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Genotypic characterization of enterotoxigenic *Bacillus cereus* isolates associated with spoilage and drug resistance from milk in Mizoram

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ABSTRACT

Bacillus cereus, a ubiquitous food borne pathogen and spoilage bacterium, poses significant risk to dairy safety as it's spores can withstand pasteurization and forms persistent biofilms on dairy equipment and utensils. With the recent rise of small-scale dairy farming in Mizoram, this study assessed contamination levels, genotypic traits, toxigenic gene distribution, spoilage potential, and multidrug resistance of *B. cereus* isolated from dairy milk across the state. A total of 108 samples of farm raw, bulk, and retail milk, six samples each, were collected from six different milk co-operative societies, namely Durtlang, Sihphir, Durtlang-leitan, Thuampui, Phunchawng, and Mualpui. The *B. cereus* group count exceeded the regulatory limit of $5.7x10^4$cfu/ml or 4.76 log cfu/ml in farm raw, bulk, and retail milk without significant variations among different milk co-operative societies. In PCR based detection of housekeeping *panC* gene, the prevalence of *B. cereus* was found to be 19.45 percent (7 isolates) in farm raw milk, however, the organism could not be confirmed from bulk and retail milk. In terms of pathogenic traits, all the seven *B. cereus* isolates carried at least one enterotoxigenic gene except *hblA* and the emetic *ces* gene was absent. The study strains belonged to Clade III with 99.72 percent homology, which are mesogenic and cytotoxic in nature. Two *B. cereus* strains bearing NCBI accession numbers, OR786090 (AS09LM) and OR786089 (AS09TM) from Durtlang - leitan and Thuampui, respectively carried highest numbers of enterotoxigenic genes, three each. Upon phylogenetic analysis, these two strains were homologous to the reference strains sourced from milk product, soil and patient's vomit. A proportion of 85.71-100 percent of *B. cereus* strains exhibited lipolytic and proteolytic spoilage potential and none of the strains were psychrotolerant. The *B. cereus* isolates showed 97.05percent resistance to β-lactams and 97.05percent sensitivity to ciprofloxacin and erythromycin. Five *B. cereus* strains were multidrug-resistant, spanning 3-4 antibiotic classes. The prevalence of *B. cereus* in farm raw milk from Mizoram, with counts exceeding permissible limits and exhibiting enterotoxigenic, spoilage, and multi drug resistance potential, underscores the need for scientific interventions in dairy farming suitable to local environment of the land locked hilly state to ensure dairy safety and shelf-life stability.

Keywords: *Bacillus cereus*, Genotypic characterization, MDR, Milk Co operative societies, Phylogenetic analysis

World Health Organization has classified *Bacillus cereus sensulato* (*sl*) as a riskgroup 2 pathogen of human and animals that has food borne pathogenic potential and spoilage abilities thereby lowering the shelf life.Food containing at least 10^3 -10^5 cfu m^{-1} *B. cereus sl* provides sufficient cells to colonize in the gut of the host and produce enough enterotoxin to cause diarrhoeal syndrome (Ehling-Schulz *et al*. 2019).The organism is ubiquitously found in dairy farm environment and particularly abundant as thermo resistant spores in the soil which can also withstand desiccation and food preserving actions (Buckley and Grotticelli, 2024). These spores of *B. cereus* can contaminate the raw milk during milking and forms biofilms on many types of substances including stainless steel surfaces of dairy equipments and utensils (Meng *et al*. 2022).

B. cereus spores are ubiguitous in raw food and can be easily carried by dairy cows, making the dairy farm environment one of the leading sources for raw milk contamination with the organism during milking. Earlier studies suggested that pasteurization of raw milk might not be effective in reducing *B. cereus* contamination, instead could act as activating factor for *B. cereus* spores and associated risk may continue in subsequently produced dairy products (Tirloni *et al*. 2022).

