Journal of Applied Biological Sciences 7 (2): 33-36, 2013
ISSN: 1307-1130, E-ISSN: 2146-0108, www.nobel.gen.tr

Isolation of Cold Shock Protein profilles from *Pseudomonas sp*. Strains and Analyses of SDS-PAGE

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Received: 18 November 2012
Accepted: 27 December 2012

Abstract

In this research, *Pseudomonas aeruginosa* balcalı-1, *Pseudomonas aeruginosa* balcalı-2 which taken Çukurova Univercity Balcalı Hospital Laboratory for cold shock protein isolation and *Pseudomonas aeruginosa* ATCC 27853 strains were used. For cold shock protein synthesis had choosen 20, 10, 5 ºC and the temperature values selected for 60 and 480 minutes incubation were carried out. Cold shock proteins to determine the molecular weight, SDS-PAGE gel system was used.

P. aeruginosa ATCC 27853 strain isolated in 1700 and 18130Da weight proteins, were synthesized more than other proteins. These two proteins have occuranced with the cold shock treatment.

P. aeruginosa Balcalı 1 produced the protein in 77066Da cold shock protein at 20ºC at for 60minutes and 5, 10, 20ºC for 480 minutes incubation

SDS-PAGE analysis showed that *P. aeruginosa* Balcalı 2 strains numbered two different protein bands in 22666and 21576 Dalton molecular weight.

Strains used in the study of *Pseudomonas* balcalı-1 and balcalı-2 from Cold Shock response higher constituted than *Pseudomonas* ATCC 27853.

As a conclusion, *Pseudomonas* have three different strains Cold shock treatment when isolated the most cold shock protein, 10 ºC at for 60 minutes incubation.

Keywords: *Pseudomonas aeruginosa*. ,Cold Shock Protein, SDS-PAGE

INTRODUCTION

All live organisms have to adapt the changes in their surroundings. As many environmental changes such as cold, hot, acid shock and pressure are fatal for many organisms, adapting these changes is necessary for the organisms to survive [1].

All organisms including eukaryotic, prokaryotic, vertebrates and invertebrates have developed various adaptation mechanisms in order to survive. An important part of the cold adaptation mechanism is realized at stoplasmic membrane level. Cold shock effects the membrane composition and organization in order to continue its function. Temperature decrease cause slowdown in reproduction. During the decrease of temperature, organism changes the composition of stoplasmic membrane and structures named cold shock proteins are synthesized [2]. The reaction of bacteria against cold shock varies from species to species. On the other hand the common property observed in all of them is that they make arrangements in membrane compositions depending on the change in temperature and accordingly synthesize proteins. It is possible to group the response of microorganisms against non lethal cold shock conditions in 3 stages.

First stage is named as adaptation and is an indicator of temporary cold shock response and right after that cold shock response occurs. Due to the decrease in reproduction speed enzyme activity and membrane viscosity decreases as a characteristic of this stage.

In the second stage, cells start to reproduce and advanced modifications are seen within the protein compared with the first stage. Therefore this stage is named as cold adaptation phase.

Third stage is named as stationer phase and cells change their protein content in advanced level [3].

Pseudomonas type bacteria are the most problematic bacteria of the food industry. If the cold shock which causes food spoilage emerges in bacteria, a long shelf life can be obtained in food, especially diary products.

In this study, cellular proteins belonging to *Pesudomonas sp*. strains that will be isolated from products kept in refrigerator (4 °C) and mesophyle environment are analyzed in SDS-PAGE ambient so as to determine the molecular weights of their protein structures that differ in organisms.

METHOD

Preparation of Bacteria Stock Cultures

P. aeruginosa ATCC 27853 and *P. aeruginosa* strains obtained from Cukurova University Central Lab are cultivated in line shape on GSP Agar broth to obtain stock culture. After that cultivation was made to N1 broth medium from stock cultures and left to incubation in the 30 ºC shaking incubator at 200 rpm.

Pseudomonas sp samples taken from C.U. University Central Lab are defined by using API 20E identification kit. *P. aeruginosa* ATCC 27853 strain is used in order to confirm the accuracy of the identification and identification is made by API 20E test and wild type samples are compared with ATCC strain (*P. aeruginosa* balcalı-1 and balcalı-2) [4, 5].

Bacteria Incubation Conditions for Cold Shock Protein Isolation

For cold shock protein production the temperatures of 20, 10 and 5 ºC are selected. Incubation is realized for 60 and 480 minutes at those temperatures. For control, bacteria used for cold shock protein production are produced at same time periods at 37 ºC.

