studies. PY79 was obtained from United States (Youngman *et al*., 1983). All the strains used (PY79, 5, 19, 96 and 99) were gram-positive spore former rods.

Soluble Protein Estimation

For protein estimation the bacterial strains PY79 (wild type), 5, 19, 96 and 99 (*div* mutants) were incubated at 37 and 45°C for 24 and 96 h. Following Laemmli (1970) the soluble proteins contents were estimated. The strains were collected, washed and centrifuged at 14,000 rpm. Cells pellets were crushed in 0.1 M phosphate buffer both by freezing and thawing and by mechanical shearing (Gerhardt *et al*., 1994). Crushed bacterial cells were centrifuged (at 14,000 rpm for 10 min) at 4°C. Supernatant was taken and used for protein estimation.

To the supernatant, 2 mL of reagent A and 200 µL of reagent B (Folin and Ciocalteu's phenol reagent) were added and the tubes were left for 30 min to equilibrate at the room temperature. Optical density was monitored at 750 nm on spectrophotometer. Amount of soluble protein was calculated by standard curve. Egg albumin (45 kDa) Bovine serum albumin (65 kDa) and Lysozyme (14.5 kDa) were used as standard (Steel and Torrie, 1981).

Polyacrylamide Gel Electrophoresis

Bacterial cells were collected and pelleted from 24 h old culture. Pellets were resuspended in 40 µL of final sample buffer. The samples were stored at -20°C and the protein analyses of the samples were accomplished with SDS polyacrylamide gel electrophoresis (PAGE).

RESULTS

Soluble Protein Estimation

After 24 h of incubation, the protein content of strain PY79 was more at both temperatures (37 and 45°C) at pH 7. While after 96 h of incubation it was more at 37°C and pH 8 of 45°C. In strain 5, after 24 and 96 h of incubation at both temperatures (37 and 45°C) the protein content was maximum at neutral pH (Table 2). In case of strain 19 maximum protein contents were observed after 24 and 96 h at pH 7. Same trend was observed in case of strain 99 at all temperatures and pH. The protein content was more in bacterial strains PY79 and 5 while the remaining strains (19, 96 and 99) showed less protein content as compared to PY79 and 5 (Table 2).

Polyacrylamide Gel Electrophoresis

In strain PY79, at 37 and 45°C the polypeptides of 60-65, 55-59 kDa were observed at pH 7, 8 and 9. At 37°C the polypeptide of 40-44 kDa were observed at pH 6, 7 and 8 and these bands were not observed at pH 9 (37°C) while at high temperature they were present at pH 6, 7, 8 and 9 (Fig. 1).

Table 2: Effect of temperatures (37 and 45°C) and varying pH (6, 7, 8 and 9) on soluble protein content of bacterial strains (PY79, 5, 19, 96 and 99)

Strains	Time (h)	Temperature (°C)	pH 6	7	8	9
PY79	24	37	350	640	393	390
		45	387	398	392	385
	96	37	356	360	334	331
		45	362	384	398	393
5	24	37	241	331	320	240
		45	240	380	234	203
	96	37	362	384	324	290
		45	270	360	264	191
19	24	37	121	205	152	71
		45	94	179	108	103
	96	37	134	181	81	80
		45	77	107	65	59
96	24	37	115	178	128	83
		45	107	147	117	63
	96	37	87	191	121	80
		45	68	109	85	62
99	24	37	76	145	120	88
		45	70	108	88	75
	96	37	140	183	149	90
		45	107	152	106	86

Table 3: Effect of varying pH (6, 7, 8 and 9) and temperatures (37 and 45°C) on protein profile of PY79 and 5

	pH															
	PY79								5							
	37°C				45°C				37°C				45°C			
Polypeptide kDa	6	7	8	9	6	7	8	9	6	7	8	9	6	7	8	9
65-60	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
59-55	-	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+
54-50	-	-	-	-	-	-	-	+	-	-	-	+	+	+	+	+
49-45	-	-	-	-	-	-	-	+	-	-	-	+	+	+	-	+
44-40	+	+	+		+	+	+	+	+	+	-	+	+	-	+	-
39-35	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
34-30	-	-	-	-	-	-	-	+	+	+	+	+	-	+	-	+
29-25	-	-	-	-	-	-	-	+	+	+	+	+	-	+	+	-
24-20	-	-	-	-	-	-	-	+	+	-	+	+	-	+	+	-
19-15	-	+	-	-	+	+	-	+	+	+	+	+	+	+	+	-
14-10	-	-	-	-	-	-	-	+	+	+	-	+	+	+	+	-
9-5	-	-	-	-	-	-	-	+		-	-	-	-	-	-	-
4-0	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-

+: Polypeptide synthesized; - : Polypeptide inhibited

When polypeptide in strain5 was compared with wild type (PY79) 60-65 kDa bands were absent in case of strain 5 while 55-59, 50-54 kDa sized polypeptide were absent in strain 5 (pH 9) at 37°C but present at 45°C. 55-59 kDa band were present at pH 7 and 8 at 37°C in PY79 but were absent in 96 (Fig. 1 and Table 3).