Although *B. cereus* group count is indicative of compromised hygiene and potential contamination of milk, it is important to analyze the toxigenic and spoilage potential of the bacterium from dairy safety and shelf-life

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point of view. The *panC* gene, encoding pantothenate-β-alanine ligase, based sequencing approach can be used for its association with the growth temperature and heat resistance (Guinebretiere *et al.* 2010) and its ability to cause food poisoning (Kindle *et al*. 2019).

India now has the world's largest dairy industry and produced nearly 230.58 million tonnes of milk during 2022-23 contributing to 24.64% of the global milk production (APEDA, 2022-2023). The population of indigenous breeds of cattle has steadily been decreasing, while that of the more productive exotic and cross-bred dairy cattle has been increasing after implementation of National Dairy Development Programme (Sharma, 2019). However, about 46 percent of the milk produced is a household venture and sold to the non-producers in the rural areas and only the remaining 54 percent is made available for sale to the organized and un-organized sector (DAHD, 2020-2021).

In Mizoram, the production of milk was 24 thousand tonnes in the year 2019-20 and Aizawl was the top milk producing district (NDDB, 2019-2020). The small dairy farmers usually sell 90 percent of the milk directly to the home consumers and the remaining is collected by Mizoram Milk Producer Co-operative Society Ltd (MULCO) through its different branches for organized selling of milk and milk products (Ralte and Chhawna, 2021).

So, keeping in view the recent rise of the state in the small-scale dairy industry and alongside probable compromising quality of milk produced in land locked hilly state with scarcity of clean water in the dairy farms, this research aimed to investigate the level of *B. cereus* contamination, its toxigenic and spoilage potential and multi drug resistance pattern of farm raw, bulk and retail milk from six Milk Co-operative societies Mizoram.

MATERIALS AND METHODS

Collection of milk samples: A total of 108 samples of farm raw, bulk, and retail milk, six samples each, were randomly collected from six Milk Co-operative societies of Mizoram, namely Durtlang, Sihphir, Durtlang-leitan, Thuampui, Phunchawng, and Mualpui.

*Enumeration, isolation and molecular confirmation of B. cereus:*The enumeration of *B. cereus* group in all the 108 samples was done by spread plate method (Abraha *et al*. 2017). *Bacillus cereus* agar (BCA) plates having colonies between 30-300 were considered for enumeration and the samples having ≥ $5.7x10^4$ cfu/ml or 4.76 log cfu/ml count were recorded as above legal limit (FSSAI, 2023).

Isolation of *B. cereus* was done by inoculating the homogenized Brain Heart Infusion (BHI) broth culture from all the 108 samples on BCA plates and incubated at 37ºC for 24 hours. The peacock blue colour colonies were next presumptively identified by positive reactions in Gram's stain, Voges-Proskauer, citrate, catalase and motility test (Meena *et al*. 2019).

For molecular confirmation of *B. cereus*, the DNA lysates were prepared from the pure culture of presumptively identified *B. cereus* isolates by using a commercial DNA extraction kit (MB568A-25PR, HiMedia). The housekeeping *panC* gene was detected from the extracted DNA in a PCR assay as per the method of Candelon *et al*. (2004). The oligonucleotide primers used in the present study are given in Table 1. The specific 521bp band was visualized on agarose gel and documented using a gel documentation system (Alpha Imager) and UV transilluminator.

Genotypic characterization of *B. cereus:* The *panC* gene positive *B. cereus* strains were examined for the presence of virulence genes, namely haemolytic (*hblA* and *hblC*) and non-haemolytic (*nheA* and *nheB*) enterotoxins and emetic toxin (*ces*) as per Fricker *et al*. (2007) and Gao *et al*. (2018) The oligonucleotide primers used in the present study are given in Table 1.

