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Characterization and Pathogenicity of Two Bacteria Isolated from A Local Chinese Hospital

Liang Chen[1], Shuang Lu[1], Weiping Cao[2], Peng Lü[1], Chunxia Zhang[1], Hongzheng Gu[2*]and Keping Chen[1*]

[1]Institute of Life Sciences, Jiangsu University, Zhenjiang, 212013, P R China

[2]The Fourth People's Hospital of Zhenjiang, 212013, P R China

Short Communication

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***For Correspondence**

Hongzheng Gu,
The Fourth People's Hospital of Zhenjiang,
212013 Jiangsu, China;
Tel:+86-551-84425604.

E-mail: jszjghz@163.com

Keping Chen,
Institute of Life Sciences,
Jiangsu University, Zhenjiang, 212013
Jiangsu, China;
Tel and Fax:+86-551-88791923

E-mail: kpchen@ujs.edu.cn

E-mail: galzi@unistra.fr

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ABSTRACT

Contamination and antimicrobial resistance levels in hospitals raise increasing concern not only in the fields of medicine and healthcare, but also in environment protection. In this experiment, two strains of bacteria were isolated from a Chinese local hospital, and their physiological and biochemical characteristics and drug resistance were investigated. We identified these two strains of bacteria as *Bacillus subtilis* and *Bacillus cerecus* using 16S rRNA sequence and phylogenetic analysis as well as proteomic tandem mass spectrometry. The pathogenicity of the two strains was studied using silkworms, and we found that *B. cereus* had a strong pathogenicity, with half of the lethal dose being 4×10^4 cfu/ml. Moreover, the supernatant of *B. cereus* also had a strong lethality to silkworm, possibly due to the presence of flagella protein and metal proteases. These two proteins may play an important role in the pathogenicity of *B. cereus*.

INTRODUCTION

In recent years, the problem of multidrug-resistant (MDR) bacteria has gradually become the focus of healthcare facilities [1-3]. The MDR bacteria are not only a healthcare problem but also an environmental problem [4-6]. In particular, the emergence and rapid spread of and pan-drug resistance bacteria can make clinical treatment difficult [7-9]. China is a developing country, and hospitals in large cities have made remarkable improvements in their environments, including the disinfection, sterilization, supervision and monitoring [10]. Many hospitals also investigate and research environmental and pathogenic microorganisms. A study of device-associated, healthcare-associated infection rates and microorganism profiles in a hospital in Shanghai, China, showed that the overall infection rate was 5.3%, and the number of infections per 1000 intensive care unit days reached 6.4% [11]. Recent investigation of 94 medical hospitals of Shandong province in China, show the qualified rate was 36.6%, 55.7% and 68.1% respectively, from year 2013 to 2015 [12]. The bacterial sedimentation assay shown that the qualified rate of operation room was 61.1%, 79.5% and 82.5% respectively. There is an upward trend in cleaning effect qualified rate of medical equipment, but the figures are still worthy of vigilance.

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Bacillus cereus, a Gram-positive bacteria species capable of forming spores, is a conditional pathogenic bacteria that can produce a variety of toxins [13]. It can cause clinical endophthalmitis sepsis, most commonly seen as food poisoning [14]. *B. subtilis* is a Gram-positive rod-shaped bacterium that can produce endogenous spores and is widely distributed among a variety of different environments. *B. subtilis* is usually treated as non-pathogenic bacteria and is widely used in fermentation engineering to produce a variety of enzymes, antibiotics, and so on.

Mass spectrometry is widely used in the identification of bacteria [15]. Over the years, it has been demonstrated that bacteria can be identified by specific proteins [16]. In the development of protein libraries, the bacterial protein two-dimensional electrophoresis tandem mass spectrometry method effectively identifies bacterial species. Moreover, this method can distinguish between species that are highly similar. This method identifies species by identifying the source of the corresponding protein spot on a two-dimensional map.

