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PROTEOMIC RESPONSE OF HIMALAYAN PSYCHROTROPHIC BACTERIUM *PSEUDOMONAS LURIDA* NPRP15

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Abstract: Low temperature proteomic response of psychrotrophic bacterium *Pseudomonas lurida* NPRp15 was investigated. Culture was subjected to cold shock (28°C to 4°C) and cold acclimatization at 4°C and 15°C. Subsequently bacterial cellular proteins were extracted and separated by SDS-PAGE. Statistical tools (Principal component analysis) were employed to analyze the cold induced and cold acclimatize proteins based on their variations in their expression level. Noticeable variations were observed in protein expression levels for cold shock and cold acclimatized bacterial cells. Similarly PCA analysis clearly classified four groups of proteins depending upon their expression levels. Group I contain 4 proteins that expression level was instantly higher in cold shock and referred as cold shock proteins (Csps). While, Group II contains 5 proteins categorically expressed immediately after cold shock and continuously expressed at the end suggested their role as both cold shock proteins (Csps) and cold acclimation proteins (Caps). Whereas group III contained those proteins whose expression level was not affected or slightly lowered during cold shock. Fourth group proteins expression level was found lowered and suggested their negligible roles in bacterial cold adaptation. The findings of present study indicated that psychrotrophic *Pseudomonas lurida* NPRp15 expressed considerably some sets unique higher molecular weight cold induced proteins that are playing vital role in bacterial cold adaptation.

Key words: Psychrotrophic, Cold shock protein, cold acclimation protein, PCA, Cold temperature, *Pseudomonas*

INTRODUCTION

Microbial growth contains various series of biochemical reactions and severely affected by external temperature variations. Proteins are most inclined living cell components and slightly configuration changes could cause their functional inactivation (Berger *et al.,* 1996; Bisht *et al.,* 2013ab; Bisht *et al.,* 2014; Bisht *et al.,* 2015). To cope with the temperature shift in natural or artificial environment, regulative proteins and key metabolic enzymes changes with response to new temperature. In the same way cold shock proteins (Csps) and cold acclimation proteins (Caps) in microorganisms describe a specific pattern of gene expression in response to abrupt shifts to low temperatures. Such type of cold temperature impact on bacterial growth could be explained by using the Arrhenius equation. It was found that for most psychrotrophic microorganisms, the slope of Arrhenius plots is linear over a wide temperature range viz; 10-30°C (Harder and Veldkamp, 1967; Ingraham *et al.,* 1963; Bisht *et al.,* 2013b; Bisht *et al.,* 2014). Temperature variation within this range cause rapid changes in bacterial growth rate and the proteins expression even contents of cells remain comparatively constant. Temperature higher and below than the Arrhenius linear range, microorganisms growth ceased immediately and this early temperature variation affects microorganism physiology and induce the synthesis of specific sets of stress regulating proteins (Herendeen *et al.,* 1979). Adaptation to upset conditions is important for survival, especially once stress causes major alterations within the metabolism. In the same case abrupt decrease in temperature cause severe effects, the foremost vital ones being the reduction of membrane thinness, organelle potency and will increase the stabilization of secondary structures of nucleic acids, which will have an effect on transcription, translation and DNA replication (Phadtare *et al.,* 2000).

Cold shock protein synthesis induces in response to a shock or a shift to a low positive temperature (Lottering and Streips, 1995; Balhesteros *et al.,* 2010). Whereas Caps are proteins specifically synthesized throughout continuous growth at cold temperatures (acclimation) (Whyte and Inniss, 1990; Bisht *et al.,* 2013; Bisht *et al.,* 2014; Bisht *et al.,* 2015). However, a new term, cold-induced proteins (CIP) has been projected by Graumann and Marahiel (1996) to outline Csp and Cap. Cold-induced proteins were antecedently known as Csps and restricted to the minute acidic proteins. While it's opposite the Caps are clearly distinct from Csps in psychrotrophic and psychrophilic microorganisms as compare to mesophiles (Berger *et al.,* 1996; Michel *et al.,* 1997; Bisht *et al.,* 2014).