Next, the *panC* gene PCR products of the most pathogenic *B. cereus* strains containing maximum number of virulence genes were processed for nucleic acid sequencing. The PCR products were purified using the GeneJET PCR purification kit and then cloned by using InsTAclonePCR Cloning Kit # K1213, #K1214(Thermo Scientific, USA). The vector containing the insert was propagated in *E. coli* (DH5-α) host. The cloned PCR products were sequenced at

Table 1. Genes, primers and annealing temperatures used in PCR assay for confirmation of *B. cereus* and detection of its toxigenic traits

Sl. No	Gene	Primer sequence	Base pair	Annealing Temperature	Reference
1	*panC*	*panC*-F: CGATATCCTCGTGATATTGATAGAG *panC*-R: TCCGCATAATCTACAGTGCCTTTC	521	59ºC for 30 sec	Candelon *et al.* (2004)
2	*nheB*	*nheB*-F:CTATCAGCACTTATGGCAG *nheB*-R:ACTCCTAGCGGTGTTCC	770	57.5ºC for 30 sec	Gao*et al.* (2018)
3	*nheA*	*nheA*-F:TACGCTAAGGAGGGGCA *nheA*-R:GTTTTTATTGCTTCATCGGCT	500	57ºC for 30 sec	Gao*et al.* (2018)
4	*hblC*	*hblC*-F:AATGGTCATCGGAACTCTAT *hblC*-R:CTCGCTGTTCTGCTGTTAAT	750	58.4ºC for 30 sec	Gao *et al.* (2018)
5	*hblA*	*hblA*-F :GTGCAGATGTTGATGCCGAT *hblA*-R:ATGCCACTGCGTGGACATAT	320	57.5ºC for 30 sec	Gao *et al*. (2018)
6	*ces*	*ces*-F:CACGCCGAAAGTGATTATACCAA *ces*-R: CACGATAAAACCACTGAGATAGTG	176	60ºC for 30 sec	Fricker *et al*. (2007)

Table 2. Viable count of *Bacillus cereus* group (log cfu/ml) (mean± SE) in farm raw, bulk and retail milk samples collected from various milk co-operative societies of Mizoram

Milk sample	Sihphir		Durtlang		Durtlang-leitan		Thuampui		Mualpui		Phunchawng	
	(10^{-3})	(10^{-5})	(10^{-3})	(10^{-5})	(10^{-3})	(10^{-5})	(10^{-3})	(10^{-5})	(10^{-3})	(10^{-5})	(10^{-3})	(10^{-5})
Farm raw milk	5.78± 0.03	7.50± 0.03	5.70± 0.04	7.56± 0.03	5.70± 0.03	7.59± 0.03	5.73± 0.04	7.60± 0.03	5.66± 0.03	7.78± 0.03	5.59± 0.04	7.57± 0.04
Bulk milk	5.62± 0.05	7.50± 0.03	5.56± 0.03	7.50± 0.03	0.0	0.0	5.59± 0.04	7.51± 0.04	0.0	0.0	0.0	0.0
Retail milk	5.98± 0.04	7.70± 0.03	5.86± 0.04	7.75± 0.04	5.73± 0.04	7.62± 0.04	5.66± 0.04	7.62± 0.04	5.87± 0.04	7.58± 0.04	0.0	0.0

Delhi University (India) sequencing facility and the NCBI GenBank accession numbers were received after analysis of obtained sequences. The phylogenetic trees of the study strains were prepared.

Spoilage potential of B. cereus: The proteolytic activity of *B. cereus* was ascertained by growing the organism in nutrient agar supplemented with Ultra-High Temperature (UHT) milk and then incubating at 7°C, 25°C, and 37°C for 7 days. The halos around the inoculation sites were considered as positive indicating the hydrolysis of milk protein (Porcellato *et al*. 2021).

Next, the lipolytic activity of the organism was assessed by growing the organism in Tributyrin agar at 37°C for 24, 48 and 72 hours, respectively and development of a clear zone around the inoculation site indicated positive reaction (Meng *et al*. 2022).

Further, the psychrotolerant activity of *B. cereus* isolates was observed by growing the organism in BCA plates at 4ºC and the growth abundance after 24 hours indicated the potential of the organism to spoil dairy products stored at 0-4°C temperature (Meng *et al*. 2022).

