4

Results

4.1. Sampling

A total of 105 samples of six kinds of most popular and commonly used legume-based traditional fermented foods were purchased from 83 retail outlets scattered over selected 16 (out of 19) districts of West Bengal, a State in the eastern part of India (Fig. 1). The quality of the outlets, including sweet-meat parlours, restaurants, stationary and grocery shops, and roadside cafés, represented a cross-section of the standards available in the State. Amriti is a deep fat-fried pretzel-looked RTE product (Fig. 2a) which is stored even up to 5 days at ambient temperature and picked up using bare hands at the time of selling. Dosa is a fried pancake (Fig. 2c), prepared when ordered by the customers. Dhokla (Fig. 2b) and idli (Fig. 2d) are steamed cakes and kept even up to 2 days at ambient temperature. Grated coconut, coriander leaves, chilly, spices and leaves of *Muraya koennigii* are used for seasoning dhokla. Papad is a flat, thin product (Fig. 2e) which is sold mostly in packaged form. Wadi is a hollow, brittle, cone-shaped product (Fig. 2f) which is sold either locally packaged or open.

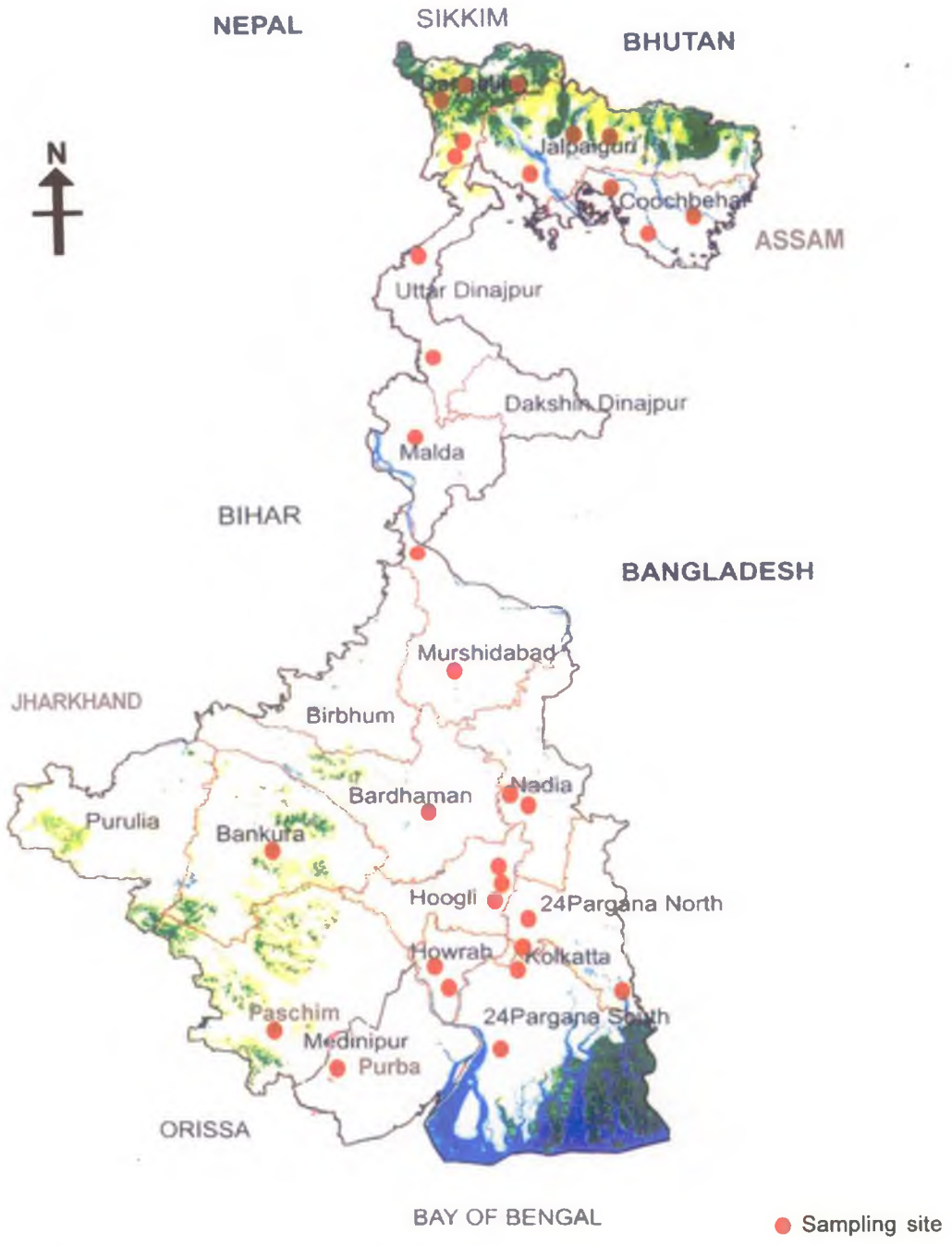


Fig. 1. Sampling sites of legume-based fermented foods in the State of West Bengal

The generalized traditional methods of production of these six foods are shown in Fig. 3. The collected information revealed that, now-a-days, in order to shorten the production time, in most cases dhokla is prepared from unfermented batter made up of Bengalgram flour (*besan*), lime juice and salt. Although dosa is prepared just prior to consumption, amriti, dhokla and idli are stored at ambient temperature even on open rack. Both raw and parboiled rice are added in different ratios in preparing dosa and idli batter. Most of the papad samples were branded, many of which were manufactured in other States of India. Packaged papads of different brands, namely Baba, Baba Lokenath, Bahurani, Duta, Ganesh, Jay, Kisan, Lazeez, Lijjat, Madhuri 777, Mahesh, MTR, Munmun, Nandan, Nimashi, Rajdhani, Ruchi, Sadhika, Shakti, Shrimati, Sona, Sonam, Super Shiv Ganga and Supreme, retailed in different markets of the State were analysed.