The pathogenic environment in hospital is likely to endanger the health of the patient; therefore, the assessment of the pathogenicity of environmental microbes is important. However, it is relatively expensive to assess bacterial pathogenicity using animal models. Kaito et al. reported a silkworm infection model in which silkworms were killed by injection with microorganisms that are virulent in humans, such as *Staphylococcus aureus, Pseudomonas aeruginosa, Vibrio cholerae*, and pathogenic strains of *Escherichia coli*, whereas the silkworms were not killed by nonpathogenic strains of *E. coli*. This silkworm model has been used to identify *S. aureus* virulence genes [17]. It has also been reported that silkworms are useful for evaluating the pathogenicity of a *Bacillus* soil bacterium, as well as for purifying an exotoxin secreted from this bacterium by monitoring its toxicity [18]. Furthermore, bacteria isolated from fish and shellfish were tested for pathogenicity in silkworms. The results showed that there were 10 strains having a strong pathogenicity in silkworms [19]. Exotoxins secreted by pathogenic microorganisms can also be identified by silkworms [20]. Similarly, we can also view some current silkworm infection models [21-23].

In this study, two strains were isolated and purified from a hospital in Zhenjiang City, Jiangsu Province, China. These two strains were assessed in terms of their physiological and biochemical characteristics by 16S rRNA sequencing using two-dimensional electrophoresis tandem mass spectrometry. Then, the pathogenicity of the two strains was evaluated by examining silkworm infection patterns. Additionally, the pathogenicity of the supernatant of *B. cereus* was evaluated. Finally, we identified two kinds of secreted proteins that may be related to the pathogenicity of *B. cereus*.

MATERIALS AND METHODS

Bacterial Strains

For the experiments, two wild-type strains of Bacillus spp. were randomly collected, strain 41-1 isolated from an ophthalmology room and strain 65-5 isolated from an operating theater and gastroscopy room of the Fourth People's Hospital of Zhenjiang, Jiangsu, China. The samples were suspended in sterilized water, and the supernatants were spread onto nutrient agar plates. Colonies were isolated after overnight incubation.

Morphologic and Biochemical Characterization of Strains 41-1 and 65-5

The colony characteristics of 41-1 and 65-5 were observed after growing on a nutrient agar plate at 37°C for 36 h, and cellular morphology was determined using light microscopy and Gram staining [24]. Physiological and biochemical analyses were performed by referring to Bergey's Manual [25] and the Manual for the Microbiology Experiment [26]. Antibiotic sensitivity tests were performed as before [27].

PCR Amplification of 16S rRNA Sequences

The 16S rRNA region was amplified by colony PCR (95°C for 5 min, followed by 30 cycles of 95°C for 45 s, 55°C for 45 s, and 72°C for 100 s, followed by 10 min at 72°C) using the primer pair 27F (AGAGTTTGATCATGGCTCAG) and 1492R (TACGGTTACCTTGTTACGACTT) [28], and was subsequently sequenced. The sequence was deposited in GenBank. The sequence was analyzed online (http://rdp.come.msu.edu). Homology comparisons of the nearly-complete 16S rRNA gene sequences of 41-1 and 65-5 were performed using the BLAST database on the NCBI website (http://www.ncbi.nlm.nih.gov/Blast.cgi). Sequence alignment was carried out using CLUSTAL X software. A phylogenetic tree was built using the MEGA 5.0 software package [29,30] and was constructed using the neighbor-joining method.

Evaluation of the Toxicity of the Bacterial Strains using Silkworms

Silkworm infection experiments were performed in accordance with previously established methods [17]. Silkworm strain 306 was raised from fertilized eggs to fifth-instar larvae in our laboratory. The newly exuviated fifth-instar larvae were fed with antibiotic-free mulberry leaves for 1 d. Then, a total of 5 µl of an overnight culture of the hospital bacteria strains was injected

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into the silkworm hemolymph through the intersegment membrane using a sterile micro syringe (Shanghai Micro plate, Shanghai, China). Pressure was immediately applied for 10 s at the injection site using alcohol wipes to stop any bleeding. Control larvae were injected with saline (0.6% NaCl). The larvae were maintained at room temperature. Each experimental group contained 10 silkworm larvae, and the number of living silkworm larvae was counted every 12 h. All experiments were independently performed at least three times.

Sample Preparation

After 20 h incubation in *Luria Bertani* (LB), *B. subtilis* and *B. cereus* were collected and washed several times, after which the bacteria were sonicated, and the total cell extract (supernatant) was mixed with aliquots of 20% tetrachloroacetic acid (TCA) acetone at -20ºC for 12 h to precipitate the protein. The protein was isolated by centrifugation at 13,000 g, 4°C for 20 min, was washed twice with acetone containing 20 Mm Dithiothreitol (DTT) and 1 mM phenylmethylsulfonyl fluoride (PMSF), and dried and stored at −80°C until use. The protein content of the pellet was determined by the Bradford method [31].