Antibiotic sensitivity test (AST) of B. cereus: The AST of the *panC* gene positive *B. cereus* strains was done against a panel of 12 antibiotics from 5 classes, β-lactam (ampicillin and penicillin G), cephalosporin (ceftriaxone and cefazolin), aminoglycosides (gentamicin, amikacin and streptomycin), fluoroquinolones (ciprofloxacin, levofloxacin and ofloxacin) and macrolides (azithromycin and erythromycin) by using disk diffusion method (Bauer *et al*. 1966). The bacterial suspension having McFarland 0.5 turbidity ($1.5x10^8$cfu/ ml) was spread on Mueller Hinton agar (MHA) plates and standard antibiotic disks (6 mm diameter) were placed on the medium and the plates were incubated at 37^0C for 24hours. The results were observed by measuring the zone of inhibition as per EUCAST (2023).

Statistical analysis: The data obtained from the study were statistically analyzed using ANOVA and Chi square test on SPSS version 27.0.

RESULTS AND DISCUSSION

Viable count, isolation and molecular detection of B. cereus: The mean viable count of *B. cereus* group was highest in raw farm milk with the counts of 5.73 ± 0.04 log cfu/ml at10^{-3}dilution and 7.78 ± 0.03 log cfu/ml at10^{-5} dilution without any significant variation among the milk samples collected from the six Milk Co-operative societies, namely Durtlang, Sihphir, Durtlang-leitan, Thuampui, Phunchawng, and Mualpui. The mean bulk milk *B. cereus* counts were 5.56± 0.03logcfu/ml at10^{-3}dilution and 7.50 ± 0.03log cfu/ml at10^{-5} dilution from Sihphir, Durtlang and Thuampui and the organism was absent in Durtlang-leitan, Mualpui and Phunchawng. Although the organism was absent in retail milk from Phunchawng, the mean count was 5.66 ± 0.04 logcfu/ ml at$10^{-3}$and 7.58 ± 0.04 logcfu/ml at10^{-5} dilution among other five milk co -operative societies (Table 2). The *B. cereus* load in milk samples exceeding the acceptable limit was also reported by Abraha *et al*. (2017), Kupradit *et al*. (2020) and Osama *et al*. (2020). The present findings of *B. cereus* group count exceeding the legal limit in farm raw milk samples ($5.7x10^4$ cfu/ml or 4.76 log cfu/ml: EFSA, 2005) might be due to washing of farms by surface runoff during monsoon and unhygienic milking practice owing to scarce supply of clean water. The probable contamination of farm raw milk might have occurred from the floors soiled with dungs, use of contaminated stored rain water, animal body, and improper personal hygiene of dairy men. The absence of *B. cereus* group in bulk milk from Durtlang-leitan, Mualpui and Phunchawng Milk Co -operative societies located in the town areas might be attributed to the dilution effect contributed by systematic collection of milk from the farms and easy transportation of milk to final collection points coupled with the appropriate cleaning and disinfection of milk cans and bulk tanks, and regular quality testing. Conversely, the prolonged holding of milk without refrigeration at retail outlets might have contributed to the contamination exceeding the legal limit.

Upon bacteriological isolation, overall 29.62 percent (32/108) milk samples were found to be presumptively positive for *B. cereus* which included 61.11percent (22/36) from farm raw milk, 19.44percent (7/36) in retail milk and 8.33percent (3/36) in bulk milk with significant ($p \leq 0.05$) variation in the detection rate among raw, bulk, and retail milk in Chi square test (Table 3).