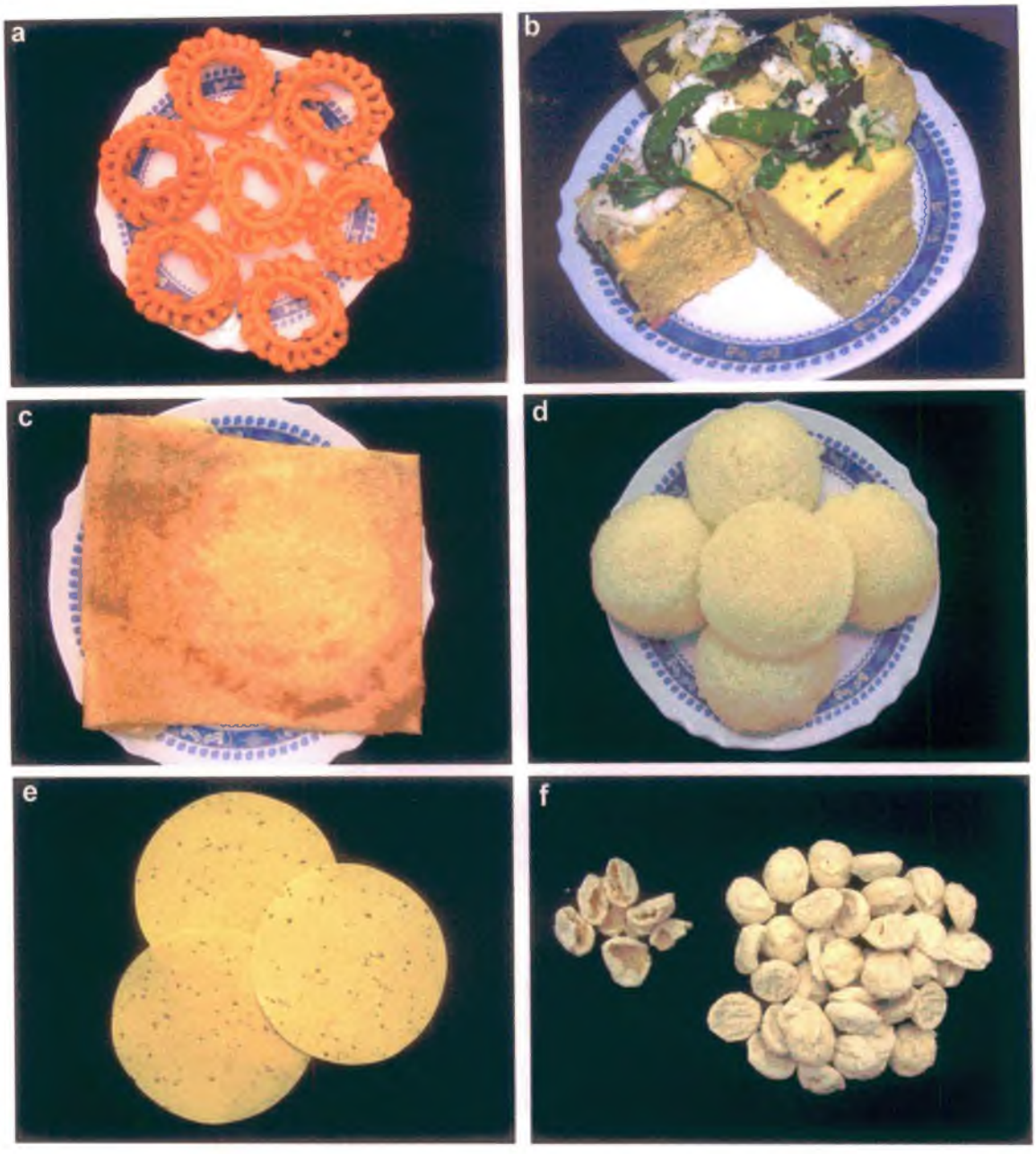
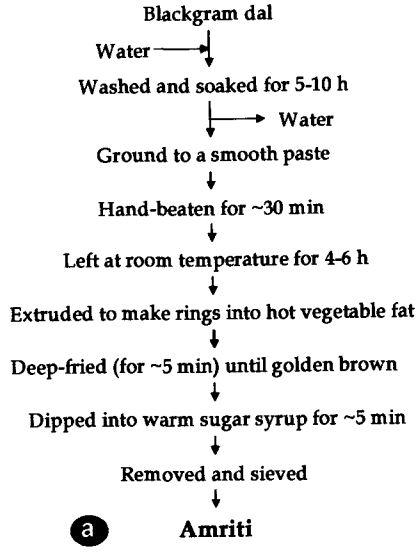
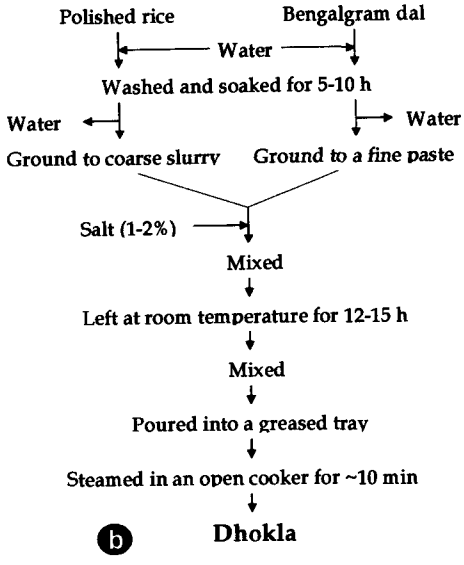
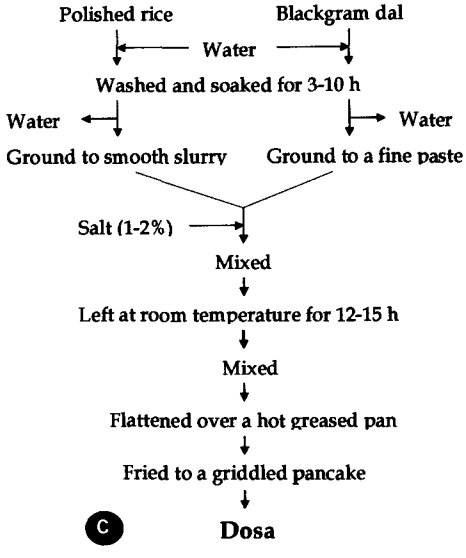


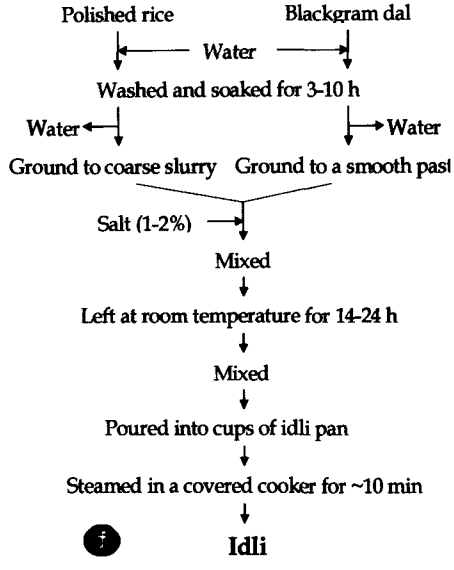
Fig. 2. Market samples of amriti (a), dhokla (b), dosa (c), idli (d), papad (e) and wadi (f)

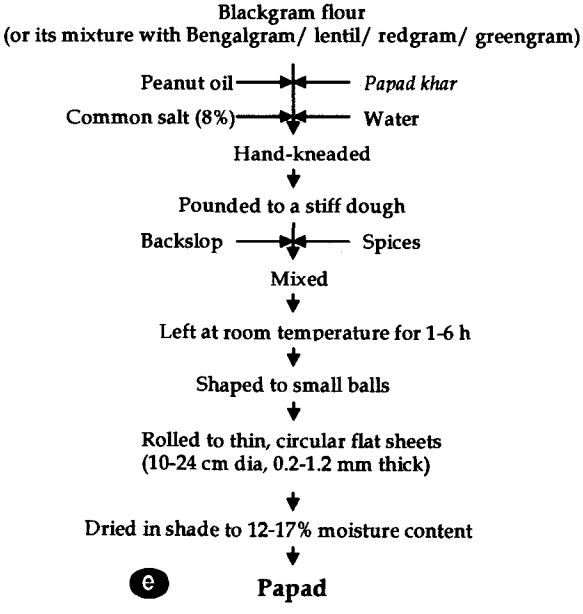
The general hygienic status of the retailers was not satisfactory. Amriti pieces, in all cases, were picked up with bare hands at the time of selling. Though spatula or forceps were used in some cases to pick up idli and dhokla pieces, in most cases bare hands were used. In some shops wadis were kept open in buckets, plastic jars or polyethylene bags. Those were picked up using bare hands to weigh for selling. In villages, wadis were commonly sold in periodic markets (locally called *haats*) where these were even kept displayed along with vegetables on polyethylene sheets. They used bare hands to handle both food and money simultaneously. Sixty-six samples were unpacked; and among the 39 packaged samples, 12 samples were locally packaged and the rest were branded packs (Table 2). Altogether 8 amriti, 5 dhokla, 16 dosa, 13 idli, 29 papad and 34 wadi samples were analysed for the presence of total aerobic mesophilic bacteria , mesophilic bacterial spores (aerobic as well as anaerobic), *B. cereus*, *C. perfringens*, *S. aureus* and Enterobacteriaceae (coliform, faecal coliform, *E. coli*, *Salmonella* and *Shigella*).