Immediately after harvesting, the culture supernatants were centrifuged at 8000 rpm for 20 min at 4°C. The supernatant of the centrifugation was rapidly filtered through a membrane (pore size=0.2 um; Nalgene Sterilization Unit, Nalge Company). The protein was then precipitated using the deoxycholate-tetrachloroacetic acid method [32]. The pellet was washed twice with ethanol:ether (1:1) and was dried and stored at −80°C until use. The protein content of the pellet was determined by the Bradford method [31].

Two-Dimensional Electrophoresis

Two-dimensional electrophoresis (2-DE) was performed with a 17-cm (linear, pH 4-7) immobilized pH gradient (IPG) gel strip (Bio-Rad, USA), as described by Liang et al. [33]. Total protein (3 mg) was loaded onto the IPG strip using active rehydration (50 V for 13 h), and isoelectric focusing (IEF) was performed at 17°C with a voltage gradient of 250 V for 30 min, 1000 V for 1 h, 10,000 V for 5 h, and then continued for a total of 60 kVh. The IPG gel strip was equilibrated for 15 min with equilibration buffer (6 M urea, 0.375 M Tris-HCl, 20% (v/v) glycerol, 2% (w/v) SDS, and 2% [(w/v) DTT), and then was equilibrated for another 15 min with the same equilibration buffer without DTT, containing 2.5% (w/v) iodoacetamide. The equilibrated strip was sealed on the top of a 12% SDS-PAGE gel for electrophoresis. The gel was visualized with 0.1% Coomassie brilliant blue R-250 and was scanned with a high precision scanner (Scan Maker 9700XL, Microtek) at a resolution of 300 dpi. Spot analysis was performed using PDQuest (version 8.0.1, Bio-Rad). Triplicate experiments were carried out for each sample.

In-gel Digestion and Mass Spectrometry Analysis

In-gel digestion was performed as previously reported. The protein spots were excised from the stained gels, washed twice in milli-Q water, destained by sonication in 25 mM ammonium bicarbonate and 25% acetonitrile, dehydrated with acetonitrile, and dried in a vacuum. The dried proteins spots were treated with 10 mM DTT for 1 h at 56°C, alkylated with 40 mM iodoacetamide for 45 min at room temperature, washed with 25 mM ammonium bicarbonate, dehydrated with acetonitrile, and incubated with 5 µL trypsin solution (20 µg/mL) at 37ºC overnight to completely digest the proteins.

Protein Identification

Mass spectrometry data were analyzed using MASCOT (Matrix Science, London, UK) and the NCBInr bacterial protein sequence database. The parameters were set as missed cleavage: 1, fixed modification: acetylation of carbamidomethyl (C), variable modification: Glu → pyro-Glu (N-term Q) or oxidation of methionine (M), mass tolerance: 0.3 Da, and mass value: MiH+. Proteins with a minimum ion score of 49 ($P<0.05$) were considered to be reliably identified.

RESULTS

Physiological and Biochemical Characteristics

The 41-1 and the 65-5 colonies grown on LB medium can see **(Figure 1)**. All colonies were positive for catalase, the deoxidization of nitrate, and the Voges-Proskauer reaction **(Table 1)**. On the basis of these observations, we made a preliminary judgment that both the 65-5 and 41-1 strains belonged to the Bacillus genus. The results of the antibiotic analysis are shown in **(Table 2)**. The antibiotic sensitivity of these two bacteria was clearly different. Bacillus sp. 65-5 was sensitive to Kan, Gen, Tet, and Chl.

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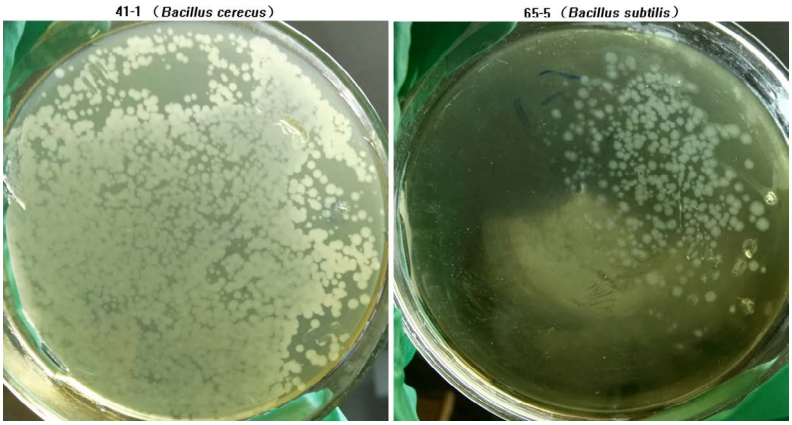

Figure 1. The morphologic characterization of 41-1 and 65-5.