In the past, plenty of studies were allotted primarily with mesophilic organisms and the cold shock response in *Escherichia coli* and *Bacillus subtilis* has been studied in detail (Ermolenko and Makhatadze, 2002; Bisht *et al.,* 2014). Contrary to this relatively less attention has been given to native's bacterial adaptation mechanisms viz; psychrotrophic bacterium once subjected to cold

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temperatures. This would be able to conjointly result in the identification of the distinctive adaptation mechanisms in various types of organisms for growing at low temperatures. In spite of a wealth of knowledge accumulated in recent years, the cold shock and cold acclimation response is not fully elucidated for psychrotrophic microorganisms belong to Himalayan regions. Therefore an attempt was made in this direction to carry out proteomic response of psycrotrophic Himalayan bacterium *Pseudomonas lurida* NPRp15 at low temperature.

MATERIALS AND METHODS

Bacterial strain and culture conditions

The psychrotrophic plant growth promoting *Pseudomonas lurida* NPRp15 was previously isolated from rhizospheric soil of Pea plant (Mishra *et al.,* 2011; Bisht *et al.,* 2013). The culture 16S RNA gene sequence was submitted to gene bank (Gene Bank accession number EU601177) and deposited in Microbial culture collection under the accession number MTCC 9246. The bacterial culture was maintained in Kings B Agar (KB) slants and preserved in 60% glycerol at -80°C. Bacterial culture was revived and sub-cultured before use in the present study.

Effect of temperature on growth pattern

In order to set the optimal and cold shock temperatures, respectively, an Arrhenius plot of the growth of isolate was computed. For this, cells were grown at temperatures from 4°C to 33°C in 100 ml of the LB broth medium broth (initial population 6.5 log CFU ml^{-1}). The cell population was determined after each 2h intervals. The numerical values were log transformed and plotted against time. The k (expressed in generations per hour) was determined as the slope of a semi-logarithmic plot of the CFU ml^{-1} versus time. Arrhenius plots of the growth of test isolate were established by plotting the log of the specific growth rate constant μ (per hour) against the reciprocal of absolute temperature K (in Kelvin's) (Berger *et al.,* 1996).

Detection of *cold shock (Csps) and cold acclimation proteins (Caps)*

For cold shock experiment, culture was grown in 100 ml LB broth at 28°C in incubator shaker. After 28 h of incubation, culture was transferred to preset incubator shaker at 4°C. After 0, 2, 4, 8, 12, 16, 20 and 24h of cold shock, 2 ml culture was taken out for extraction of total cellular protein and 1 ml culture was taken for estimation of population density. For cold acclimation proteins, culture was grown in 100 ml LB at 4°C and 15°C for 48 h.

Extraction and Quantification of protein of total cellular protein

Total cell protein was extracted by using sonication (Soni Prep 150, Sanyo). For this, cells were harvested by centrifugation at 6,000 rpm for 5 min at 4°C. The cell pellet was washed three times by using 0.85% saline phosphate buffer (pH 7.2). Then cells were suspended in 0.5 ml of extraction buffer containing 2.5% SDS, 4 mM EDTA in 15 mM Tris-HCl (pH 8.8) by vortexing. Then cells were disrupted by sonication (Soni Prep 150, Sanyo) in 3 cycle at 6 μm (amplitude) for 2 min after 45 second cooling interval in -20°C cooling box. Sonicated cell lysate were purified by precipitation by adding 4 volumes of cold acetone in and incubated at -20°C for 16h and acetone was removed by centrifugation at 10,000 for 15 min at 4°C and pellet was washed with 85% ethyl alcohol and followed by air dried. The pellet was resuspended in 200 μl extraction buffer containing 1 mM PMSF and samples were stored at -20°C till further use. The protein concentration was determined in each sample by Bradford method with Bovine Serum Albumin as standard (Bradford, 1976). The final concentration of protein in each samples were reduced to 20 μg ml^{-1}.