All the 32 presumptive *B. cereus* isolates from farm raw, bulk and retail milk were subjected to PCR assay for the detection of the housekeeping *panC* gene. However, the prevalence rate of *B. cereus* was calculated based on the total number of samples subjected to preliminary bacteriological examination to avoid the false interpretation of higher

Table 3. Bacteriological and Molecular detection of *B. cereus* from farm raw, bulk and retail milk samples collected from various milk Co-operative societies of Mizoram

Sl. No.	Type of milk	Milk Co- operative Society												ST	Total	
		Sihphir		Durtlang		Durtlang-leitan		Thuampui		Mualpui		Phun chawng			BD (%)	MD (%)
		BD	MD	BD	MD	BD	MD	BD	MD	BD	MD	BD	MD			
1	Farm raw (n=6)	3	1	3	1	4	1	5	2	4	1	3	1	36	22[b] (61.11)	7 (19.4)
2	Bulk (n=6)	1	0	1	0	0	0	1	0	0	0	0	0	36	3[a] (8.33)	0 (0)
3	Retail (n=6)	2	0	2	0	1	0	1	0	1	0	0	0	36	7[a] (19.44)	0(0)
Total No (N)=18 (%)		5 (27.77)	1 (5.55)	5 (27.7)	1 (5.55)	5 (27.70)	1 (5.55)	7 (38.80)	2 (11.10)	5 (27.77)	1 (5.55)	3 (16.66)	1 (5.55)	108	32 (29.62)	7 (6.48)

Bacteriological method: $\chi 2ac = 0.00^{**}$ (**=p≤0.01), $\chi 2abc = 0.00^{**}$(**=p≤0.01), ST=Sample tested, n= number of samples, N= Total number of samples from each Milk Co-operative societies, BD: Bacteriological detection, MD: Molecular detection

prevalence of the organism. Accordingly, the prevalence of *B. cereus* in farm raw milk was recorded as19.44 percent (7/36). The organism could not be confirmed molecularly in bulk and retail milk (Table 3). Studies across the world have recorded variable prevalence of *B. cereus* in raw and pasteurized milk, such as 47 percent from Ghana (Owusu-Kwarteng *et al*. 2017), 97.50 percent from Iran (Banaei *et al*. 2020), 20 percent from South Korea (Rajalingam *et al*. 2022) and 34.38 percent (Pheepakpraw *et al*. 2025). From India, Kumari and Sarkar (2014) and Meena *et al*. (2019) had reported that *B. cereus* was highly prevalent both in pasteurized milk (55 percent) and raw milk (30 percent).

Abubaker *et al*. (2023) stated that the microbial contamination of raw milk with *B. cereus* even from a healthy cow with appropriate cleanliness could not be ruled out and found to be periodically contaminating the raw milk. The present findings correlated with the farming conditions in the state and were supported by the earlier findings of Meng *et al.* (2022) from China with 100 percent prevalence of *B. cereus* in cattle faeces followed by 61.11percent in raw milk.

Genotypic characterization of B. cereus: All the seven *panC* positive *B. cereus* strains from farm raw milk possessed at least one enterotoxigenic (diarrhoeal) gene except haemolytic gene (*hblA*) and emetic (*ces*) gene (Fig. 1 & 2).Among the diarrhoeal genes, two *B. cereus* strains exhibited the non-haemolytic genes (*nheA* and *nheB*) and a combination of non-haemolytic and haemolytic (*nheA*, *nheB* and *hblC*) genes manifesting 28.57 percent each, followed by a combination of non-haemolytic and haemolytic genes (*nheB* and *hblC*), haemolytic gene (*hblC*) and non-haemolytic gene (*nheA*) evincing 14.07percent, each.

In terms of pathogenic potential, two *B. cereus* strains, AS09LM from Durtlang-leitan and AS09TM from Thuampui Milk Co-operative societies had possesed highest three numbers of virulence genes. These two strains were sequenced and provided with accession number, OR786090 and OR786089, respectively from NCBI GenBank. The results from phylogenetic analysis of *panC* gene sequences had a homology of 99.72 percent with the strains belonging to Clade III under mesophilic group. The *B. cereus* belonging to the mesophilic group