Table 1. Physiological and biochemical characteristics of 41-1 and 65-5

Tested items	41-1	65-5	Tested items	41-1	65-5
Oxidase	+	+	semi-solid agar	+	_
Deoxidization of nitrate	+	+	Lysozyme patience	+	_
Glucose fermentatiom	+	+	V-P test	+	+
D-mannitol	_	_	Parasporal crystal	_	_
(+,Positive;-,negative)					

Table 2. Antimicrobial susceptibility and resistance pattern of 41-1 and 65-5

Bacterial	Kalamycin	Gentamycin	Tetracycline	Chloromycetin
41-1	R (≥ 64 μg/ml)	R (≥ 16 μg/ml)	R (≥ 16 μg/ml)	R (≥ 32 μg/ml)
65-5	S (≤ 16 μg/ml)	S (≤ 4 μg/ml)	S (≤ 4 μg/ml)	S (≤ 8 μg/ml)

16S rRNA Gene Sequence and Phylogenetic Analysis

The 16S rRNA gene sequence of strain 41-1 was 1,516 bp in length and that of strain 65-5 was 1,515 bp in length. The 16S rRNA sequences of the two strains were analyzed by BLAST, which showed that the two strains belonged to the *Bacillus sp*. This conclusion agrees with the result of physiological and biochemical analyses. The 16S rRNA gene sequences of 17 strains of *Bacillus* were selected and compared in a phylogenetic tree **(Figure 2)**. It can be seen that the 41-1 strain had a high similarity to the generalized *B. cereus* sensu lato, while the 65-5 strain had a high similarity to *B. subtilis*. Thus, we concluded that the strain 41-1 is a generalized *B. cereus* sensu lato, and 65-5 strain is *B. subtilis*.

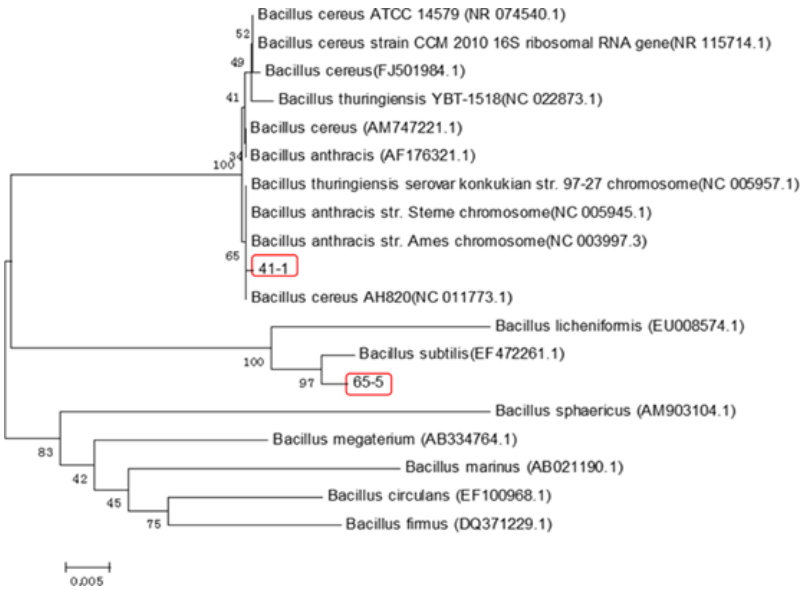

Figure 2. Phylogenetic tree of the 16S rRNA sequences of two bacterial. The tree was constructed by the neighbor-joining method.Numbers in parentheses represent the sequences accession numbers in GenBank. The number at each branch point represents the percentage of bootstrap values.

