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Survey the role of *in vivo* and *in vitro* condition to expiration of nano structure para crystalline layer gene in bacteria

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Abstract

Para crystalline layer is a monomolecular outermost protein layer in bacteria and archiae, credit of protein or glycoprotein subunits and has crystalline biopolymer structure. Para crystalline -layer protects bacteria to phagocytosis and prohibit the entry of some biomolecule -for example antibiotics- and adhesion to matrix proteins, is one of virulence agents and layer producer. Para crystalline layer have application potential in biotechnology, molecular nanotechnology, and biomimetics. The research was performed with laboratory method in 2005-2007 years, in Azzahra hospital and Isfahan University. In this research, 26 *Bacillus cereus* strains were studied. Identification of bacteria, was performed with microbiological methods: staining, chemical test, using differential and selective media. Isolation of *Bacillus cereus* strains was performed on Selective Agar, and cultured in TSA, for 16 hours, under aerobic condition. Then subjected to the separation of surface proteins and electrophoresed along with molecular weight marker. Para crystalline layer in *B. cereus* has 97 KD MW. Out of 26 *B. cereus* strain, 14 strain produced Para crystalline layer and 12 strain don't have Para crystalline -layer. Out of 13 isolates from staff hand, 11 sample (84/6%) and from 13 isolates from hospital surfaces, 1 sample (7/7%) have produced Para crystalline layer.

Keywords: Para Crystalline Layer, Bacillus cereus, nosocomial infections

Introduction

Nosocomial infections (NIs) remain a major global concern. Overall, national prevalence rates have been described as ranging between 3.5 and 9.9%. They lead to additional days of treatment, increase the risk of death, and increase treatment costs. Staff hands and hospital surfaces have important role in NIs (Kamp & Kramer, 2004).The health-care environment contains a diverse population of microorganisms (Sehulster & Raymond, 2003).

Microorganisms are present in great numbers in moist, organic environments, but some also can persist under dry conditions. Environmental source or means of transmission of infectious agents, the presence of the pathogen does not establish its causal role; its transmission from source to host could be through indirect means, e.g., via hand transfer. The surface would be considered one of the potential reservoirs for the pathogens. The most important and frequent mode of transmission of nosocomial infections, is divided into two subgroups: direct-contact transmission and indirect-contact transmission (Sehulster & Raymond, 2003).

Direct-contact transmission involves a direct body surface-to-body surface contact and physical transfer of microorganisms between a susceptible host and an infected or colonized person. Direct-contact transmission also can occur between two patients, with one serving as the source of the infectious microorganisms and the other as a susceptible host. Indirect-contact transmission involves contact of a susceptible host with a contaminated intermediate object, usually inanimate, such as contaminated instruments, needles, or dressings, or contaminated gloves that are not changed between patients and staff hands.

Bacillus cereus bacteria are large spore forming, Gram-positive rod-shaped, facultative anaerobes. *B. cereus* strains are common in the environment and can be found in soil, dust, air, water, and on decaying. It has been regarded as a relatively nonpathogenic opportunist commonly associated with enterotoxin mediated diarrheal food poisoning. This organism has been increasingly isolated from serious nongastrointestinal infections including endocarditis, wound infection, osteomyelitis, oral cavity associated with infected root canals, periodontal pockets, bovine mastitis, severe systemic, pyogenic infections, gangrene, septic meningitis, cellulitis, panophyhalmitis, lung abscesses, infant death, and endocarditis and now *B. cereus* regarded one of nosocomial infections bacteria (Van der Zwet *et al.,* 2000; Hilliard *et al.,* 2003; Washington *et al.,* 2006).

Survival spore forming bacteria on hands and surfaces in vegetative cells of can survive for at least 24 h on inanimate surfaces, and spores survive for up to 5 months. Surface structures are an important structural component of prokaryotic organisms and essential for many aspects of their life (Jalalpoor *et al.,* 2007). *B. cereus* produces several potential virulence factors in addition to the toxins associated with gastrointestinal infections, and these factors are thought to playa role in non-gastrointestinal infections. These virulence factors include three hemolysins, three phospholipases, three different beta lactamases, extracellular collagenases, membrane-bound proteases, and para crystalline layer (Jalalpoor *et al.,* 2007, Washington *et al.,* 2006).

Nosocomial outbreaks of Bacillus infections have involved common-source spread from contaminated reservoirs in the environment. These sources have included contaminated hemodialyzers, bronchoscopes,

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Ommaya reservoirs, manual ventilation balloons, multiple-unit injectables, and contaminated diapers, gloves, and surgical bandages (Jalalpoor *et al.,* 2007; Van der Zwet *et al.,* 2000; Washington *et al.,* 2006). All of the various surface components of a bacterial cell are important in its ecology since they mediate the contact of the bacterium with its environment, the only senses that a bacterium possesses result from its immediate contact with its environment.