Isolation of Protein

2ml Sol-1 (Glucose 50 mM, Trisma-Base 25 mM, EDTA 10 mM, 100 mL distilled water) solution is added to the bacteria pellets that are produced by precipitation at different temperatures and incubation is realized for 60 minutes at 37 ºC. The samples are precipitated at 2000 rpm and +4 ºC for 10 minutes. The upper phase is conveyed into a clean tube and was centrifuged at 8000 rpm and +4 ºC' for the protoplast structures in the liquid to be precipitated. The upper phase is poured and 2ml distilled water is added on it for the protoplasts to be exploded and freezing was applied at -33 ºC for 60 minutes and resolving was applied at 70 ºC for 60 minutes. This process was repeated 3 times for each sample.

Cold ethanol at same volume is added on the exploded sample (by freeze/resolve method) and was kept for one night at -33 ºC. The samples are precipitated at +4 ºC for 20 minutes with 10000 rpm, upper phase is removed and total protein is obtained [6].

Determination of Molecular Weight of the Proteins

The molecular weight of the proteins was determinated using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) with 5% stacking gel and 10% separating gel. After electrophoresis the gel was stained with Coomasie Brillant Blue R-250 and destained with methanol-acetic asid-water solution (1:1:8) [7, 8].

RESULTS AND DISCUSSION

Cold shock proteins which are incubated and isolated from strain no *P. aeruginosa* ATCC 27853 at 5, 10, 20 and 37 ºC for 60 and 480 minutes are analyzed in SDS-PAGE gel having 12% concentration and protein fractions with different molecular weights shown in table 4-1 and figure 4-1 are obtained.

When the temperature is raised to 20 ºC, at the end of 60 minutes, 3 protein bands with weights of 20920, 15110 and 14620 Da are initially detected. These proteins are considered to come out as a response to living in cold environment.

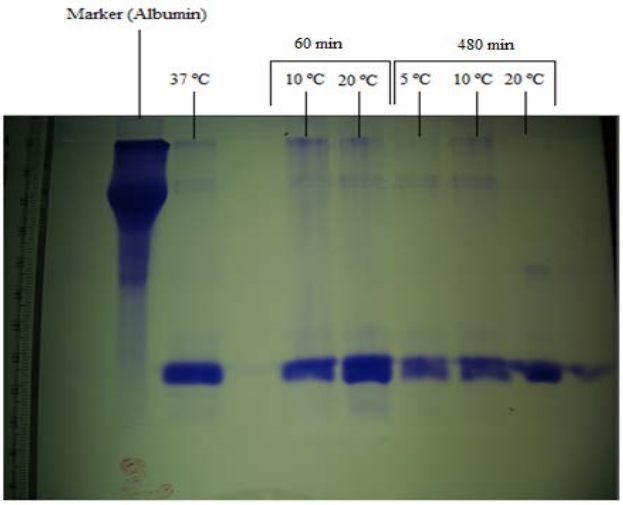

Figure 4.1. Cold shock proteins profiles in 12% SDS-PAGE gel

Table 4.1. Cold shock proteins profiles in 12% SDS-PAGE gel

		60 min			480 min		
Marker	37 ºC CONTROL	5 ºC	10 ºC	20 ºC	5 ºC	10 ºC	20 ºC
68000 Da	97140 Da	18133 Da	90660 Da	90660 Da	85000 Da	90660 Da	30220 Da
	85000 Da		80000 Da	85000 Da	75550 Da	85000 Da	17430 Da
	75550 Da		71570 Da	75550 Da	18130 Da	75550 Da	17000 Da
	17210Da		20000 Da	20920 Da	17000 Da	18130 Da	
	15632 Da		18130 Da	18130 Da		17000 Da	
	15111 Da		16790 Da	17000 Da			
				15110 Da			
				14620 Da			

Low temperature stress and its effect on protein synthesis due to the stimulation of the genes are examined in detail in various mesophylic microorganisms. For example while *E. coli* shows optimum reproduction at 37 ºC, when it si produced at an environment of 10-15 ºC, more than 12 new protein is synthesized. These proteins are named cold shock proteins and are mentioned to be necessary for the organism to adapt low temperature conditions.

Moreover it is also observed that bands having weights of 85000 and 75500 Da come out in the sample produced at 37 ºC. These bands being observed at 37 ºC makes us consider that switching to mesophilic occurs at 20 ºC.

Considering that two proteins (85000 and 75550 Da) come out at the end of incubation at 37 ºC, it is understood that new protein bands obtained after incubations of 60 and 480 minutes (5, 10 and 20 ºC) are cold shock proteins.