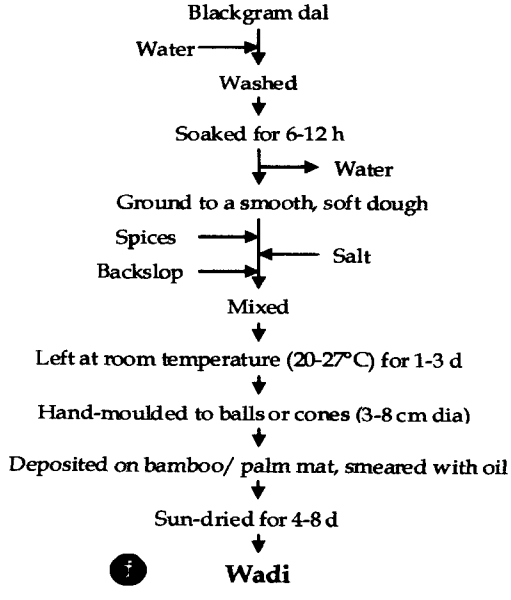


Fig. 3. Flow sheets for the preparation of amriti (a), dhokla (b), dosa (c), idli (d), papad (e) and wadi (f)

Table 2. Sampling of legume-based fermented foods from retailed outlets in West Bengal

Sple No.	Kind of sample	Date of purchase	Purchased from (locality, district)	Open/Pkd (L/B)*
S1	Wadi	20.10.03	Shivmandir, Darjiling	Open
S2	Wadi	28.10.03	Haldibari, Coochbehar	Pkd (L)
S3	Wadi	10.11.03	Jalpaiguri, Jalpaiguri	Pkd (L)
S4	Wadi	17.11.03	Malda, Malda	Open
S5	Wadi	22.12.03	Sealdah, Kolkata	Open
S6	Wadi	29.12.03	Tomluk, Purba Medinipur	Pkd (L)
S7	Wadi	29.12.03	Tomluk, Purba Medinipur	Pkd (L)
S8	Wadi	25.01.04	Kalimpong, Darjiling	Open
S9	Wadi	09.02.04	Siliguri, Darjiling	Pkd (L)
S10	Wadi	09.02.04	Bagdogra, Darjiling	Open
S11	Wadi	16.02.04	Mohitnagar, Jalpaiguri	Pkd (L)
S12	Wadi	16.02.04	Matigara, Darjiling	Open
S13	Idli	23.02.04	Bagdogra, Darjiling	Open
S14	Dosa	23.02.04	Bagdogra, Darjiling	Open
S15	Idli	01.03.04	Siliguri, Darjiling	Open
S16	Dosa	01.03.04	Siliguri, Darjiling	Open
S17	Wadi	08.03.04	Raiganj, Uttar Dinajpur	Open
S18	Papad	08.03.04	Shivmandir, Darjiling	Pkd (B)
S19	Idli	14.04.04	New Jalpaiguri, Jalpaiguri	Open
S20	Dosa	14.04.04	New Jalpaiguri, Jalpaiguri	Open
S21	Idli	22.04.04	Coochbehar, Coochbehar	Open
S22	Dosa	22.04.04	Coochbehar, Coochbehar	Open
S23	Papad	22.04.04	Coochbehar, Coochbehar	Pkd (B)
S24	Amriti	22.04.04	Coochbehar, Coochbehar	Open
S25	Amriti	22.04.04	Coochbehar, Coochbehar	Open
S26	Dosa	28.04.04	Raiganj, Uttar Dinajpur	Open
S27	Dhokla	28.04.04	Raiganj, Uttar Dinajpur	Open
S28	Amriti	28.04.04	Raiganj, Uttar Dinajpur	Open
S29	Dosa	05.05.04	Siliguri, Darjiling	Open
S30	Dosa	05.05.04	Siliguri, Darjiling	Open
S31	Dosa	05.05.04	Siliguri, Darjiling	Open
S32	Idli	05.05.04	Siliguri, Darjiling	Open
S33	Dhokla	12.05.04	Siliguri, Darjiling	Open
S34	Dhokla	12.05.04	Siliguri, Darjiling	Open
S35	Dhokla	12.05.04	Siliguri, Darjiling	Open
S36	Dosa	19.05.04	Jalpaiguri, Jalpaiguri	Open
S37	Dhokla	19.05.04	Siliguri, Darjiling	Open
S43	Wadi	31.05.04	Coochbehar, Coochbehar	Open
S44	Wadi	01.06.04	Maynaguri, Jalpaiguri	Pkd (L)
S45	Wadi	04.06.04	Garifa, Bardhaman	Pkd (L)
S46	Wadi	26.06.04	Aranghata, Nadia	Open
S47	Wadi	26.06.04	Krishnanagar, Nadia	Open
S48	Wadi	28.06.04	Shyamnagar, 24 Parganas (North)	Open
S49	Wadi	01.07.04	Bishnupur, Bankura	Open
S50	Wadi	04.07.04	Uttarpara, Hoogli	Open
S51	Wadi	04.07.04	Uttarpara, Hoogli	Open
S52	Papad	11.07.04	Islampur, Uttar Dinajpur	Pkd (B)
S53	Papad	11.07.04	Raiganj, Uttar Dinajpur	Pkd (B)
S54	Papad	16.07.04	Alipurduar, Jalpaiguri	Pkd (B)
S55	Dosa	27.07.04	Siliguri, Darjiling	Open
S56	Papad	27.07.04	Haldibari, Coochbehar	Pkd (B)
S57	Papad	27.07.04	Haldibari, Coochbehar	Pkd (B)
S58	Papad	27.07.04	Siliguri, Darjiling	Pkd (B)
S59	Idli	09.08.04	Sealdah, Kolkata	Open
S60	Dosa	09.08.04	Sealdah, Kolkata	Open

Sple No.	Kind of sample	Date of purchase	Purchased from (locality, district)	Open/Pkd (L/B)*
S61	Idli	09.08.04	Sealdah, Kolkata	Open
S62	Dosa	09.08.04	Sealdah, Kolkata	Open
S63	Idli	09.08.04	Sealdah, Kolkata	Open
S64	Dosa	09.08.04	Sealdah, Kolkata	Open
S65	Wadi	09.08.04	Howrah, Howrah	Open
S66	Wadi	09.08.04	Howrah, Howrah	Open
S67	Wadi	10.08.04	Jadavpur, Kolkata	Pkd (L)
S68	Wadi	10.08.04	Tolleyganj, Kolkata	Pkd (L)
S69	Papad	10.08.04	Vadreswar, Hoogli	Pkd (B)
S70	Papad	10.08.04	Srerampore, Hoogli	Pkd (B)
S71	Wadi	10.08.04	Nungi, 24 Parganas (South)	Open
S72	Wadi	10.08.04	Titagarah, 24 Parganas (North)	Open
S73	Papad	10.08.04	Nungi, 24 Parganas (South)	Pkd (B)
S74	Papad	10.08.04	Nungi, 24 Parganas (South)	Open
S75	Papad	11.08.04	Howrah, Howrah	Pkd (B)
S76	Papad	11.08.04	Howrah, Howrah	Pkd (B)
S77	Papad	11.08.04	Naihati, 24 Parganas (North)	Pkd (B)
S78	Papad	11.08.04	Titagarah, 24 Parganas (North)	Pkd (B)
S79	Papad	11.08.04	C.R.Avenue, Kolkata	Pkd (B)
S80	Papad	11.08.04	Krishnanagar, Nadia	Pkd (L)
S81	Papad	11.08.04	Srerampore, Hoogli	Pkd (B)
S82	Papad	11.08.04	Santoshpur, Kolkata	Pkd (B)
S83	Papad	11.08.04	Titagarah, 24 Parganas (North)	Pkd (B)
S84	Papad	11.08.04	Howrah, Howrah	Pkd (B)
S85	Papad	11.08.04	Naihati, 24 Parganas (North)	Pkd (B)
S90	Wadi	18.12.04	Kharagpur, Paschim Medinipur	Open
S91	Wadi	18.12.04	Kharagpur, Paschim Medinipur	Open
S92	Papad	18.12.04	Kharagpur, Paschim Medinipur	Pkd (B)
S93	Papad	18.12.04	Kharagpur, Paschim Medinipur	Pkd (B)
S94	Idli	27.12.04	Siliguri, Darjiling	Open
S95	Dosa	27.12.04	Siliguri, Darjiling	Open
S96	Idli	27.12.04	Siliguri, Darjiling	Open
S97	Dosa	27.12.04	Siliguri, Darjiling	Open
S98	Dosa	18.01.05	Malda, Malda	Open
S99	Amriti	18.01.05	Malda, Malda	Open
S100	Idli	18.01.05	Malda, Malda	Open
S101	Idli	18.01.05	Malda, Malda	Open
S102	Idli	18.01.05	Malda, Malda	Open
S103	Amriti	24.01.05	Siliguri, Darjiling	Open
S104	Amriti	24.01.05	Siliguri, Darjiling	Open
S105	Amriti	24.01.05	Siliguri, Darjiling	Open
S106	Amriti	24.01.05	Siliguri, Darjiling	Open
S107	Wadi	31.01.05	Malda, Malda	Open
S108	Wadi	31.01.05	Malda, Malda	Open
S119	Papad	31.01.05	Malda, Malda	Pkd (B)
S110	Papad	31.01.05	Malda, Malda	Pkd (B)
S111	Wadi	15.02.05	Farakka, Murshidabad	Pkd (L)
S112	Wadi	15.02.05	Farakka, Murshidabad	open
S113	Papad	15.02.05	Baharampur, Murshidabad	Pkd (B)
S114	Papad	15.02.05	Baharampur, Murshidabad	Pkd (B)

*Pkd, packaged; L, locally packaged; B, branded.