Detection of proteins by SDS-PAGE

The extracted and quantified protein (whole cell) was resolved in a 12% SDS- Polyacrylamide gels according to the method of Laemmli (1970) at 50 Vcm^{-1} for 20 h. A medium molecular weight protein marker (Range 18.4 to 97.4 kDa) was also loaded for the determination of molecular weight of unknown protein. The protein bands were developed by immersing the gel in 0.05% Coomassie Brilliant Blue R250 stain for 5 h followed by destaining with 3% NaCl until the background is clear. The resolved protein bands, after separation on the gel, were visualized and documented using a gel-documentation imaging system (Alpha Imager TM 1200).

SDS-PAGE Image and statistical analysis

The quality of the gels was ensured using the quality control (QC) application of the software. This includes an analysis of image compression, image saturation (spot exposition), image bit depth, available dynamic range and intensity levels in use, alteration due to edited image and intensity level resolution. Only those images which passed the available quality control were included in the analysis. To determine molecular weight MW (kDa) of protein, molecular analysis tools of Alpha Imager EP software were used by putting known molecular weight protein marker as standard. Spot quantifications were realized by computing scanning densitometry by using analysis tools of Alpha Imager EP software. The densities of individual proteins were determined by surface integration. The background corrected abundance of each spot was

calculated and the abundance ratio was determined by dividing the sample abundance by the reference abundance. Proteins with increased expression level (compared with the whole-spot density) at cold temperature compared to the optimum growth temperature were referred to as Csps or Caps, depending on the experiments.

Principal component analysis (PCA) was used to separate the proteins according to variations in synthesis to display the correlations between the various proteins and the affinities between the different cold treatments. For this, a PCA was performed for all detected expressed and under expressed proteins. PCA analysis was carried out using the XLSTAT Addinsoft SARC version 2011.

RESULT AND DISCUSSIONS

In the present study, various tools were applied to investigate low teprature proteomic response in psychrotrophic *Pseudomonas lurida* NPRp15. In the same direction growth range of bacterium was found to be between 0 to 35°C and optimum growth temperature was recorded 28°C (Figure 1). In psycrotrophic bacterium similar growth pattern was earlier observed for *Pseudomonas* sp. NARs9 and *Pseudomonas* sp. PGERs17 (Berger *et al.,* 1996; Mishra *et al.,* 2008; Mishra *et al.,* 2009). Analysis of optimal and threshold stress (cold condition) temperature for the growth of test strain *Pseudomonas lurida* NPRp15, an Arrhenius plot was made (Figure 2) by expressing log of the specific growth rate constant μ (per hour) against the reciprocal of absolute temperature (K). The plot was linear from 30 to 7°C (the normal growth temperature range) and at temperatures below 7°C the slope decreased sharply. While maximum upper growth limiting temperature was recorded 35°C. The linear Arrhenius plot part of this bacterium corresponding to the normal growth range spanning 30 to 7°C (Figure 2), whereas below and above this range were less favorable for growth for most of the psychrotrophs (Berger *et al.,* 1996; Bisht *et al.,* 2013; Bisht *et al.,* 2014; Bisht *et al.,* 2015). Based on the observation of Arrhenius plot for the growth of *Pseudomonas lurida* NPRp15 three temperature ranges can be defined as under (i) an Arrhenius zone from 7°C (critical temperature, $T_{critical}$) to 30°C (including optimal temperature, $T_{optimum}$), within which the activation energy of growth is constant; (ii) a cold shock sub-range (below 7°C) or stress temperature and (iii) heat shock sub-range (above 30°C). According to Arrhenius plot, a temperature of 28°C corresponds to the optimal temperature ($T_{optimum}$) for *Pseudomonas lurida* NPRp15. A temperature of 4°C, below the critical temperature ($T_{critical}$) was chosen for cold shock experiments.