DISCUSSION

In bacterial strains 19, 96 and 99 the soluble protein content significantly decreased when compared with PY79. In general, the soluble protein content (except in PY79 at pH 8, 9 after 96 h; in 5 at pH 7 after 24 h; in 19 at pH 9 after 24 h) are more at 37°C as compared to 45°C. Ahmed and Sabri (2004) also reported that protein content are more at 25 and 42°C in wild type (PY79) as compared

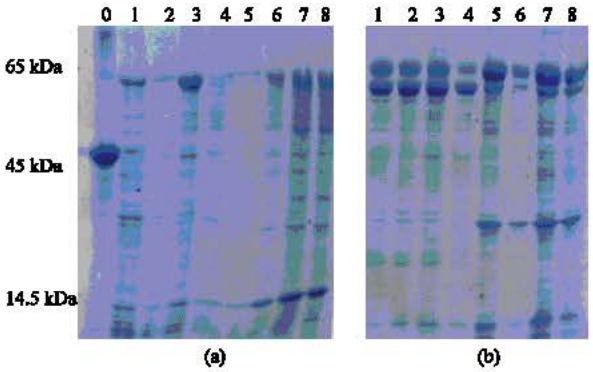

Fig. 1: Polypeptide profile of strains 5 and PY79 at varying pH (6, 7, 8 and 9) and temperatures (37 and 45°C). (a) Lane 0) Marker, 1) 37°C, pH 6, 2) 37°C, pH 7, 3) 37°C, pH 8, 4) 37°C, pH 9, 5) 45°C, pH 6, 6) 37°C, pH 7, 7) 37°C, pH 8, 8) 37°C, pH 9 and (b) 1) 37°C, pH 6, 2) 37°C, pH 7, 3) 37°C, pH 8, 4) 37°C, pH 9, 5) 45°C, pH 6, 6) 37°C, pH 7, 7) 37°C, pH 8, 8) 37°C, pH 9

to *divI* and *divII* mutants. At 37 and 45°C (except in PY79 at pH 8) all strains preferred pH 7 for maximum soluble proteins. A change in ambient temperature is a common stress condition experienced by free-living organisms; the response to heat shock represents a protective and homeostatic response to counteract temperature-induced damage in cells (Yura *et al*., 1993). The ubiquitous response to this stress condition is marked by the large-scale induction of heat shock proteins (HSPs) which include molecular chaperones that assist folding nascent proteins and repairing damaged proteins and ATP-dependent proteases that degrade misfolded proteins. Escherichia coli and Bacillus subtilis have long served as paradigms for heat shock response in gram-negative and gram-positive bacteria (Arsene *et al*., 2000; Schumann, 2003). In general protein's function is defined to great extent by its interaction with other proteins (Winters and Day, 2003).

The expression of proteins was changed with change in pH (Table 3 and Fig. 1). The 60-65 kDa polypeptide was only observed in PY79 (except at pH 6, 37°C) at all pH while for other polypeptides variation exist at all pH at both temperatures. In strain PY79 at high temperature (45°C) some stress proteins (50-54, 30-34, 10-14 and 5-9 kDa) were appeared at pH 9. In strain 5 it appeared that polypeptides of 55-59 kDa (pH 6, 7, 8), 50-54 kDa (pH 6, 7, 8), 45-49 kDa (pH 8), 20-24 kDa (pH 7) and 10-14 kDa (pH 8) were the heat stress proteins. In bacterial strain 19, 50-54 kDa (at pH 8, 9), 45-49 (at pH 8, 9), 40-44 (at pH 8, 9), 35-39 (at pH 8, 9), 30-34 (at pH 8), 25-29 (pH 9), 20-24 (at pH 9) and 15-19 kDa (pH 9) appeared to be heat stress proteins. It appeared that 55-59, 50-54, 45-49 kDa (at pH 6, 7, 8); 40-44 kDa (pH 7, 9) and 20-24 kDa (pH 8) were heat stress proteins in strain 96. In strain 99, at high temperature different stress proteins appeared especially at pH 6 (30-34, 25-29 kDa), pH 7 (25-29 kDa), pH 8 (50-54, 30-34 kDa) and at pH 9 (45-49, 30-34, 20-24 kDa). At temperature 37°C the polypeptides of the 55-59 kDa at pH 6, 7 and 9 were observed which were disappeared at high temperature. Sabri and Hasnain (1994) reported that 70 kDa at alkaline pH and 30 kDa under acidic pH are stress specific proteins at 45°C. pH specific stress proteins 60-63 kDa was observed at 45°C by Sabri and Hasnain (1994). pH specific proteins were also produced which may vary with temperature and strain. In general many polypeptide products are present at acidic pH and were not present at alkaline pH. Alkaline shifts in pH also induce the production of several heat shock proteins. So from the above results and discussion we hypothesized that signal specific proteins fall in two categories, one with rather general specificity and other with restricted specificity.

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