4.2. Isolation and confirmation of bacterial pathogens

Bacteria were isolated from the foods using selective media, and the presumptive isolates were confirmed morphologically and biochemically. Characteristic turquoise to peacock blue colonies

surrounded by a zone of precipitate of the same colour on *B. cereus* selective agar were regarded as presumptive *B. cereus* (Fig. 4). Confirmation of *B. cereus* was done according to Claus and Berkeley (1986). All the 81 presumptive isolates were endospore-forming (Fig. 5), and fermented glucose, however differed in their ability to reduce nitrate to nitrite, motility and production of acetylmethylcarbinol. A total of 48 strains were confirmed as positive following all these reactions (Table 3).

Fig. 4. *B. cereus* on *B. cereus* selective agar

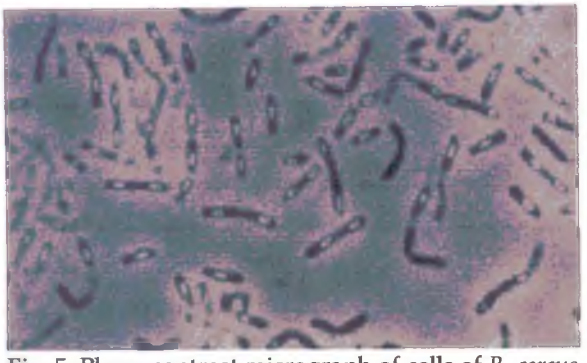


Fig. 5. Phase-contrast micrograph of cells of *B. cereus* 37-B1 (x 1250)

Table 3. Confirmation of the presumptive *B. cereus* strains grown on *B. cereus* selective agar plates*

Isolate code	Nitrate reduction	Motility	VP reaction	% +ve
1-B1, B2, B3	+, +, +	+, +, +	-, -, -	0
2-B1, B2, B3, B4, B5	+, +, +, +, +	+, +, +, +, +	+, -, +, -, -	40
3-B1, B2, B3, B4, B5	+, +, +, +, +	+, +, +, +, +	-, -, -, -, -	0
5-B1, B2, B3, B4, B5	+, +, +, +, +	+, +, +, +, +	-, -, -, -, -	0
6-B1, B2	+, +	+, +	-, +	50
16-B1	+	+	+	100
18-B1, B2, B3, B4, B5	-, +, +, -, +	+, +, +, +, +	+, +, +, +, +	60
33-B1, B2, B3	+, +, +	-, -, -	+, +, +	0
34-B1, B2, B3	+, +, +	+, +, -	+, -, +	33
35-B1, B2, B3	+, +, +	+, +, +	+, -, -	33
37-B1, B2, B3	+, +, +	+, +, +	+, -, -	33
46-B1, B2	+, +	-, +	+, +	50
49-B1, B2	+, +	+, +	+, +	100
52-B1, B2	+, +	+, +	+, +	100
54-B1	+	+	-	0
55-B1	+	+	+	100
57-B1, B2, B3, B4, B5	+, +, +, +, +	+, +, +, +, +	-, +, +, +, +	80
66-B1, B2, B3, B4, B5	+, +, +, +, +	+, +, +, +, +	+, +, +, +, +	100
70-B1, B2	+, +	+, +	+, +	100
80-B1, B2	+, +	+, +	-, -	0
93-B1, B2, B3	+, +, +	+, +, +	+, +, +	100
94-B1, B2, B3	+, +, +	+, +, +	+, +, +	100
98-B1, B2, B3	+, +, +	+, +, +	+, +, +	100
104-B1, B2, B3	+, +, +	+, +, +	+, +, +	100
105-B1, B2, B3	+, +, +	+, +, +	+, +, +	100
111-B1, B2, B3	+, +, +	+, +, +	+, +, +	100
113-B1, B2, B3	+, +, +	+, +, +	+, +, +	100

*All the isolates were endospore-forming, and fermented glucose.
+, positive reaction; -, negative reaction.

Perfringens agar (PA; OPSP) plates, incubated in an anaerobic jar containing AnaeroHiGas pack (Fig. 6), showed characteristic black colonies of presumptive *C. perfringens* (Fig. 7). Isolates, stored in cooked meat medium (Fig. 8), were examined microscopically and confirmed by the absence

Fig. 6. Anaerobic cultures for *C. perfringens*

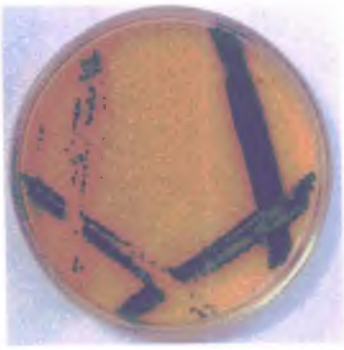


Fig. 7. *C. perfringens* in perfringens agar (OPSP)

Fig. 8. Storage of *C. perfringens* (Cp) in cooked meat medium. C, control

of motility, reduction of nitrate, fermentation of raffinose and lactose, and liquefaction of gelatin (Adams and Moss 1995). None of the 59 presumptive isolates was confirmed as *C. perfringens* (Table 4).

Table 4. Confirmation of the presumptive *C. perfringens* strains grown on perfringens agar (OPSP) plates*

Isolate code	Motility	Nitrate reduction	Raffinose fermentation	Gelatin liquefaction	% +ve
17-C1, C2, C3, C4, C5	+, +, +, +, +	+, +, -, -, -	+, +, +, +, +	+, -, -, -, -	0
18-C1	+	-	-	-	0
33-C1, C2, C3	+, +, +	-, -, -	-, -, -	-, -, -	0
35-C1, C2, C3	+, +, +	-, -, -	+, +, +	-, -, -	0
37-C1, C2, C3	+, +, +	-, -, -	+, +, +	-, +, +	0
46-C1, C2, C3	+, +, +	-, +, -	+, +, +	-, -, -	0
48-C1, C2	-, -	-, -	+, -	-, +	0
49-C1, C2	-, +	-, -	+, -	-, +	0
50-C1, C2	+, -	+, -	+, +	-, -	0
51-C1	+	-	+	-	0
52-C1, C2, C3	+, +, +,	+, +, +	+, +, +	+, +, -	0
54-C1	-	+	-	+	0
57-C1, C2, C3	+, +, -	-, +, -	+, +, -	+, -, +	0
65-C1, C2, C3	+, +, +,	+, +, +	+, +, -	+, +, -	0
66-C1, C2, C3	+, -, -	-, -, -	-, -, -	+, -, -	0
67-C1	+	+	-	-	0
69-C1	+	+	-	-	0
90-C1, C2, C3	+, +, +,	+, +, -	-, -, -	-, +, -	0
91-C1, C2, C3	+, -, -	+, +, +	+, +, +	+, -, -	0
92-C1, C2, C3	+, +, +,	-, -, +	+, +, +	-, +, +	0
93-C1, C2, C3	+, +, +,	+, +, -	-, +, +	+, -, +	0
103-C1, C2, C3	+, +, +,	+, -, +	+, +, -	+, +, +	0
108-C1	+	+	-	-	0
109-C1	+	+	-	-	0
110-C1, C2	+, -	-, -	-, -	+, -	0

*All the presumptive isolates fermented lactose.
+, positive reaction; -, negative reaction.