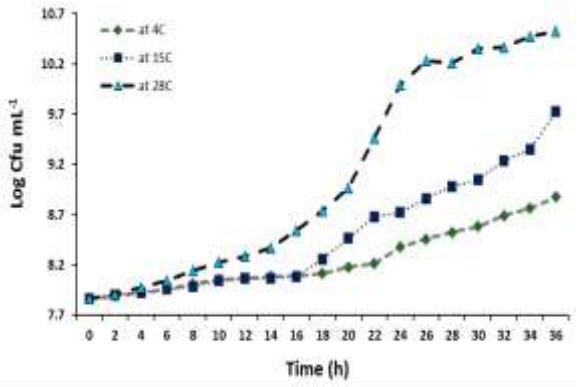


Figure 1 Growth curves of *Pseudomonas lurida* NPRp15 at three different temperatures.

Mid-exponential-phase cultures of *Pseudomonas lurida* NPRp15 were subjected to cold shock from 28 to 4°C, growth ceased immediately for 2 to 4h as measured by cell population (Figure 3). Psychrotrophic bacteria respond according to the amplitude of the cold shock to temperature downshifts by a lag period before growth as reported earlier (Mishra *et al.,* 2008; Mishra *et al.,* 2009; Michel *et al.,* 1997; Garnier *et al.,* 2010). In the present study, rapid change from high to low temperature, when applied to mid-exponential growing cells of test strain, induced a lag period (4h) whose duration increased with the magnitude of the shift. After the 4h lag phase, growth resumed with a characteristic growth rate as observed for previously grown culture at 4°C. Such results are determined earlier in the mesophile like *E. coli* (Jones *et al.,* 1987) the psychrotrophic *Lactococcus piscium* Strain CNCM I-4031 (Garnier *et al.,* 2010), *Pseudomonas fragi* (Michel *et al.,* 1997), *Arthrobacter globiformis* (Berger *et al.,* 1996), *Listeria monocytogenes* and *Listeria innocua* (Phan-Thanh and Gormon, 1995),On the contrary, other microorganisms such as psychrotrophic *Vibrio* sp. (Araki, 1991) and the mesophilic *Lactococcus lactis* subsp. *Lactis* (Panoff *et al.,* 1995) and *Bacillus subtilis* (Lottering and Streips, 1995) had no lag phase consequent to a cold treatment. Their growth continued at an intermediate rate followed by a growth rate characteristic as commonly observed at low temperature. Thus, the time to readapt to the low temperature is not directly dependent on the growth temperature range of bacterium, i.e., of its psychrophilic, psychrotrophic, or mesophilic nature.

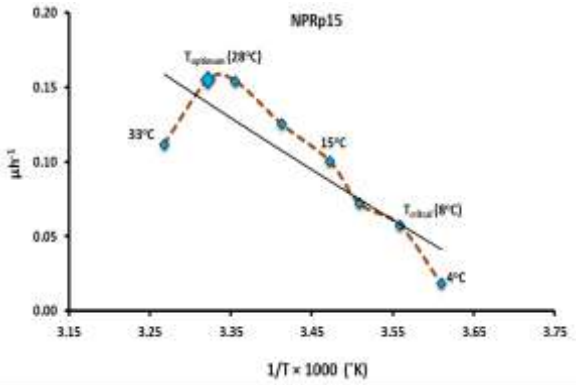


Figure 2: Arrhenius plot of the relationship between growth rate (k) and temperature (Kelvin's) for *Pseudomonas lurida* NPRp15. (Some datum points are marked with the corresponding temperature in degrees Celsius.)