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Pathogenicity Assessment of the Two Bacterial Strains to Silkworms

As shown in **(Table 3)**, when the bacterial cells were injected, all the silkworms were killed by bacteria strain 41-1 within 12 h, and by bacteria strain 65-5 within 108 h. This indicates that the lethality of bacteria strain 41-1 is stronger than strain 65-5. Moreover, the supernatant of the 41-1 strain was also able to kill all the silkworms within 24 h. To calculate the half-lethal dose of the 41-1 strain, the number of living silkworms was counted, as listed in **(Table 3)**. The death probability of silkworms treated with different doses of strain 41-1 was evaluated by the Bliss method, and the median lethal dose (LD50) was 4×10^4 cfu/ml **(Table 4)**.

Table 3. Killing of silkworms by bacterial strains.

	Survival rate of silkworms after injection										
Sample	**0h**	**12 h**	**24 h**	**36 h**	**48 h**	**60 h**	**72 h**	**84 h**	**96 h**	**108 h**	**≥ 120 h**
0.6% NaCl	100	100	100	100	100	100	100	100	100	100	100
65-5	100	100	60	60	60	40	20	20	20	0	0
41-1	100	0	0	0	0	0	0	0	0	0	0
LB	100	100	100	100	100	100	100	100	100	100	100
supernatant of the 41-1 strain	100	70	0	0	0	0	0	0	0	0	0
Fifth instar larvae of silkworms (n=10) were injected with overnight cultures of two bacteria strains isolated from the hospital, or with supernatant of the 41-1 strain. 0.6% NaCl and LB were used as a control. The percentages of surviving larvae post-infection are shown.											

Table 4. The lethal effect of bacteria 41-1 on silkworm

Injected bacterial cell number/cfu	Survival rates of silkworms after injection/%						
	0 h	**2 h**	**4 h**	**6 h**	**8 h**	**10 h**	**12 h**
0. NaCl	100	100	100	100	100	100	100
1×10^2	100	100	80	80	60	40	0
1×10^3	100	100	70	70	50	30	0
1×10^4	100	90	70	60	50	20	0
1×10^5	100	90	60	50	20	0	0
1×10^6	100	70	50	30	10	0	0
1×10^7	100	60	40	20	0	0	0

When the larvae of silkworms were injected with pathogenic bacteria, they looked normal during the first few hours and then their activity decreased and they gradually stopped eating. Then, part of their bodies started to swell. Finally, their bodies became soft, and their skin turned dark or released a black, watery discharge. This indicates that the larvae suffered from septicemia due to the bacterial infections, and resulted from melanization. The incidence of larvae is shown in **(Figure 3)**.

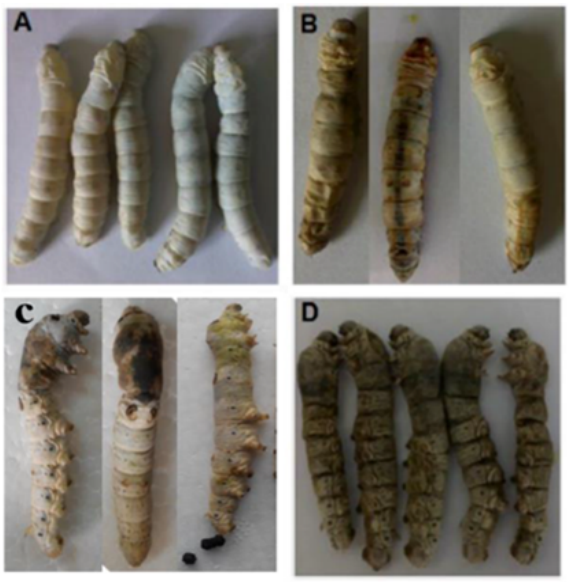

Figure 3. Silkworms that were injected with bacteria isolated from the hospital. A: Silkworms 5 d post-injection with saline (0.6% NaCl). B: After bacterial infection with strain 41-1, the silkworms' bodies expanded, and the silkworms exhibited diarrhea and vomiting. C: After infection with Culture supernatant of strain 41-1.D: After bacterial infection with strain 65-5, the black color of the larvae killed by bacteria was cause by melanization.

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Comparative Proteomics Analysis and Identification of the Two Strains of *Bacillus sp*.