Characteristic grey-black shiny colonies surrounded by a clear zone on Baird-Parker agar were regarded as presumptive *S. aureus* (Fig. 9) which were confirmed by studying morphology (Fig. 10), and production of coagulase and thermostable nuclease (Fig. 11) (Adams and Moss 1995), fermentation of mannitol (HiMedia 1998) and production of acetylmethylcarbinol (Schleifer 1986). Out of three presumptive isolates, two were confirmed as *S. aureus* (Table 5).

Fig. 9. *S. aureus* on Baird-Parker agar

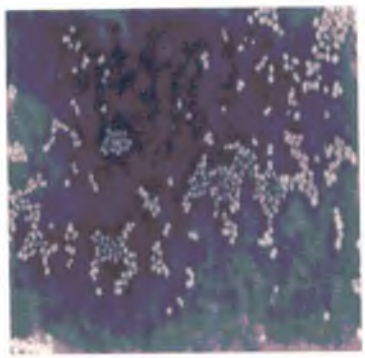


Fig. 10. Phase-contrast micrograph of cells of *S. aureus* 34-S1 (x 1200)

Fig. 11. *S. aureus* on DNase test agar

Table 5. Confirmation of the presumptive *S. aureus* strains grown on Baird-Parker agar plates

Isolate code	Coagulase	Mannitol fermentation	Thermostable DNase	VP reaction	% +ve
34-S1, S2, S3	+, +, -	+, +, -	+, +, -	+, +, +	67

+, positive reaction; -, negative reaction.

Pink colonies on tryptone soya agar-violet red bile glucose agar without lactose plates were considered as presumptive Enterobacteriaceae (Fig. 12). Those presumptive isolates were confirmed by testing for ability to ferment glucose and absence of oxidase (Nout *et al.* 1998). Out of 214 isolates, 154 were found confirmed members of Enterobacteriaceae. The isolates were then tested for their identity as coliform and faecal coliform by checking the production of gas at 37/44 °C in bile broth (Fig. 13). *Escherichia coli* (Fig. 14) was confirmed by the production of indole from tryptophan. A total of 72 strains of coliform and 19 strains of faecal coliform were found, of which only two were confirmed *E. coli* (Table 6).

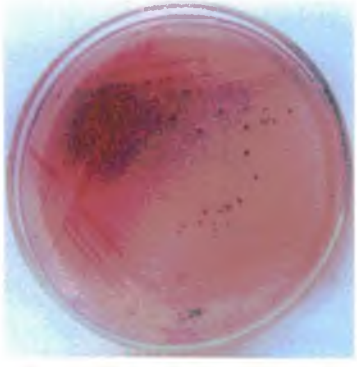


Fig. 12. Enterobacteriaceae on TSA-VRBGA

Fig. 13. Coliform/faecal coliform (2) in BGBB against control (1)

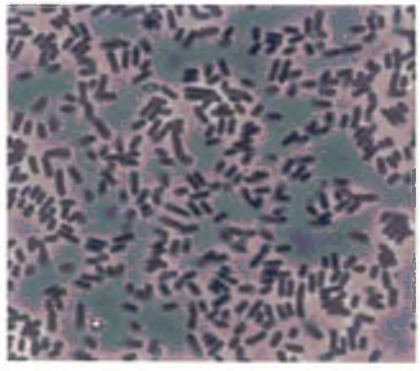


Fig. 14. Phase-contrast micrograph of cells of *E. coli* 7-E2 (x 1120)

Characteristic black colonies with metallic sheen (Fig. 15) and brown colonies (Fig. 16) on bismuth sulphite agar plates were regarded as presumptive *Salmonella* and *Shigella*, respectively. A total of 190 strains of *Salmonella/ Shigella* from bismuth sulphite agar plates were differentiated using their response to triple sugar iron agar (Fig. 17) and lysine iron agar (Fig. 18) (Adams and Moss 1995). The 33 strains of *Salmonella* were further confirmed by studying morphology (Fig. 19), the presence of motility, ability to reduce nitrate to nitrite, and inability to produce indole from tryptophan (Table 7). None of the isolates was confirmed as *Shigella* (Table 7).

Table 6. Confirmation of the presumptive Enterobacteriaceae strains isolated from TSA-VRBGA plates

Isolate code	Enterobacteriaceae			Coliform		Faecal coliform	
	Glucose fermentation	Oxidase	% +ve	Gas at 37°C	% +ve	Gas at 44°C	% +ve
2-E1, E2, E3, E4, E5	+, +, +, +, +	-, -, -, -, -	100	-, +, -, -, -	20	x, -, x, x, x	0
3-E1, E2, E3	+, +, +	-, -, -	100	-, -, +	33	x , x, +	33
5-E1, E2, E3, E4, E5	+, +, +, +, +	-, -, -, -, -	100	+, +, -, +, +	80	-, -, x, -, +	20
6-E1, E2, E3	+, +, +	-, -, -	100	-, -, -	0		
7-E1, E2, E3, E4, E5	+, +, +, +, +	-, -, -, -, -	100	+, +, +, -, -	60	+, +, +, x, x	(60)
8-E1, E2, E3, E4, E5	+, +, +, +, +	-, -, -, -, -	100	-, -, -, +, -	20	x , x, x, -, x	0
9-E1, E2, E3, E4, E5	+, +, +, +, +	-, -, -, -, -	100	+, -, -, -, -	20	-, x, x, x, x	0
10-E1, E2, E3, E4, E5	+, +, +, +, +	-, +, -, -, -	80	+, x, +, -, -	40	+, x, +, x, x	40
13-E1, E2, E3, E4, E5	+, +, +, +, +	-, -, -, +, -	80	-, +, +, x, +	60	x , -, -, x, -	0
14-E1, E2, E3, E4, E5	+, +, +, +, +	-, +, -, -, -	80	+, x, -, +, +	60	-, x, x, -, -	0
15-E1, E2, E3, E4, E5	+, +, +, +, +	-, -, -, -, -	100	-, -, -, -, +	20	x , x, x, x, -	0
18-E1, E2, E3, E4, E5	+, +, +, +, +	-, -, -, -, -	100	+, +, -, -, -	40	-, -, x, x, x	0
19-E1, E2, E3, E4, E5	+, +, +, +, +	-, -, -, -, -	100	+, +, +, +, +	100	-, -, -, -, -	0
24-E1, E2	+, +	-, -	100	-, -	0		
25-E1, E2, E3, E4, E5	+, +, +, +, +	-, +, +, -, +	40	-, x, x, -, x	0		
32-E1, E2, E3	+, +, +	-, -, -	100	-, -, +	33	x, x, -	0
37-E1, E2, E3	+, +, +	-, -, -	100	+, +, +	100	+, +, -	67
43-E1, E2, E3, E4, E5	+, +, +, +, +	-, -, -, -, -	100	+, +, +, +, +	100	-, -, -, +, -	20
44-E1, E2, E3, E4, E5	+, +, +, +, +	-, -, -, -, -	100	-, -, -, +, +	40	x, x, x, -, -	0
45-E1, E2, E3, E4, E5	+, +, +, +, +	-, -, -, -, +	80	-, -, -, -, x	0		
46-E1, E2, E3, E4, E5	+, +, +, +, +	+, +, +, -, +	20	x, x, x, -, x	0		
47-E1, E2, E3, E4, E5	+, +, +, +, +	-, -, +, +, +	40	+, +, x, x, x	40	+, -, x, x, x	20
48-E1, E2, E3, E4, E5	+, +, +, +, +	-, -, -, -, -	100	+, -, +, +, +	80	-, x, -, -, -	0
49-E1, E2, E3, E4, E5	+, +, +, +, +	+, -, +, -, -	60	x, +, x, +, +	60	x, -, x, -, -	0
51-E1, E2, E3, E4	+, +, +, +	+, +, +, -	25	x, x, x, +	25	x, x, x, +	25
52-E1, E2	+, +	-, +	50	-, x	0		
54-E1, E2	+, +	-, -	100	-, -	0		
61-E1, E2, E3, E4, E5	+, +, +, +, +	-, -, -, -, -	100	+, +, -, +, +	80	+, +, x, +, +	(80)
65-E1, E2	+, +	-, -	100	-, -	0		
66-E1, E2	+, +	-, -	100	-, -	0		
67-E1, E2, E3, E4, E5	+, +, +, +, +	-, +, -, -, -	80	-, x, -, -, -	0		
68-E1, E2, E3, E4, E5	+, +, +, +, +	-, -, -, -, -	100	+, +, +, -, +	80	-, +, -, x, -	20
72-E1, E2, E3, E4, E5	+, +, +, +, +	-, -, +, -, -	80	-, -, x, -, -	0		
73-E1, E2, E3, E4, E5	+, +, +, +, +	-, -, -, -, -	100	+, +, +, -, -	60	-, -, -, x, x	0
75-E1, E2, E3	+, +, +	-, -, -	100	-, +, +	67	x, -, -	0
76-E1, E2, E3	+, +, +	-, -, -	100	+, +, +	100	-, +, -	33
78-E1, E2, E3	+, +, +	-, -, -	100	-, -, -	0		
83-E1, E2, E3	+, +, +	-, -, -	100	+, +, +	100	+, -, -	33
84-E1, E2, E3	+, +, +	-, -, -	100	-, -, -	0		
90-E1, E2, E3, E4	+, +, +, +	+, +, -, +	25	x, x, -, x	0		
91-E1, E2, E3, E4	+, +, +, +	+, +, -, +	25	x, x, -, x	0		
94-E1, E2, E3	+, +, +	-, -, +	67	+, +, x	67	-, -, x	0
96-E1, E2, E3	+, +, +	+, +, -	33	x, x, +	33	x, x, -	0
97-E1, E2, E3	+, +, +	-, +, -	67	+, x, -	33	-, x, x	0
98-E1, E2, E3	+, +, +	+, +, +	0				
104-E1, E2, E3, E4	+, +, +, +	+, -, +, -	50	x, -, x, +	25	x, x, x, -	0
105-E1, E2, E3, E4	+, +, +, +	+, +, +, -	25	x, x, x, -	0		
107-E1, E2, E3, E4, E5	+, +, +, +, +	+, -, -, +, +	40	x, -, -, x, x	0		
108-E1, E2, E3, E4, E5	+, +, +, +, +	-, +, -, -, +	60	-, x, -, -, x	0		
109-E1, E2, E3, E4, E5	+, +, +, +, +	+, +, +, +, +	0				
111-E1, E2, E3, E4, E5	+, +, +, +, +	+, +, +, +, +	0				
112-E1, E2, E3, E4, E5	+, +, +, +, +	+, +, +, +, +	0				