In cold shock application made with *P. aeruginosa* ATCC 27853 strain, it is determined that cold shock proteins are synthesized at 5 and 10 ºC at 60 minutes of incubation and at 20 ºC at 480 minutes of application. (they did not come out at other temperatures) [9, 10, 11] Cold shock proteins which are incubated and isolated from strain no *P. aeruginosa* balcalı-1 at 5, 10, 20 and 37 ºC for 60 and 480 minutes are analyzed in SDS-PAGE gel having 12% concentration and protein fractions with different molecular weights shown in table 4.2 and figure 4.2 are obtained.

Table 4.2. Cold shock proteins profiles in 12% SDS-PAGE gel

	Control	60 min			480 min		
Marker	37 °C	5 °C	10 °C	20 °C	5 °C	10 °C	20 °C
68000Da	85629 Da	85629 Da	85629 Da	88923 Da	85629 Da	85629 Da	92480 Da
	72250 Da	72250 Da	74580 Da	77066 Da	77066 Da	77066 Da	85629 Da
	64222 Da	62486 Da	23591 Da	24083 Da	21407 Da	21811 Da	77066 Da
	60842 Da	25688 Da	21081 Da	21407 Da			25130 Da
	23835 Da	23835 Da	20104 Da	20280 Da			21607 Da
	21407 Da	21407 Da	19260 Da	19260 Da			19931 Da
	19760 Da	19760 Da	18645 Da	18796 Da			19266 Da
	19593 Da	19260 Da					
	18796 Da	18796 Da					

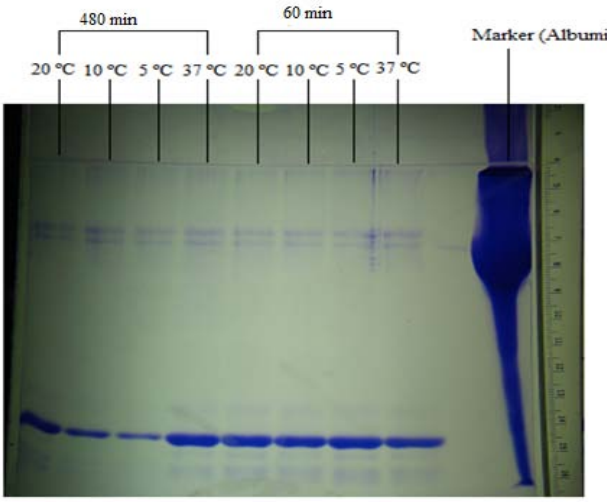

Figure 4.2. Cold shock proteins profiles in 12% SDS-PAGE gel

Protein structures at weight of 77066 Da synthesized at *P.aeruginosa* balcalı-1 strain for 60 minutes at 20 ºC and for 480 minutes at all temperatures are evaluated as cold shock proteins.

All protein structures coming out at 5, 10 and 20 ºC except proteins obtained from organism at 37 ºC for control purpose are cold shock proteins. Considering this strain, it is observed that 5 ºC and 10 ºC for incubations of 60 minutes and 20 ºC for incubations of 480 minutes are temperatures which cold shock response is realized most [12] Cold shock proteins which are incubated and isolated from strain no *P. aeruginosa* balcalı-2 at 5, 10, 20 and 37 ºC for 60 and 480 minutes are analyzed in SDS-PAGE gel having 12% concentration and protein fractions with different molecular weights shown in table 4.3 and figure 4.3 are obtained.

The molecular weights of proteins synthesized during cold shock response formation made with *P. aeruginosa* balcalı-2 strain are found to be lower than the ones obtained from *P. aeruginosa* ATCC 27853 and *P. aeruginosa* balcalı-1 strains.

When organism is produced at 10 ºC, it was found out that 7 different proteins with weights 83111, 77379, 68000, 24391, 22666, 22000 and 21576 Da are produced. The proteins with weights 83111, 77379 and 68000 Da are synthesized at 37 ºC. While the proteins with weights 22666 and 21576 Da are produced at 5 ºC, the structures with weights 24391 and 22000 Da are observed for the first time at this temperature. Accordingly the fractions with weights 22666 and 21576 Da being observed at both temperatures (5 and 10 ºC) shows that these structures are cold shock proteins.