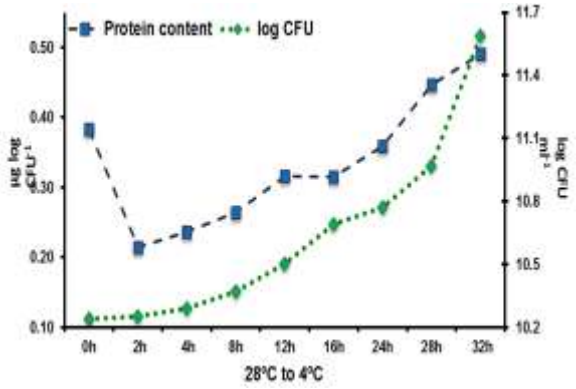


Figure 3: Effect of cold shock on *Pseudomonas lurida* NPRp15 growth and protein content

The cold shock in present study characterized by the reduced protein content led to cessation of growth immediately for 2 to 4h. Total protein content after the early 2h cold shock was dramatically lowered down. However as growth resumed the protein content was also found corresponding to growth. It was interesting to observe that the cell survival was 100% because there was no change in cell population before the shift and after the shift. In the same way, this criterion of classification according to temperature range of growth fails to predict the consequences of cold shocks on protein synthesis. Indeed, after cold shock treatments, the relative rate of synthesis of most cellular proteins was maintained in psychrotrophic *Pseudomonas lurida* NPRp15. Similar results were observed previously for psychrotrophs such as *Bacillus psychrophilus* (Akaki, 1991) *Arthrobactor globiformis* (Berger *et al.,* 1996) and *Pseudomonas* (Bisht *et al.,* 2014; Bisht *et al.,* 2015). Also there are reports from mesophiles such as *Bacillus subtilis* (Lottering and Streips, 1995). Therefore it could be assumed that the test strain respond to the cold stress by the overexpression of a subset of proteins called Csps (cold shock proteins).

Protein steadiness is known to vary greatly with temperature change. Therefore, the protein content of bacteria is expected to change with incubation temperature. The mesophilic *E. coli* cells growing outside the normal temperature range are characterized by pronounced modifications of the levels of most proteins (Herendeen *et al.,* 1992). In the current study, we found that the SDS-PAGE protein pattern of cold tolerant pseudomonad cells growing at 28°C and 4°C showed highly altered levels of few proteins (Figure 4, Figure 5).

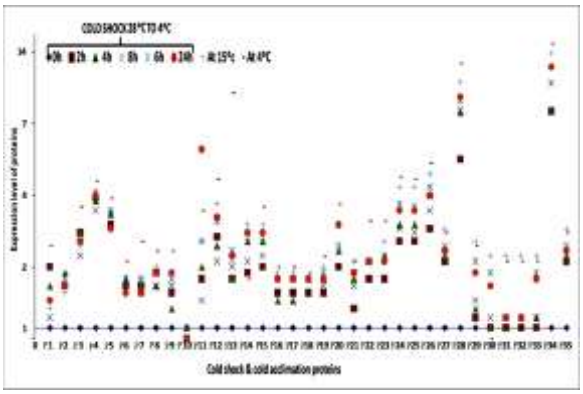
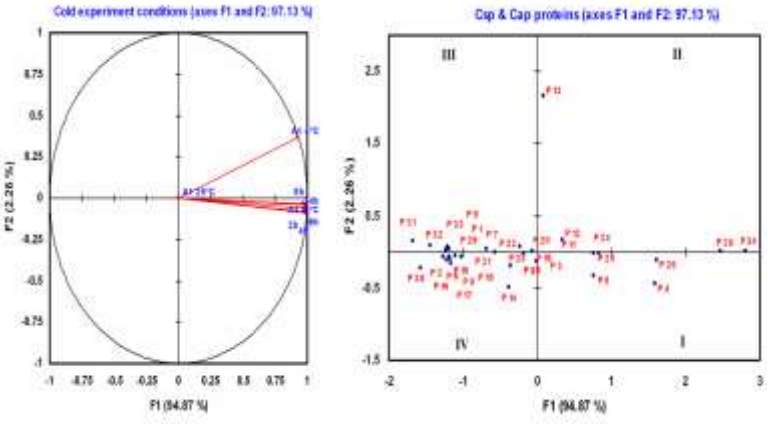