+, positive reaction; -, negative reaction; x, not determined; Circle indicates the presence of *E. coli*.

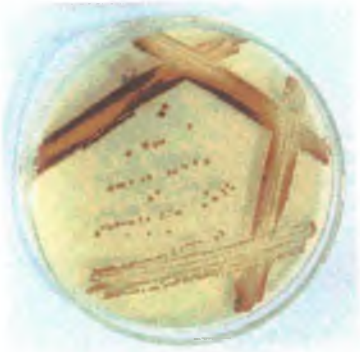


Fig. 15. *Salmonella* on bismuth sulphite agar

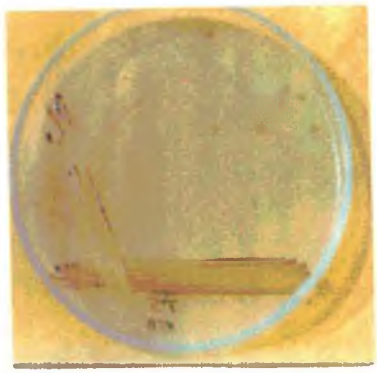


Fig. 16. *Shigella* on bismuth sulphite agar

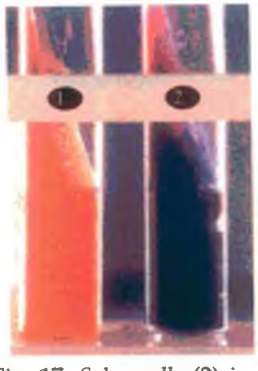


Fig. 17. *Salmonella* (2) in TSI agar against control (1)

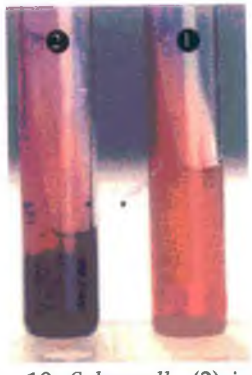


Fig. 18. *Salmonella* (2) in lysine iron agar against control (1)

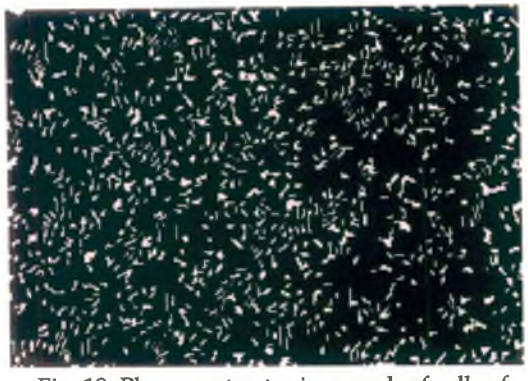


Fig. 19. Phase-contrast micrograph of cells of *Salmonella* 1-S4 (x 1200)

4.3. Biochemical profile and bacteriological quality of fermented foods

The results of moisture, pH and microbial analyses of 105 samples of the six kinds of foods are summarized in Table 8. While dhokla and idli were the high moisture-content (62 g (100 g)$^{-1}$) foods, others contained less moisture (14-27 g (100 g)$^{-1}$). Papad was alkaline (pH 8.7), whereas all the other foods were acidic (pH 4.4-5.8).

Thirty-eight percent (40 of 105) of the samples contained total aerobic mesophilic bacterial cells at a level of > 10^6 cfu g^{-1} (Table 8). Majority of the samples of each of the six foods, except dosa, had a high count (> 10^4 cfu g^{-1}) of these bacteria. While most of the samples of amriti, dosa, idli and papad contained total aerobic mesophilic bacteria in the range of 10^2-10^6 cfu g^{-1}, in most of the samples of dhokla and wadi their count was at a higher level (> 10^6 cfu g^{-1}).

Aerobic mesophilic bacterial spores were found in 88% (92 of 105) of the samples. All the samples of amriti, dhokla and papad contained these spores. A high count (> 10^5 cfu g^{-1}) of them was found in papad and wadi (10 of 63 samples). As dosa samples were freshly prepared ones, their load in the product was never more than 10^5 cfu g^{-1}. On the other hand, the load of their anaerobic counterpart was less; they occurred in 39% of the tested samples (41 of 105). Amriti, dosa and idli were free of them.

All the six types of foods contained *B. cereus*; this organism occurred in 20% of the samples (21 of 105). The potentially hazardous level (> 10^4 cfu g^{-1}) was observed in dhokla, papad and wadi. *C. perfringens* could not be detected from any of the 105 samples, and only one sample was found contaminated (at the load of 4 x 10^4 cfu g^{-1} dhokla) with *S. aureus*.

