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Identification of Salinity Induced Polypeptide in Moderately halophilic bacteria

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ABSTRACT

Moderately and halophilic bacteria are known to grow well in the environments with high salt concentration. In order to investigate osmo tolerance in moderately halophiles; *Paenibacillus sp*. strain XII was chosen and grown in the media with different concentration of salt (NaCl), total protein was extracted and analyzed by electrophoresis. A new polypeptide with molecular weight of 21.4 kDa was observed to be expressed by the bacterium in response to salt. The polypeptide was purified and isoelectric point (pI) of which was determined which indicates the protein to be highly acidic. N-terminal sequencing was carried out and sequence homology search was done. A strong homology was found between N-terminally sequenced polypeptide and a segment from the N-terminus of spore coat–associated protein; CotN of *Oceanobacillus iheyensis* an extremely halotolerant of deep-sea.

INTRODUCTION

Moderately halophilic bacteria include some heterogeneous group of bacteria, which are dependent and adapted well to survive in high-salt environments (1-2). To respond hyper saline environment moderately halophiles have evolved different adaptive mechanisms to sense and respond to hypersalinity (3). Among these mechanisms are Production and accumulation of high concentration of a wide range of compounds collectively known as compatible solutes or osmolytes. These compounds are synthesized de novo, or when present externally, accumulate in cytoplasm by transport systems (4). This capability of moderately halophiles has made them useful for production of osmolytes, such as glycine-betaine and ectoine (5). Moderately halophiles must have numerous potentials for application in biotechnology among which are; being as a cheaper alternative for the current chemically based approaches for removal of phosphate from saline environments, could be used for recovery of hyper saline waste brines derived from different industrial processes, their possible application in production of bioactive compounds such as antibiotics or biological surfactants that have a number of applications in the industry (6) and yet many other possible applications.

Our goal for undertaking the present study was based on the investigation of the molecular aspects of resistance to the osmotic stress. Moderately halophilic bacterium was used and its response to osmo-stress was studied. This study has focused on proteins which might be involved in osmo-protection and conferring the bacterium to endure the hypersaline condition. Our results indicate that such osmo-protection could be attributed to the expression of specific proteins.

MATERIALS AND METHODS

Bacterial Growth Media Bacterial strains were grown in nutrient broth (10 g/*l* meat extract, 10 g/*l* peptone, 5 g/*l* NaCl, pH 7.5-7.6), containing different amount of NaCl (% W/V: 1%, 2.5% , 5% , 7.5% , 10% , 12.5% and 15%, final concentration). Bacterial growth rate was monitored by applying measurement of OD at 620 nm at different time intervals (0-45 h).

Protein preparation and Electrophoresis Total bacterial protein was prepared according to Souza *et al*. (7) and used for protein assay and analysis by SDS-PAGE according to Laemmli on 13% SDS-polyacrylamide gel (8).

Polypeptide purification To isolate specific polypeptide band(s) preparative gel was used (8). Following to electrophoresis, staining and destaining the target polypeptide band was cut out and transferred into dialysis bag containing elution buffer (192 mM glycine, 25 mM Tris.HCl, pH 8.3). The polypeptide was eluted from the gel by applying constant current (5 mA at 4^{o}C overnight).

Isoelectric focusing Capillary Isoelectric focusing gel was performed according to method describe by OFarell (9), at the end of focusing, gels were extruded from each tube for staining and pI determination. For determining the pI of polypeptide, gels were cut into 0.5 cm long

pieces, each piece was placed in a separate tube containing 2 ml of distilled H_2O, crushed and incubated at room temperature for 3 h then the pH of each tube was measured (9). For staining gels were impregnated in distilled H_2O until ampholine was completely diffused out, stained and destained as above.

Protein sequencing N-terminal amino acid sequencing of protein was performed in the laboratory of biochemistry of protein, University of Gent, Belgium using Applied Biosystems 494 automated sequencer according to the method of Edman.

RESULTS AND DISCUSSION

Moderately halophilic bacteria are defined as those that grow well in presence of 0.5–2.5 M of salt. It is thus rational to suggest that such organisms to be capable of undertaking mechanisms that enable them to apply, sustain, grow and reproduce in such a condition. we found a strong similarity between spore coat associated protein; CotN of this bacillus and our identified polypeptide in *Paenibacillus* sp. strain XII both in pI, molecular weight and partial amino acid sequence.

Growth characterization of Paenibacillus To study proteins that are possibly involved in osmo resistance in moderately halophiles; *Paenibacillus* sp. strain XII was grown in appropriate medium supplemented with different salt concentrations. As shown in figure 1 little or no bacterial growth could be observed in nutrient broth which indicates salt dependency of bacterial growth. Growth starts at 1% of NaCl and reaches to the most optimal at 2.5% of salt indicating that the bacterium is a moderately halophyte. In fact growth starts at 1% of salt and increases up to 5% with the best growth at 2.5%. This observation does agree well with what has been defined for moderately halophytes which suggests growth capability in media with salt concentration ranging from 0.5 M up to 2.5 M (1, 2). Following to determination of the optimal salt requirement (0.5 – 0.9 M of salt) a gradual increase of salt was applied to determine the uppermost salt tolerance of the bacterium. Increasing salt concentration up to 15% (2.6 M of salt) has completely arrested bacterial growth while for lower concentrations of salt (10% and 12.5%) a minimal growth could be observed after 28 h (Fig. 1).