Figure 4: Scatter plot of cellular protein profile of *Pseudomonas lurida* NPRp15 subjected to cold shock from 28 to 4°C and cold acclimation proteins at 4°C and 15°C

Note: Proteins values: (>1) indicates decrease in rate of synthesis; (<1) indicates increase in rate of synthesis; (=1) indicates no change in rate of synthesis



[A] **[B]**

Figure 5: Principal component analysis (PCA) of cold shock and cold acclimatization proteins profile of *Pseudomonas lurida* NPRp15. (Factor map of rows (i.e., proteins); proteins with similar distributions of appearance with increases in cold shocks duration occur in similar positions on the map, while positions of some Csp showing as role of cold acclimation proteins.)

Consequently the comparative SDS-PAGE analysis of whole cell protein profile of the test isolate revealed that there is a marked variation in the expression of majority of the genes products at growth temperature of 4 and 28°C (Figure 4, Figure 5). The test strain responded to cold shock by over expressing a total of 29 proteins. Interestingly, expression of all these proteins was higher when test isolate was cold acclimated at 4°C. Nine proteins (numbered P3, P4, P5, P12, P24, P25, P26, P28 and P34) presented an increased level of expression only 2h after cold shifts (Figure 5) and their syntheses continue to increase with the cold shock duration. In general there was more expression of proteins at 4°C compare to the growth at 28°C. On the other hand, few proteins whose expression was not much altered while growing the isolates at these temperatures can be regarded as the product of housekeeping genes. Similar observation was found in present study for two proteins (P10 & P30), those were almost similarly expressed at 28°C and 4°C temperatures and could be regarded as housekeeping proteins. Whereas 10 proteins expressed under 2 fold level, while rest of 23 proteins showing more than 2 fold expression at 4°C. Conversely 13 proteins (numbered P3, P4, P5, P11, P12, P13, P15, P20, P24, P25, P26, P28 and P34 with molecular masses ranged from 18.6 to 101.6 kDa) presented an increased level of expression (3.1 to 15.5 fold) at the cold temperature (4°C) and considered to be Caps (Figure 5). The expression of these Caps was noticed to be higher at 15°C also. Nine of these Caps were found more than 2 fold expressions after early cold shocks (2h after shift). Three Caps proteins P13, P28 and P34 were found to be highly expressed at 4°C (Figure 4, Figure 5). Earlier studies on *A. globiformis* SI55 have revealed that 18 peptides are newly synthesized or present at increased level at 4°C (Berger *et al.,* 1996). In relation to this finding we can suggest that these pseudomonad strains synthesize higher level of proteins to cope with low temperatures. Similar proteins called Caps (cold acclimation proteins), have been found to be produced by other psychrotrophic bacteria, *B. psychrophilus* and *Pseudomonas fragi* when grown at 0 and 4°C, respectively (Michel *et al.,* 1997; Whyte and Inniss, 1992; Hebraud *et al.,* 1994). In present study, two proteins P13, P15 with molecular weight 34.53 KDa, 38.51 KDa were absent at 28°C but got over expressed following shifts to cold shock and their expression increased with the cold shock duration (Figure 4, Figure 5). These proteins may, therefore, be involved in maintaining some metabolic functions at low temperature by replacing cold-denaturated peptides. A cold temperature specific proteolytic system has been described for several psycrotrophic bacteria, and some of these Caps could act as cold-specific proteases that eliminate denaturated proteins whose accumulation would be deleterious for the cells (Berger *et al.,* 1996; Michel *et al.,* 1997; Garneir *et al.,* 2010; Bisht *et al.,* 2014). Others could be involved in maintaining membrane fluidity at low temperature or may act either as antifreeze proteins or as anabolic enzymes involved in the synthesis of putative antifreeze substances.