As shown in **(Figure 4)**, comparing the proteomic maps of the 65-5 strain and 41-1 strains, we found that the two proteomes had a different distribution of protein spots. To compare 65-5 with 41-1, we selected 12 protein spots from the protein map of the 65-5 strain and 11 protein spots from the protein map of the 41-1 strain. These protein spots were then identified by mass spectrometry. The results are shown in **(Table 5)**. The proteins of the 65-5 strain were all identified as *B. subtilis* proteins, and all of the proteins of the 41-1 strain were identified as belonging to *B. cereus*. Thus, strain 65-5 and 41-1 isolated from the hospital were conclusively identified as *B. subtilis* and *B. cereus*, respectively. *B. anthracis*, *B. thuringiensis*, and *B. cereus* can be distinguished from each other by their overall 2-DE protein patterns.

Table 5. Proteins identified by MALDI-TOF MS

Spot no.	Accession	Protein name	pI[a]	MW[b]	AA[c]	No. peptide matched[d]	Seq Cov(%)[e]	Score
Expressed in 65-5								
A-1	P38021	Ornithine aminotransferase [*Bacillus subtilis subsp. subtilis str. 168*]	5.07	44133	401	11	2%	47
A-2	CUB27349.1	Malate dehydrogenase [*Bacillus subtilis*]	4.99	33632	312	39	13%	228
A-3	924003915 CUB32558	Alkyl hydroperoxide reductase subunit C [*Bacillus subtilis*]	4.5	20713	187	14	7%	97
A-4	BAA12507.1	YqgD [*Bacillus subtilis*]	5.15	25332	226	49	21%	266
A-5	AFQ59382.1	Ribosome-associated sigma 54 modulation protein [*Bacillus subtilis QB928*]	5.36	22097	190	21	11%	74
A-6	WP_033881820	fructose-6-phosphate aldolase, partial [*Bacillus subtilis*]	5.73	22414	208	34	16%	145
A-7	WP_019714429	elongation factor Ts [*Bacillus subtilis*]	5.17	32462	293	15	5%	102
A-8	EHA32564.1	triosephosphate isomerase [*Bacillus subtilis subsp. subtilis str. SC-8*]	4.93	27418	256	29	11%	85
A-9	AID00070	heme peroxidase [*Bacillus subtilis subsp. subtilis str. OH 131.1*]	5.23	28970	249	12	4%	81
A-10	WP_025853878	ABC transporter ATP-binding protein[*Bacillus subtilis group*]	4.77	29144	261	14	5%	70
A-11	WP_015483183	pyruvate dehydrogenase E1 component subunit beta [*Bacillus subtilis*]	4.79	35466	325	15	4%	74
A-12	WP_003221888	ATP synthase subunit alpha [*Bacillus subtilis*]	5.22	54709	502	23	4%	155
Expressed in 41-1								
B-1	SCC63043	Alanine dehydrogenase [*Bacillus cereus*]	5.22	40141	377	62	16%	346
B-2	EEL50644	Butyryl-CoA dehydrogenase [*Bacillus cereus Rock3-44*]	5.61	40659	371	27	7%	178
B-3	CUB10128	Pyruvate dehydrogenase E1 component subunit beta [*Bacillus cereus*]	4.76	35221	325	13	4%	108
B-4	EEL03554	Triosephosphate isomerase 1 [*Bacillus cereus BDRD-ST196*]	5.01	27081	254	11	4%	75
B-5	WP_000054174	Superoxide dismutase [Mn] 1 [*Bacillus cereus*]	5.34	24753	218	30	13%	152
B-6	WP_060488430	ribosome-recycling factor [*Bacillus cereus*]	6.45	20579	185	10	6%	74
B-7	ADK04225	nucleoside diphosphate kinase [*Bacillus cereus biovar anthracis str. CI*]	5.26	18849	166	14	8%	116
B-8	EAL11933	fructose-1,6-bisphosphatase, class II [*Bacillus cereus G9241*]	5.52	33346	315	39	12%	182
B-9	WP_000101972	Electron transfer flavoprotein subunit alpha [*Bacillus cereus*]	4.83	34475	326	23	7%	68
B-10	WP_000186155	pyridoxal 5'-phosphate synthase subunit PdxS [*Bacillus cereus*]	5.25	31789	295	23	7%	77
B-11	EJR09352	ATP-dependent Clp protease proteolytic subunit 2, partial [*Bacillus cereus MSX-D12*]	5.17	5907	50	14	28%	80
Supernatant of the 41-1 strain								
C-1	WP_000048982	50S ribosomal protein L10 [*Bacillus cereus group*]	5.38	18044	166	27	16%	151
C-2	CUB10128	Pyruvate dehydrogenase E1 component subunit beta [*Bacillus cereus*]	4.76	35221	325	15	4%	91