Table 7. Confirmation of the presumptive *Salmonella/ Shigella* strains isolated from bismuth sulphite agar plates

Isolate code	In triple sugar iron agar[a]				In lysine iron agar[b]			Motile	Indole production	Nitrate reduction	Confirmed identity
	Slant	Butt	Gas	H_2S	Slant	Butt	H_2S				
1-S1, S2, S3	A, A, A	A, A, A	+, +, +	-, -, -	R, R, R	K, K, K	-, -, -	x[c], x, x	x, x, x	x, x, x	
1-S4, S5, S6	K, K, K	A, A, A	+, +, +	+, + +	K, K, K	A, A, A	+, +, +	+, +, +	-, -, -	+, +, +	*Salmonella*
1-S7, S8, S9	A, K, A	A, A, A	+, +, +	+, +, +	R, R, R	A, A, A	-, -, -	x, +, x	x, x, x	x, -, x	
1-S10, S11, S12	A, A, A	A, A, A	+, +, +	-, -, -	R, R, R	K, K, K	-, -, -	x, x, x	x, x, x	x, x, x	
2-S1, S2, S3	A, A, A	A, A, A	+, +, +	-, -, -	R, R, R	A, A, A	-, -, -	x, x, x	x, x, x	x, x, x	
2-S4, S5, S6	A, A, A	A, A, A	+, +, +	-, -, -	R, R, R	K, K, K	-, -, -	x, x, x	x, x, x	x, x, x	
3-S1, S2, S3	K, A, K	A, A, A	+, +, +	-, -, -	K, K, K	A, K, A	-, -, -	+, x, +	x, x, x	-, x, -	
3-S4, S5, S6	A, A, A	A, A, A	+, +, +	-, -, -	R, R, R	K, K, K	-, -, -	x, x, x	x, x, x	x, x, x	
4-S1, S2, S3	A, A, A	A, A, A	+, +, +	-, -, -	R, R, R	A, A, A	-, -, -	x, x, x	x, x, x	x, x, x	
4-S4, S5, S6	A, A, A	A, A, A	+, +, +	-, -, -	R, R, R	A, A, A	-, -, -	x, x, x	x, x, x	x, x, x	
4-S7, S8, S9	A, A, A	A, A, A	+, +, +	-, -, -	R, R, R	A, R, A	-, -, -	x, x, x	x, x, x	x, x, x	
5-S1, S2, S3	K, A, A	A, A, A	+, +, +	-, -, -	R, R, R	A, A, A	-, -, -	-, x, x	x, x, x	-, x, x	
5-S4, S5, S6	A, A, A	A, A, A	+, +, +	-, +, +	R, R, R	A, A, A	+, +, +	x, x, x	x, x, x	x, x, x	
5-S7, S8, S9	A, A, A	A, A, A	+, +, +	-, -, -	R, R, R	A, A, A	-, -, -	x, x, x	x, x, x	x, x, x	
5-S10, S11, S12	A, A, K	A, A, A	+, +, +	-, -, -	R, R, R	R, A, A	-, -, -	x, x, +	x, x, +	x, x, +	
6-S1, S2, S3	K, K, K	A, A, A	+, +, +	-, -, -	K, K, K	A, A, A	-, -, -	+, +, +	x, +, +	-, +, +	
6-S4, S5, S6	A, K, A	A, K, A	+, -, +	-, -, -	K, K, K	A, K, A	-, -, -	x, x, x	x, x, x	x, x, x	
7-S1, S2, S3	K, K, K	A, A, A	+, +, +	+, +, +	K, K, K	A, A, A	+, +, +	+, +, +	+, -, -	+, +, +	*Salmonella*
7-S4, S5, S6	A, K, A	A, A, A	+, +, +	-, -, -	K, K, K	K, A, A	-, -, -	x, +, x	x, -, x	x, +, x	*Salmonella*
8-S1, S2, S3	K, K, A	A, A, A	+, +, +	-, -, -	K, K, K	A, A, A	-, -, -	-, -, x	x, x, x	-, -, x	
8-S4, S5, S6	A, A, A	A, A, A	+, +, +	-, -, -	K, K, K	K, A, A	-, -, -	x, x, x	x, x, x	x, x, x	
9-S1, S2, S3	K, K, A	A, A, A	+, +, +	-, -, -	K, K, K	A, A, A	-, -, -	-, -, x	x, x, x	-, -, x	
9-S4, S5, S6	A, A, K	A, A, A	+, +, +	-, -, -	K, K, K	A, A, A	-, -, -	x, x, +	x, x, x	x, x, -	
10-S1, S2, S3	A, A, A	A, A, A	+, +, +	-, -, -	K, K, K	A, A, A	-, -, -	x, x, x	x, x, x	x, x, x	
10-S4, S5, S6	A, A, A	A, A, A	+, +, +	-, -, -	K, K, K	K, K, K	-, -, -	x, x, x	x, x, x	x, x, x	
11-S1, S2, S3	A, K, A	A, A, A	+, +, +	-, -, -	K, K, K	K, A, A	-, -, -	x, +, x	x, -, x	x, +, x	*Salmonella*
11-S4, S5, S6	A, A, A	A, A, A	+, +, +	-, -, -	K, K, K	A, K, K	-, -, -	x, x, x	x, x, x	x, x, x	
12-S1, S2, S3	A, A, A	A, A, A	+, +, +	+, +, +	K, K, K	A, A, A	+, +, +	x, x, x	x, x, x	x, x, x	
12-S4, S5, S6	A, A, A	A, A, A	+, +, +	-, -, -	K, K, K	K, K, K	-, -, -	x, x, x	x, x, x	x, x, x	
13-S1, S2, S3	K, K, K	A, A, A	+, +, +	-, -, -	K, K, K	K, K, A	-, -, -	+, +, +	x, x, -	-, -, +	*Salmonella*
13-S4, S5, S6	R, R, R	K, A, K	-, -, -	- + -	K, K, K	K, K, K	-, -, -	x, x, x	x, x, x	x, x, x	
14-S1, S2, S3	A, A, A	A, A, A	+, +, +	-, -, -	K, K, K	A, R, A	-, -, -	x, x, x	x, x, x	x, x, x	
14-S4, S5, S6	A, A, A	A, A, A	+, +, +	-, -, -	K, K, K	K, K, K	-, -, -	x, x, x	x, x, x	x, x, x	
15-S1, S2, S3	A, A, A	A, A, A	-, -, -	+, +, +	R, R, R	A, A, A	-, -, -	x, x, x	x, x, x	x, x, x	
15-S4, S5, S6	A, A, A	A, A, A	+, +, +	-, -, -	K, K, K	A, A, A	-, -, -	x, x, x	x, x, x	x, x, x	