It must use its surface components to assess the environment and respond in a way that supports its own existence and survival in that environment. In medical situations, the surface components of bacterial cells are major determinants of virulence for many pathogens. The surface properties of a bacterium are determined by the exact molecular composition of its membrane and cell envelope, including capsules, glycocalyx, para crystalline layer, peptidoglycan, LPS, and the other surface structures, such as flagella and pili or fimbriae. Over the past 3 decades of research, it has become apparent that one of the most common surface structures on bacteria are monomolecular Para crystalline arrays of proteinaceous subunits termed surface layer or para crystalline layer. Para crystalline layer is attached to the outermost portion of their cell wall. It consists of a single molecular layer composed of identical proteins or glycoproteins and in electron micrographs, has a pattern resembling floor tiles (Sara & Uwe, 2000; Mesnage *et al.,* 2001; Messner *et al.,* 2008; Sara, 2001).

The para crystalline layer lattices can have oblique (p1, p2) square (p4), or hexagonal (p3, p6) symmetry. Depending on the lattice type, one morphological unit consists of one, two, four, three, or six identical (glyco) protein subunits, respectively, and they exhibit center-to-center spacings of approximately 2.5 to 35 nm. Most para crystalline layers are 5 to 25 nm thick. It is now evident that para crystalline layers are the most common cell surface components of pathogen bacteria such as *Lactobacillus* sp., *Rickettsia* sp., *Serratia* sp., *Caulobacter* sp., *Campylobacter* sp., *Corynebacterium* sp., *Clostridium* sp. and *Bacillus* sp. (Sara & Uwe, 2000; Mesnage *et al.,* 2001; Messner *et al.,* 2008; Sara, 2001).

Because para crystalline layer lattices possess pores identical in size and morphology in the 2 to 8 nm range, occupying up to 70% of the surface area they work as precise molecular sieves, providing sharp cutoff levels for the bacterial cells. para crystalline layers from various Bacillaceae were shown to be suitable for the production of isoporous ultrafiltration membranes with well-defined molecular weight cutoffs. The para crystalline layer lattice and the pore areas of para crystalline layers contain functional groups (carboxylic acid, amine, and hydroxyl groups) which are aligned in well-defined positions and orientations.

The repetitive features of para crystalline layers have led to their use as immobilization matrices for binding of monolayers of functional molecules e.g., enzymes, antibodies, antibiotics and immunogens in a geometrically well-defined way. This application potential has been exploited for the production of bioanalytical sensors, immunoassays, affinity microparticles, and affinity membranes. The para crystalline layer has been associated with a number of possible functions, these include the following:

1. The para crystalline layer protects bacteria from harmful enzymes (para crystalline layer from Bacillaceae were found to function as adhesion sites for cell-associated exoenzymes) and antimicrobial agents.
2. The para crystalline layer protects bacteria from changes in pH.
3. The para crystalline layer protects bacteria from attack by bacterial parasites such as *Bdellovibrio bacteriovorus*, and from bacteriophages.
4. The para crystalline layer can function as an adhesin, enabling the bacterium to adhere to host cells and environmental surfaces, colonize, and resist flushing.
5. The para crystalline layer may contribute to virulence by protecting the bacterium against complement attack and phagocytosis.
6. The para crystalline layer may act as a as a coarse molecular sieve. para crystalline layers can contribute to virulence when they are present as a structural component of the cell envelope of pathogens (Sara & Uwe, 2000; Mesnage *et al.*, 2001; Messner *et al.*, 2008; Sara, 2001).

Spread of para crystalline layer producer *B. cereus* strains in staff hand and hospital surfaces leads to the increase of antibiotic resistant NIs. The aims of this search was to survey frequency of para crystalline layers of *Bacillus cereus* strains in hospital surfaces and staff hands.

Materials and methods

Sampling

A total of 274 bacteria, 194 bacteria from hospital surfaces and 80 bacteria from staff hand were isolated of Azzahra-hospital during of 2005-2007 years. Hospital surfaces samples were randomly collected from high and low hospital contact surfaces with swab (Effective sampling of surfaces requires moistened swabs) in Tryptone Soya Agar (Merck) and staff hand samples, were randomly collected from staff hand in Blood Agar (Merck) via Fingerprint Technique (Jalalpoor *et al.,* 2009; Sehulster & Raymond, 2003).

Bacterial strains

Specimen grown on sheep blood and chocolate agars were incubated at 37°C under aerobic conditions. Bacillus as Gram-positive bacilli, the intracellular and cell-free spores do not stain by the Gram technique but can be visualized with the malachite green stain by which the spores will appear green. On SBA, colonies of *B. cereus* usually large, with a matte or granular texture, and most strains are beta hemolytic. The strains were identified based on colony morphology, Gram stain reaction, spore

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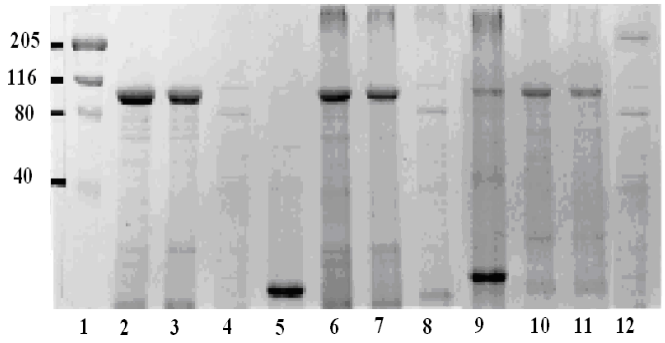
formation, and biochemical tests with the BioMerieux database system (Kotiranta *et al.,* 1998; Kotiranta *et al.,*1999).