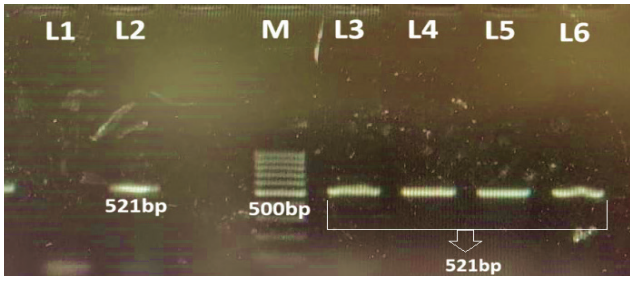

Fig.1: Agarose gel electrophoresis plate showing PCR amplicons of housekeeping *panC* (521bp) gene of *Bacillus cereus* strains in representative samples: L3, L4, L5 & L6: Sample strains from farm r aw milk, L1:Negative control, L2:Positive control, M: 100bp DNA ladder.

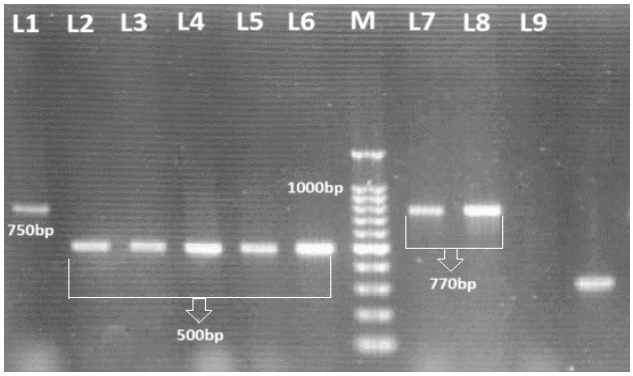

Fig. 2. Agarose gel electrophoresis plate showing PCR amplicons of enterotoxigenic genes: *hblC* (750bp), *nheA* (500bp) and *nheB* (770bp) genes of representative *B. cereus* strains: L1-L8: Sample strains from farm raw milk, L9: Negative control, M: 100bp DNA ladder.

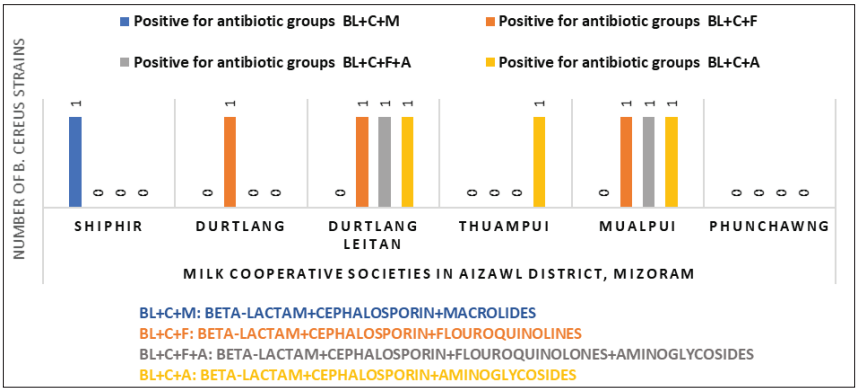

Fig. 3. Graphic representation of multi-drug resistance pattern of *B. Cereus* strains isolated from raw milk

of clade III and IV are typically cytotoxic, having the potential to thrive at temperatures ranging from 10 to 45°C. The sequenced strains, OR786090 and OR786089, were in 100 percent homology with *B. cereus* strain of Kefir, a milk product, from China, patient vomit from China and soil sample from South Korea. The *B. cereus* OR786089 strain was also found to be 99 percent homologous to *B. cereus* strain from soil of Delhi (Fig. 4).