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C-3	WP_000101972	Electron transfer flavoprotein subunit alpha [*Bacillus cereus*]	4.83	34475	326	23	7%	97
C-4	SCC05347	Flagellin [*Bacillus cereus*]	5.13	47276	446	15	3%	95
C-5	AOM08110	Zinc metalloproteinase precursor [*Bacillus cereus*]	6.02	65340	591	16	3%	84
C-6	CUB10849	1-pyrroline-5-carboxylate dehydrogenase [*Bacillus cereus*]	5.63	47811	437	14	3%	75
C-7	EEL21676	Elongation factor Ts [*Bacillus cereus Rock1-3*]	5.25	32797	298	15	5%	91
C-8	EAL11933	fructose-1,6-bisphosphatase, class II [*Bacillus cereus G9241*]	5.52	33346	315	17	5%	142

The A-11 protein spot of *B. subtilis* and the B-3 spot of *B. cereus* were both identified as pyruvate dehydrogenase E1 component subunit beta. The amino acid sequences of the two proteins had 89% similarity. The function of this protein in *Bacillus* sp is likely to be conserved.

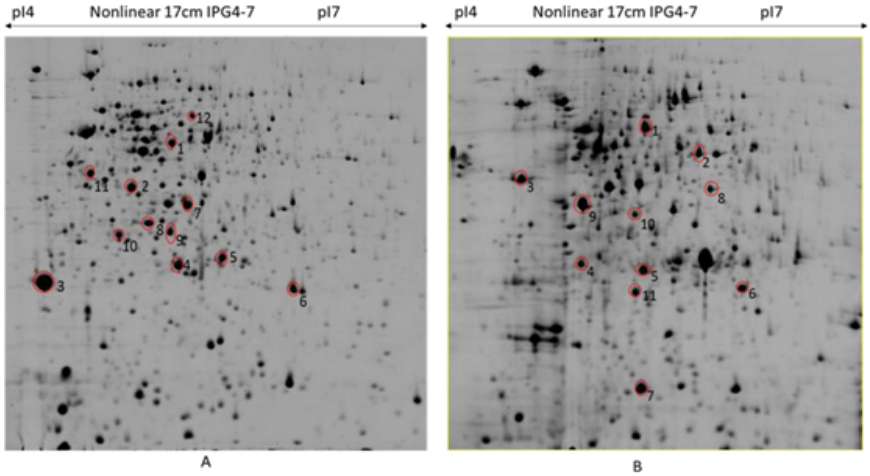

Figure 4. Comparison of 2-DE gel from bacteria strain 65-5 and bacteria strain 41-1.A:2-DE pattern of proteins from bacteria strain 65-5.B: 2-DE pattern of proteins from bacteria strain 41-1.

Comparative Analysis of the Supernatant Proteins of *B. subtilis* and *B. cereus*

As shown in **Figure 5**, we selected 11 protein spots from the protein map of the 41-1 strain and 8 spots from the protein map of the supernatant of this strain. These protein spots were then identified by mass spectrometry. The results are shown in **Table 5**. Three *B. cereus* proteins were identical to three protein spots from the supernatant of overnight culture. Namely, B-3 was the same as C-2, B-8 was the same as C-8, and B-9 was the same as C-3. As mentioned above, both B-3 and C-2 were identified as pyruvate dehydrogenase E1 component subunit beta. Studies have shown that this protein acts as a fibronectin-binding protein in mycoplasma infection and promotes bacterial infection. Flagellin and metalloproteinases were also found in the supernatant of *B. cereus*. Studies have shown that they may play an important role in the pathogenicity of *B. cereus*.