Detection of Para Crystalline Layer

For the examination of surface proteins, 16 h old bacterial cells cultured on TSA enriched with 0.6% yeast extract were collected from the agar plates, washed once in phosphate buffered saline (PBS) (pH 7.4), and suspended in the same buffer; the cell suspensions were adjusted to optical density of 0.6 (450 nm). Equal volumes (4 ml) of the cell suspensions were centrifuged (3,000 3 g, 6 min). The pellets were re-suspended in 500 ml of 1% sodium dodecyl sulfat (SDS)-Tris-HCl (pH 8) and shaken for 30 min at RT. After centrifugation, the supernatants were boiled for 5 min in sample buffer (60 mM Tris-HCl, 1% SDS, 10% glycerol, 1% mercaptoethanol, and 0.0005% bromophenol blue) (Kotiranta *et al.,* 1998, Kotiranta *et al.,* 1999) and analyzed by SDS-10% polyacrylamide gel (PAGE) electrophoresis (Sambrook *et al.,* 2001) (Fig. 1).

Fig.1. SDS PAGE of surface proteins in B. cereus strains Lane 1: Myosin 206 kDa- Betagalactosidase 117 kDa- BSA 80 kDa- Ovalbumin, 40 kDa and Lane 2- 12 : B. cereus strains isolated from staff hand and hospital surfaces



Statistical analyses

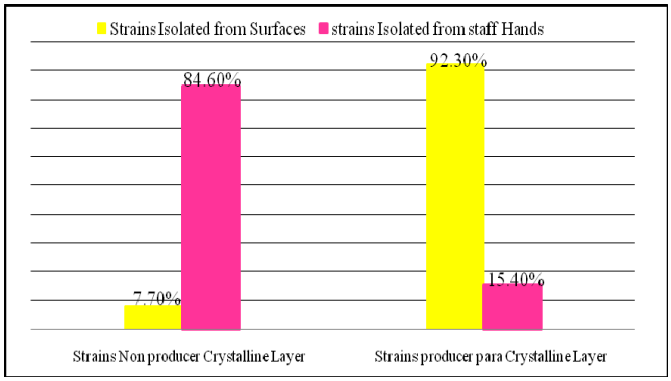
All the statistical analyses were carried out using SPSS version 14. Chi-square and fisher test was used for determination of significance of association. The p≤ 0.05 was considered significant.

Results and discussion

Based on the results obtained in this study, the frequency of *B. cereus* strains on hospital surfaces and staff hand was 6.7% and 16.25% respectively Based on the results of SDS-PAGE, 46.20% of the studied *B. cereus* strains have been para crystalline layer producer and 53.8% lack the ability to produce para crystalline layer (Fig. 2). Thus the 84.6% of *B. cereus* strains isolated from staff hand and 7.7% of the strains isolated from the hospitals surface have been para crystalline layer producer.

According to the results of other similar studies carried out in Iran, *Bacillus* species have been the most bacterial separation from the hospital environment and staff hand. Bacterial strains were isolated from hospital surface 74 (24%) and from staff hands 48 (60%) respectively (Jalalpoor *et al.,* 2009; Jalalpoor *et al.,* 2010; Sambrook & Russell, 2001).

Fig.2. Frequency of para crystalline layer in B. cereus strains



Based on the results of similar studies in other countries, the frequency of *Bacillus* species in staff hand was 37% and frequency of *B. cereus* strains on staff hand has been reported 15%. In the 1998-1999 years, Kotiranta and groups studied on four strains of *B. cereus* and the strains isolated from clinical samples could produce para crystalline layer while the standard strains could not have produced para crystalline layer (Kotiranta *et al.,* 1998; Kotiranta *et al.,*1999). Based on the results obtained in the present study, 11 (84.60%) of *B. cereus strains* isolated from staff hand have produced para crystalline layer while only 1 (7.70%) isolate from hospital surfaces was positive for para crystalline layer.

The results of this study and other similar studies, treating many of para crystalline layer in bacterial isolates from *in vivo* conditions, compared with bacterial isolates from *in vitro* conditions. Regarding this point *B. cereus* is a human pathogenic bacteria and a para crystalline layer structure is considered to be pathogenic, can be interpreted that the bacterium if considered on biological conditions, produces para crystalline layer to protect by influencing antibiotic and harmful enzymes in the human body (Jalalpoor *et al.,* 2009; Jalalpoor *et al.,* 2010; Sambrook & Russell, 2001; Schaffer & Paul, 2005).

According to results of this search and similar published study indicate spread of *B. cereus* strains resistant in hospitals, the lack of bacterial population control, leads to rapid release of resistance genes from resistance strains among sensitive bacterial population and ultimately leading to the spread of resistance nosocomial infections in hospitals and the community (Jalalpoor *et al.,* 2010).

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