In present study, we monitored the kinetics of protein variations during the early hours following the downshifts in temperature from 28 to 4°C. This analysis led us to classify the proteins in four groups according to their kinetics and ways of variations and to derive at least two classes of proteins, the well-known Csps and the cold acclimation proteins (Caps) - involved in cold adaptation. As previously defined, the term "Csps" is used here for proteins that are transiently overexpressed, and the term "Caps" is used for proteins whose production is increased continuously in cells adapted to the low temperature. While the distinction between these two classes of proteins is obvious and variability in the kinetics of induction of individual proteins within each class may exist.

In the current study, statistical analysis of the results by PCA was used to group whole cell proteins of test isolate according to their expression level. PCA revealed that two factor axes (designated F1 to F2) to represent 97.13% of the variability of the data. The factor F1 represent 94.87% and 2.26% for the factor F1 variability of the data, the factor scores of these first two axes of PCA were used to represent the data (Figure 5A). Proteins with similar distributions of appearance after cold shocks interval occur in similar positions on the map of rows (Figure 5B). The first axes (F1) of the factor map represent the highest variance of data and positive values of this axis correlated with those proteins that played important role in bacterial cold adaptation. This analysis clearly demonstrated the existence of four distinct groups of proteins (Group I to Group IV) (Figure 5). Group I proteins correspond to early Csps that were overexpressed immediately after the shift and late Csps which were overproduced only 2 h or 4h postshift. Most Csps were optimally induced during the lag phase, and their synthesis decreased with growth resumption. Csps are undoubtedly involved in the adaptation of cells to the new cold environment. As demonstrated for many psycrotrophic and mesophilic bacteria (Garnier *et al.,* 2010), the adaptive response is greatly involved in translation (Jones *et al.,* 1996). Proposed an interesting cold shock ribosome adaptation model to explain the induction and function of the cold shock response in *E. coli*. They suggested that a shift to low temperature results in a cold-sensitive block in initiation of translation of most cellular mRNAs, whereas mRNAs for the cold shock proteins (including CspA) can be efficiently translated. Cold shock proteins RbfA, CsdA, and initiation factor 2

(2α and 2β) associated with ribosomes and resulting in efficient translation of cellular mRNAs. Therefore, as cells adapt to the low temperature, there is an increase in protein synthesis accompanied by repression of the cold shock response.

In the present study, second group of proteins could also be considered Caps, indeed as Csps, that were found to be rapidly induced in our test isolate. Their relative rate of expression remained high for a number of hours under cold shock condition 4°C as well as continuous growth at the same temperature. Similar group of proteins have been previously characterized by their continuous overexpression in psycrotrophic cells adapted to low temperatures (Berger *et al.,* 1996; Michel *et al.,* 1997; Bisht *et al.,* 2013). It was also the case for the many Caps, whose increased synthesis took place later during the lag phase. In the present case, distinction between these two Csps and Caps was not always obvious and sometimes found to vary with each strain to strain. However, the common characteristic of group II proteins was their higher level of proteins expressions at 4°C than at the preshift temperature, even several hours after growth resumption. These proteins, therefore, may be involved in cellular processes allowing continuous growth in a cold environment. Some proteins could operate in maintaining metabolic pathways or membrane fluidity at low temperatures. Others could be involved in cold-specific proteolysis of denaturated proteins as described for psrcrotrophic *A. globiformis* (Potier *et al.,* 1987). Moreover, it is tempting to suggest that the rapidly induced group II proteins could be involved in similar adaptation mechanisms as Csps. The variability in the kinetics and levels of induction of Csp and Cap to the cold shock amplitude indicated common targets for these two groups of proteins. Such overlapping of the conditions of expression of some Caps which are rapidly and strongly expressed under cold shock conditions, such as Csps, have been described for both psychrophiles and psychrotrophs (Michel *et al.,* 1997).