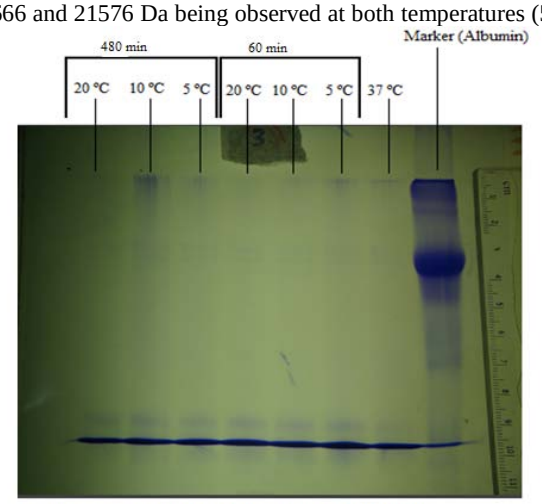

Figure 4.3. Cold shock proteins profiles in 12% SDS-PAGE gel

Table 4.2. Cold shock proteins profiles in 12% SDS-PAGE gel

	CONTROL	60 min			480 min		
Marker	37 °C	5 °C	10 °C	20 °C	5 °C	10 °C	20 °C
68000 Da	83111 Da	86300 Da	83111 Da	86400Da	86300Da	83111Da	86300 Da
	77379 Da	80142 Da	77379 Da	83111Da	80142Da	80142Da	77379 Da
	68000 Da	31170 Da	68000 Da	80145Da	70125Da	70125Da	31100 Da
	24129 Da	24391 Da	24391 Da	31166Da	25213Da	24933Da	25500 Da
	22240 Da	22666 Da	22666 Da	25500Da	23134Da	22897Da	23375 Da
		21576 Da	22000 Da	23375Da	21576Da	22000Da	22240 Da
			21576 Da	22400Da		21576Da	

At the stage when incubation period is 480 minutes, if the organism is produced at 5 ºC, protein bands with weights of 86300, 80142, 70125, 25213, 23134 and 21576 Da are detected in SDS-PAGE gel. Among these structures, fractions with weights 86300, 80142 and 21576 Da have the same molecular weights with the ones that are obtained when incubation is applied at 5 ºC for 60 minutes. This situation shows that the change in incubation period does not make any difference on cold shock response in *P. aeruginosa* balcalı-2 strain.

When organism is produced at 20 ºC, it is observed that 6 different proteins with weights 86300, 77379, 31100, 25500, 23375 and 22240 Da are synthesized. Among these proteins, the structures with weights 31100, 25500, 23375 Da are detected for the first time. On the other hand, the structures with weights 77379 and 22240 Da are produced also when organism is produced at 37 ºC. These structures coming out at 20 ºC (they are synthesized at 37 ºC) make us consider that their priority is not high in cold shock response.

Among the proteins, the structure with weight 86300 Da is realized after incubation at 5 ºC for 60 and 480 minutes. This structure being synthesized at both periods and at 5 ºC, shows that it is responsible for cold shock response. [12, 13, 14, 15, 16]

At the end of incubation performed in *P. aeruginosa* ATCC 27853 strain at 37 ºC, 6 proteins with different molecular weight are synthesized. It is observed that 15 proteins are synthesized when incubation period is selected as 60 minutes and 12 proteins are synthesized when incubation period is selected as 480 minutes. (totally 27)

At the end of incubation performed in *P. aeruginosa* balcalı-1 strain at 37 ºC, 9 proteins with different molecular weight are synthesized. It is observed that 23 proteins are synthesized when incubation period is selected as 60 minutes and 13 proteins are synthesized when incubation period is selected as 480 minutes. Totally 36 proteins are synthesized in the cold shock study made with this strain.

At the end of incubation performed in *P. aeruginosa* balcalı-2 strain at 37 ºC, 5 proteins with different molecular weight are synthesized. It is observed that 20 proteins are synthesized when incubation period is selected as 60 minutes and 19 proteins are synthesized when incubation period is selected as 480 minutes. Totally 36 proteins are synthesized in the cold shock research made with this strain.

When Cold adapted *Pseudomonas flourescens* bacteria is produced at 5 ºC, 5 cold shock proteins are synthesized. When *Pseudomonas flourescens* is produced at 0 ºC, it is mentioned that increase occurred in sythesis of 28 proteins [17].

In both 3 organisms highest amount of protein is realized in 60 minutes of incubation at 10 ºC. This result shows that cold shock response reaches to maximum level at the latent period of the organism and at 10 ºC (just like mentioned in the literature) [10]

Among the strains used in the research, *P. aeruginosa* balcalı-1 and 2 strains created a higher level of cold shock response compared to *P. aeruginosa* ATCC 27853 strain.

As also mentioned in the literature, there are researches related with possibility of cold shock protein synthesizing organisms being used in removing environmental pollution, especially in water treatment systems. In contrary with other psychotropic world, the roles of *Acinetobacter sp.* in treatment of waste water, biosurfactant production and lipolitic enzymes are excessively researched[18, 19]. In this context, especially *P.aeruginosa* balcalı-2 strain has potential to be used for this purpose. Besides, Pseudomonas type bacteria are the most problematic bacteria of food industry. If these cold shock proteins can be analyzed from bacteria, the proteins synthesized at cold by these microorganisms can be expressed and a longer shelf life in foods, especially in diary industry.

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