Benahmed *et al*. (2020) and Didouh *et al.* (2023) also isolated *B. cereus* from dairy environment, milk powder and dairy processing equipment in India and Algeria, respectively where *panC* gene sequences were positioned in phylogenetic group III and associated with food poisoning. Similar to the present findings, the enterotoxigenic genes were found to be more prevalent in *B. cereus* isolated from milk and dairy farm environment around the world when compared to the emetic genes. In Norway, Porcellato *et al*. (2021) found the *nheAB* genes in 100 percent *B. cereus* isolates from raw and pasteurized milk followed by *nheC* (98percent), *hblACD* genes (51percent) while *ces* gene was absent. Cruz-Facundo *et al*. (2023) had indicated that the 93.75 percent *B. cereus* isolated from Artisanal cheese in Mexico had at least one diarrhoeagenic gene and Didouh *et al.* (2023) found that *nheBC* gene (94.10 percent) was the highest in *B. cereus* from dairy processing equipment of Algeria followed by *nheA (*88.20 percent) and *hblC* (29.40 percent) gene.

Spoilage potential of B. cereus: The proteolytic activity of the seven *B. cereus* strains from farm raw milk was not observed at 7°C of growth temperature, whereas 85.71percent (6) and 100 percent (7) *B. cereus* strains showed proteolytic activity at 25°C and 37°C, respectively. However, the lipolytic activity was observed in 85.71percent (6), 85.71percent (6) and100 percent (7) *B. cereus* strains at 24 hours, 48 hours, and 72 hours of growth at 37°C, respectively with high raw milk spoilage potential. None of the studied *B. cereus* strains showed the psychrotolerant activity at 4°C growth temperature after 24 hours and the strains were found to be mesophilic based on the phylogenetic analysis of *panC* (pantothenate synthetase) gene. The present findings further confirmed the lower abundance of the psychrotolerant *B. cereus* in the environment.

The mesophilic nature of the *B. cereus* isolates from farm raw milk along with their spoilage potential found in the present study might complement each other to facilitate its proliferation in the dairy environment increasing the chances food safety and shelf-life issues. Yusuf *et al*. (2018) opined that such *B. cereus* generates thermostable lipase and proteases which can stay active even after pasteurization.

Multi drug resistance (MDR) pattern of B. cereus: All the seven *B. cereus* strains isolated from farm raw milk showed the resistance pattern of 100 percent to ampicillin and penicillin-G followed by 85.71 percent to ceftriaxone and 71.42 percent to cefazolin. The *B. cereus* strains were 100 percent sensitive to erythromycin followed by 85.71 percent to amikacin, ciprofloxacin, ofloxacin, streptomycin, levofloxacin and azithromycin. Intermediate sensitivity was also recorded in *B. cereus* strains against cefazolin, ceftriaxone, gentamicin and streptomycin. A total of five *B. cereus* strains from different Milk Co-operative socities except Phunchawng had shown MDR against 3 to 4 classes of antibiotics. MDR was observed in one isolate from Sihphir against the combination of β-lactam, cephalosporin and macrolides,one isolate each from Durtlang, Durtlang-leitan and Mualpui against the β-lactam, cephalosporin and fluoroquinolones combination, one isolate each from Durtlang leitan, Thuampui and Mualpui against a combination of β-lactam, cephalosporin and aminoglycosides and one isolate each from Durtlang-leitan and Mualpui to the combination of β-lactam, cephalosporin, fluoroquinolone and aminoglycosides groups. (Fig.3).

From India, Kumari and Sarkar (2014) reported that *B. cereus* strains of dairy products were resistant to β-lactam antibiotics, namely ampicillin, carbenicillin, cephalothin and penicillin G. However, Gao *et al*. (2018) from China showed that *B. cereus* from pasteurized milk were resistant to β-lactam antibiotics, cephalosporins and rifampicin, but sensitive to quinolones, aminoglycosides, and macrolides. Similarly, from Egypt, Amer *et al*. (2019) isolated the milk *B. cereus* strains showing the resistance against penicillin (100 percent) followed by ampicillin (93.33percent),