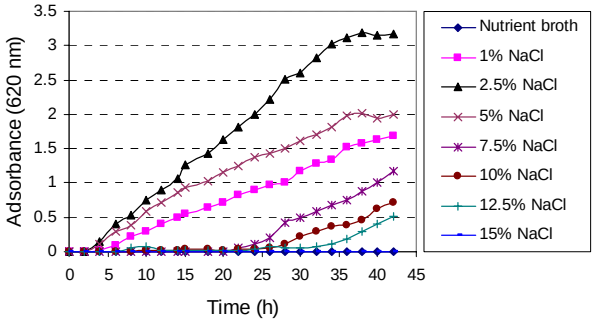
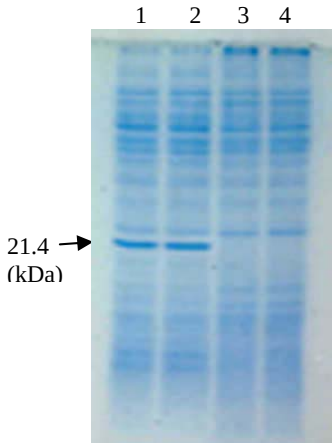


Figure 1: The growth behavior of *Paenibacillus* sp. strain XII in different media supplemented with different concentration of salt (NaCl) measured at 620nm. The bacterial growth is dependent to a basal level of salt that was determined to be 1%. The best growth was observed at 2.5 % (0.5 M) of salt and to lesser extent at 5% (0.9 M) of NaCl. Increasing salt concentration reduces growth of bacterium to a complete arrest at 15% of salt.

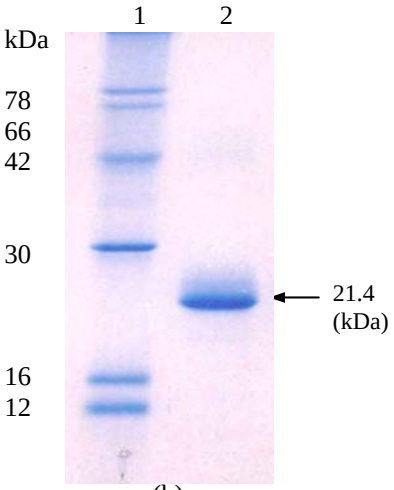
SDSPAGE analysis and characteristics of polypeptide profile in response to salt Following to this preliminary study, the bacterium was grown and total protein was extracted for analysis of protein profile in response to salt by electrophoresis. Applying different concentrations of salt, it was found that at 7.5% of salt a new polypeptide with molecular weight of 21.4 kDa is expressed by the bacterium (Fig. 2a).



(a)

Figure 2a: Electrophoretic pattern of polypeptides of *Paenibacillus* sp strain XII carried out on a 13% acrylamide gel; Lanes 1 and 2, protein profile of two separate bacterial cultures that were grown in the medium containing 7.5% NaCl (W/V); Lanes 3 and 4, the corresponding negative controls.

Figure 2b: Electrophoretic analysis of 21.4 kDa purified protein on a 13% acrylamide gel. Lane 1; molecular weight marker, Lanes 2 purified protein.



(b)

The polypeptide band was carefully removed, eluted from the gel and subjected to SDS-PAGE for both determination of molecular weight (Fig. 2b) and verifying its purity. The molecular weight of polypeptide was determined based on the comparison of relative mobility (Rf) of the polypeptide with that of molecular weight standards according to Hams and Rickwood (10).

Determination of isoelectric point (pI), N-terminal sequencing and data base analysis Further information regarding with the isolated polypeptide was obtained by determining isoelectric point (pI). Isoelectric focusing (IEF) was carried out and the pI of 4.8 was calculated (Fig. 3) which indicates the polypeptide is highly acidic.

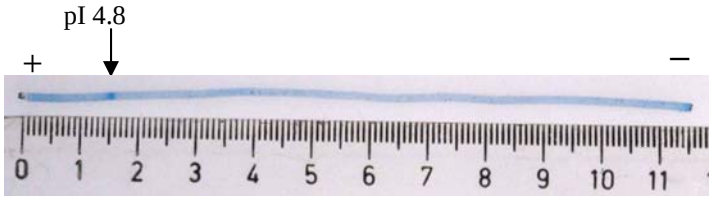


Figure 3: Isoelectric focusing and determination of pI of the 21.4 kDa polypeptide shown in figure 2.

To finalize our study N-terminal sequencing of polypeptide was carried out and ten amino acids; "YFSDAETTNN" was determined. To identify the possible identity of the polypeptide further analysis was done by applying sequence similarity searches with that of other accessible polypeptide sequences by applying BLAST program available in data banks. The ExPASy Proteomics tools (http://us.expasy.org/tools/, with accession numbers: OB1302 or AP004597), and the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/ with accession No: NP_692223) were used and the most similarity was determined. From the features of the partial amino acid sequence, similarity searches, MW and pI, the salt inducible polypeptide was found to be mostly similar to spore coat associated protein (CotN) of *Oceanobacillus iheyensis* strain HTE831 an alkalophilic and extremely halotolerant bacterium of the deep-sea (11-12) with MW 21508 kDa, 195 amino acids according to UniProt database; http://www.ebi.uniprot.org/ (accession number Q8ERK0) and estimated pI of 3.81 based on http://us.expasy.org/tools/). The sequence homology was found with a segment of amino acids starting from the amino acid 28 to 37 from the N terminus of Cot N.

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