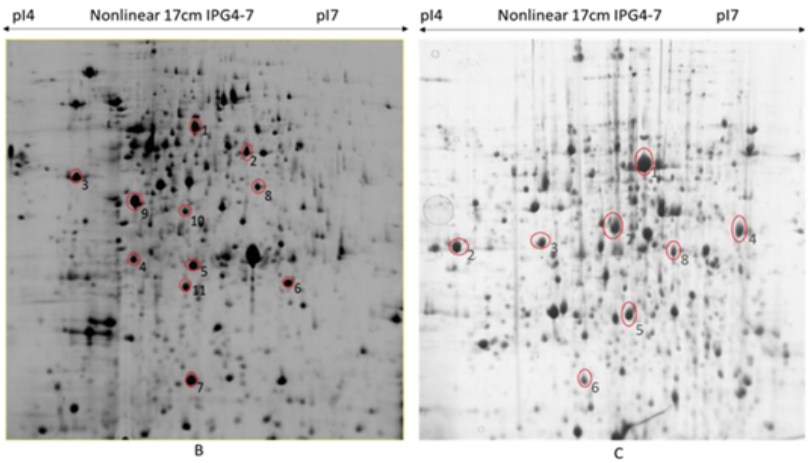

Figure 5. Comparison of 2-DE gel from bacteria strain 41-1 and supernatant of the 41-1 strain. A: 2-DE pattern of proteins from bacteria strain 41-1.B:2-DE gels of proteins from a culture supernatant of the 41-1 strain.

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DISCUSSION

In this study, two bacterial strains isolated from a hospital were studied in terms of their morphological, physiological, and biochemical characteristics, which is also a deep exploration of our previous work [27]. Based on these analyses, both bacteria strains 65-5 and 41-1 were preliminarily identified as Bacillus sp. The phylogenetic tree and 16S rRNA gene sequence analyses showed that bacteria 65-5 could be *B. subtilis* and bacteria 41-1 might be broadened *B. cereus* sensu lato. *B. anthracis*, *B. thuringiensis*, and *B. cereus* belong to the generalized *B. cereus* sensu lato group, and the similarity among them is very high. It is impossible to distinguish among them using the 16S rRNA method. Thus, one limitation of the 16S rRNA method is that only the bacterial genus can usually be identified [34]. However, bacteria with high similarity can then be distinguished from each other by two-dimensional electrophoretic tandem mass spectrometry [35,36], and we identified Bacillus sp. 65-5 and Bacillus sp. 41-1 based on 11 proteins belonging to *B. subtilis* and 10 proteins belonging to *B. cereus*. In the case of the pyruvate dehydrogenase E1 component subunit beta protein, the amino acid sequences had 89% similarity. Still, even in this case, the mass spectrometric results could distinguish the two strains as *B. subtilis* and *B. cereus*.

Both *B. cereus* (41-1) and the supernatant of *B. cereus* (41-1) showed strong pathogenicity to silkworms. The pathogenicity of *B. cereus* (41-1) was stronger than that of *B. subtilis*. Thus, *B. cereus* can infect silkworms and secrete a pathogenic protein that can kill silkworms to some extent. This study shows that *B. cereus* isolated from a hospital had a strong pathogenicity, and in the hospital environment it could easily lead to traumatic infections. Thus, the hospital should focus on protection against this bacterial strain. Proteome comparison analysis revealed that *B. cereus* (41-1) eluted flagellin and zinc protein precursors. Other studies have shown that flagellin contains a pathogen-associated molecular pattern, namely the flg22 epitope, which is recognized by the flagellin-sensitive 2 receptor kinase and functions as a pattern recognition receptor [37-39]. Among putative virulence factors are bacterial metalloproteases, with special regard to zinc containing proteases, which are known to act occasionally as toxins in many pathogenic bacteria [40,41]. Alternatively, they may play indirect roles in pathogenicity.

CONCLUSION

In summary, two bacterial strains in the hospital functional area were identified as *B. subtilis* and *B. cereus*, respectively, and their pathogenicity was described. We found that *B. cereus* and its supernatant were highly pathogenic in silkworm, while the lethal concentration was 4×10^4 cfu/ml. Silkworms is regarded not only as a good research model for human disease, but also for human pathogenic microbial infection. Silkworm is easy to feed and the growth cycle is short. In addition, the genetic background of silkworm is clear. Silkworm is used in exploring pathogenic microbial pathogenesis and antibiotic screening from the perspective of biosecurity. In recent years, MALDI-TOF-MS has been widely used as a new type of soft ionization mass spectrometry technology, which has been widely used in the rapid detection of pathogens and also provides reference for the identification of protein. In view of the above, MALDI-TOF-MS will be widely used in most clinical clinics, providing rapid and accurate clinical diagnosis. For the cleaning and health of hospital department, they should pay attention to the superimposed disinfection using a variety of drugs, to prevent the patient of bacterial infection after the trauma.

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