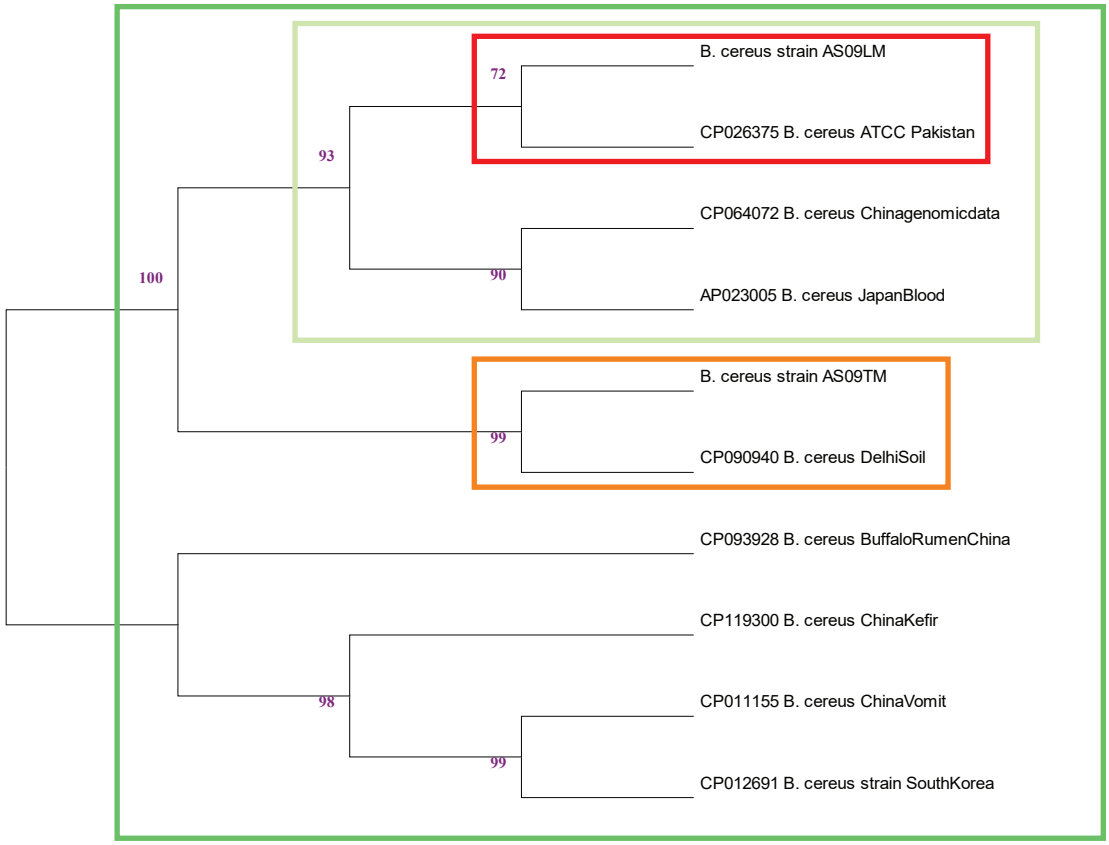

Fig. 4. Phylogenetic tree analysis of *B. cereus* strain, AS09LM (OR786090) and AS09TM (OR786089) from farm raw milk of Durtlang-leitan and Thuampui Milk Co-operative Societies of Mizoram

cefoxitin and amoxicillin (80 percent) and yet sensitive to erythromycin and enrofloxacin (100 percent), vancomycin (93.33 percent), and doxycycline (80 percent). Ahmed *et al*. (2020) also reported that 61.50 percent milk *B. cereus* isolates were resistant to 5 or more antibiotics.

This study highlights a significant risk of *B. cereus* contamination in farm raw milk of Mizoram, likely to be originating from the contaminated dairy farm environment, which is often exposed to surface runoff and faecal contamination. Poor farm hygiene and milking practices contributed by inadequate drainage, close animal contact, and limited access to clean water in the small dairy farms commonly established on hill slopes in the challenging hilly terrain of the state might have led to the prevalence of mesophilic, enterotoxigenic, and spoilage associated *B. cereus* strains along with multidrug resistance in farm raw milk indicating a potential public health risk and the reduced shelf life of the milk produced.

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