The third group (Group III) based on PCA analysis in the present study contain proteins that were rapidly repressed after early cold shock, and transiently expressed in the middle of cold shock. However their relative rate of synthesis was found high under cold acclimation (4°C). Thus these proteins can be inferred as the early Caps, whose increased synthesis initiated during the lag phase. However, the common characteristic of group III proteins were higher level of synthesis at 4°C compared to optimum temperature, even after several hours of growth at cold temperature. These proteins, therefore, be involved in cellular processes allowing continuous growth in a cold environment. Similar types of protein kinetics were described for psycrotrophic *A. globiformis* and *P. fragi* when they were exposed to mild cold shock and cold acclimation condition (Berger *et al.,* 1996; Michel *et al.,* 1996).

In the present study, fourth group included those proteins, whose synthesis decreased after the cold shock or remained the same. Such repressed proteins were previously designated as Haps (heat acclimation proteins) by previous researchers because of their optimal expression in cells grown at steady-state at high temperature (25 to 34°C) (Hebraud *et al.,* 1994; Michel *et al.,* 1996). Michel *et al.,* (1996) subsequently characterized four peptides as Hsps because of transient overexpression upon heat shock treatments. The synthesis of such proteins was found to repress in *E. coli* when the growth temperature was shifted from 37 to 15°C (Jones *et al.,* 1987). This repression indicates that cells have recovered steady-state physiological conditions. Similar observations were found in psycrotrophic bacterium *A. globiformis* and *P. fragi (Berger et al., 1996;* Michel *et al.,* 1996). These proteins previously reported in psychrotrophic and mesophilic bacteria were regarded as housekeeping proteins (Berger *et al.,* 1996*;* Hebraud and Guzzo, 2000).

In present study, continuous growth at 15°C is characterized by the reduced number of overexpressed proteins as well as a low number of repressed proteins. Since it does not cause large changes in protein expression and probably it is not a stress temperature. This transfer temperature corresponds to the linear range of growth temperature for the test isolates as indicated by the Arrhenius plot. Cold shocks are known to induce modifications in DNA super helicity that are susceptible to modification of the access of RNA polymerase to several promoters (Berger *et al.,* 1996*;* Qoronfleh *et al.,* 1992). Therefore, over-expression of some proteins can be partly explained by the fact that a small set of genes might be turned on by the temperature decrease and the products of which are directly involved in adaptation process. They would therefore appear as early Caps. Thus these proteins are distributed as their synthesis at mild cold shock kinetics. Similar protein profiles at moderate cold temperature were described for psycrotrophic bacteria *Arthrobacter globiformis* SI55 (Berger *et al.,* 1996; Michel *et al.,* 1996).

These Csps may act directly and specifically after a cold shock to prepare the cells for growth at the new temperature, as an example, by initiating the synthesis of late Caps, and/or to repair damage due to

the cold. Although there are very few data on the cold shock response in psychrotrophic bacteria, more Csps seem to be overexpressed in psychrotrophs than in mesophilic bacteria. Julseth and Inniss[31] reported the induction of 26 Csps after a 24 to 5°C cold shock in the psychrotrophic yeast *T. pullulans*. Cloutier *et al.*, (1992) showed that arctic *Rhizobium* strains respond to a very low temperature (-10°C) by synthesizing more proteins than temperate strains do at higher temperatures.

Thus, finding of present study indicates to cope with cold shock test strain regulate protein expression and particularly a subset of proteins that are possibly playing vital role in the adaptation to low temperature. The number of the new Csps (P13, P28 and P34) and Caps (P13 and P15) expressed by isolates in response to cold shock and during cold acclimation are needed to be investigated at molecular structural level so that their exact role in bacterial cold resistance mechanisms may well be depicted and strengthen our knowledge about adaptive mechanisms of cold tolerance in psychrotrophic microorganisms.

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