Isolate code	In triple sugar iron agar[a]				In lysine iron agar[b]			Motile	Indole production	Nitrate reduction	Confirmed identity
	Slant	Butt	Gas	H_2S	Slant	Butt	H_2S				
17-S1, S2, S3	K, K, K	A, A, A	+, +, +	-, -, -	K, K, K	A, A, A	-, -, -	+, +, +	-, -, -	+, +, +	*Salmonella*
17-S4, S5, S6	A, A, A	A, A, A	+, +, +	-, -, -	K, K, K	K, K, K	-, -, -	x, x, x	x, x, x	x, x, x	
21-S1, S2, S3	A, A, A	A, A, A	+, +, +	-, -, -	K, K, K	K, K, K	+, +, +	+, +, +	-, -, -	+, +, +	*Salmonella*
21-S4, S5, S6	K, K, K	K, K, K	-, -, -	-, -, -	K, K, K	K, K, K	-, -, -	x, x, x	x, x, x	x, x, x	
22-S1, S2, S3	A, A, A	A, A, A	+, +, +	-, -, -	K, K, K	K, K, K	-, -, -	x, x, x	x, x, x	x, x, x	
22-S4, S5, S6	A, A, A	A, A, A	+, +, +	-, -, -	K, K, K	K, K, K	-, -, -	x, x, x	x, x, x	x, x, x	
23-S1, S2, S3	K, K, K	A, A, A	+, +, +	-, -, -	K, K, K	K, K, K	-, -, -	+, +, +	-, -, x	+, +, -	*Salmonella*
24-S1, S2, S3	A, A, A	A, A, A	+, +, +	-, -, -	K, K, K	K, K, K	-, -, -	x, x, x	x, x, x	x, x, x	
25-S1, S2, S3	A, A, A	A, A, A	-, +, +	-, -, -	K, K, K	K, K, K	-, -, -	x, x, x	x, x, x	x, x, x	
26-S1, S2, S3	A, K, K	A, K, K	+, -, -	-, -, -	K, K, K	K, K, K	-, -, -	x, x, x	x, x, x	x, x, x	
26-S4, S5, S6	K, K, K	K, K, K	-, -, -	-, -, -	K, K, K	K, K, K	-, -, -	x, x, x	x, x, x	x, x, x	
27-S1, S2, S3	K, K, K	A, A, K	-, -, -	-, -, -	K, K, K	A, A, K	-, -, -	+, +, x	x, x, x	-, -, x	
29-S1, S2, S3	A, A, A	A, A, A	+, +, +	-, -, -	K, K, K	K, K, A	-, -, -	x, x, x	x, x, x	x, x, x	
30-S1, S2, S3	R, R, R	K, K, K	-, -, -	-, -, -	K, K, K	K, K, K	-, -, -	x, x, x	x, x, x	x, x, x	
32-S1, S2, S3	R, R, R	K, K, K	-, -, -	-, -, -	K, K, K	K, K, K	-, -, -	x, x, x	x, x, x	x, x, x	
33-S1, S2, S3	K, K, K	A, A, A	+, +, +	+, +, +	K, K, K	A, A, A	+, +, +	+, +, +	x, x, x	-, -, -	
34-S1, S2, S3	A, A, A	A, A, A	+, +, +	-, -, -	K, K, K	A, A, A	- + -	x, x, x	x, x, x	x, x, x	
36-S1, S2, S3	A, A, A	A, A, A	+, +, +	-, -, -	K, K, K	A, A, A	-, -, -	x, x, x	x, x, x	x, x, x	
37-S1, S2, S3	A, A, A	A, A, A	+, +, +	-, -, -	K, K, K	A, A, A	-, -, -	x, x, x	x, x, x	x, x, x	
43-S1, S2, S3	K, K, K	A, A, A	+, +, +	-, -, -	K, K, K	K, K, K	-, -, -	+, +, +	-, -, -	+, +, +	*Salmonella*
43-S4, S5, S6	K, K, K	A, A, A	+, +, +	-, -, -	K, K, K	K, K, K	-, -, -	+, +, +	-, -, -	+, +, +	*Salmonella*
43-S7, S8, S9	A, A, A	A, A, A	+, +, +	-, -, -	K, K, K	K, K, K	-, -, -	x, x, x	x, x, x	x, x, x	
43-S10	A	A	+	-	K	K	-	x	x	x	
62-S1, S2, S3	A, A, A	A, A, A	+, +, +	-, -, -	K, K, K	K, K, K	-, -, +	x, x, +	x, x, +	x, x, +	
68-S1, S2, S3	K, A, A	A, A, A	+, +, +	-, -, -	K, K, K	K, K, K	-, -, -	+, x, x	-, x, x	+, x, x	*Salmonella*
68-S4, S5, S6	K, A, A	A, A, A	+, +, +	-, -, -	K, K, K	K, K, K	-, -, -	+, x, x	-, x, x	+, x, x	*Salmonella*
83-S1, S2, S3	K, K, K	A, A, A	+, +, +	-, -, -	K, K, K	A, A, A	-, -, -	+, +, +	-, -, -	+, +, +	*Salmonella*
84-S1, S2, S3	K, K, K	A, A, A	+, +, +	-, -, -	R, R, R	R, R, A	-, -, -	+, +, +	-, -, -	+, +, +	*Salmonella*
85-S1, S2, S3	K, K, K	A, A, A	+, +, +	-, -, -	K, K, K	A, A, A	-, -, -	+, +, +	-, -, -	+, +, +	*Salmonella*

[a]A, acidic, yellow colour; K, alkaline, no change in colour; +, blackening (H_2S) positive reaction; -, no reaction.

[b]R, deep red, lysine deamination;, K, alkaline, no colour change; A, acidic, yellow colour; +, blackening of medium; -, no blackening of medium.

[c]x, not determined.

Enterobacteriaceae occurred in all the six types of foods studied (Table 8); these were detected in 46% (48 of 105) of the samples. Of the Enterobacteriaceae isolates, 92% were coliforms and 57% were faecal coliforms (Table 9). One sample each of idli (3.8×10^3 cfu g^{-1}) and wadi (3.2×10^4 cfu g^{-1}) were found contaminated with *E. coli*.

Salmonella was present in 11.4% (12 of 105) of the total samples analysed. It was not detected in amriti, dhokla and dosa. However, its prevalence in the other three foods is noteworthy; 15% (2 of 13), 14% (4 of 29) and 18% (6 of 34) of the samples of idli, papad and wadi, respectively, were found contaminated with this pathogen.

To demonstrate the variability of viable counts observed with single samples of different kinds of foods, the data ranges of positive samples are presented in Fig 20. The number of total aerobic mesophilic bacteria, mesophilic bacterial spores, *B. cereus*, *C. perfringens*, *S. aureus* and Enterobacteriaceae varied considerably. As to the total aerobic mesophilic bacteria, some samples of dhokla, papad and wadi contained a high load (> log 7.0 cfu g^{-1}) of them. The maximum load (log 11.4 cfu g^{-1}) with a wide range (log 4-11 cfu g^{-1}) of total aerobic mesophilic bacterial count occurred in wadi. As per aerobic mesophilic bacterial spores, some samples of papad and wadi exceeded log 5.0 cfu g^{-1}. A large variation

Table 8. Moisture content, pH and load of different components of microbiota (expressed as percentages of samples analysed) of legume-based traditional fermented foods

Parameter	Food					
	Amriti (n = 8)	Dhokla (n = 5)	Dosa (n = 16)	Idli (n = 13)	Papad (n = 29)	Wadi (n = 34)
Moisture[a], g (100 g) $^{-1}$	19.5bc±1.12	62.1a±0.86	27.3b±1.73	61.8a±1.58	18.0bc±0.41	14.4c±0.33
pH[a]	5.8b±0.07	4.9c±0.11	4.4d±0.08	4.6cd±0.07	8.7a±0.07	5.7b±0.04
Bacterial load (cfu g^{-1})[b]						
TAMB						
< DL[c]						
10^2-10^4	12.5		56.3	23.1	17.2	2.9
> 10^4-10^6	62.5		43.8	76.9	75.9	5.9
> 10^6-10^9	25	60			6.9	55.9
> 10^9 -10^{12}		40				35.3
aMBS						
< DL[d]			50	15.4		8.8
10^2-10^5	100	100	50	84.6	79.3	76.5
> 10^5-10^7					20.7	14.7
anMBS						
< DL[c]	100	40	100	100	44.8	35.3
10-10^4		60			55.2	47.1
> 10^4-10^5						17.6
B. cereus						
< DL[d]	75	40	81.3	92.3	79.3	82.4
10^2-10^3		20	12.5	7.7	10.3	8.8
> 10^3-10^4	25		6.3		6.9	2.9
> 10^4-10^6		40			3.4	5.9
Enterobacteriaceae						
< DL[c]	50	80	87.5	46.2	69	26.5
10-10^3	25			7.7	17.2	17.6
> 10^3-10^5	12.5		12.5	46.2	13.8	23.5
> 10^5-10^8	12.5	20				32.4

[a]Values are mean with standard error of measurements. Means with the same following letters, within rows, are not significantly different (P <0.05).

[b]TAMB, total aerobic mesophilic bacteria; aMBS, aerobic mesophilic bacterial spores, anMBS, anaerobic mesophilic bacterial spores.

[c]DL (detection limit), 10 cfu g^{-1}.

[d]DL, 100 cfu g^{-1}.

Table 9. Percentage of the different components of Enterobacteriaceae

Enterobacteriaceae component	Food						
	Amriti	Dhokla	Dosa	Idli	Papad	Wadi	Total
Coliform	49.1	100	58.7	82.6	49.2	59.8	91.9
Faecal coliform	0	66.7	0	15.5	12.5	18.6	56.9
E. coli	0	0	0	3.9	0	0.6	0.1

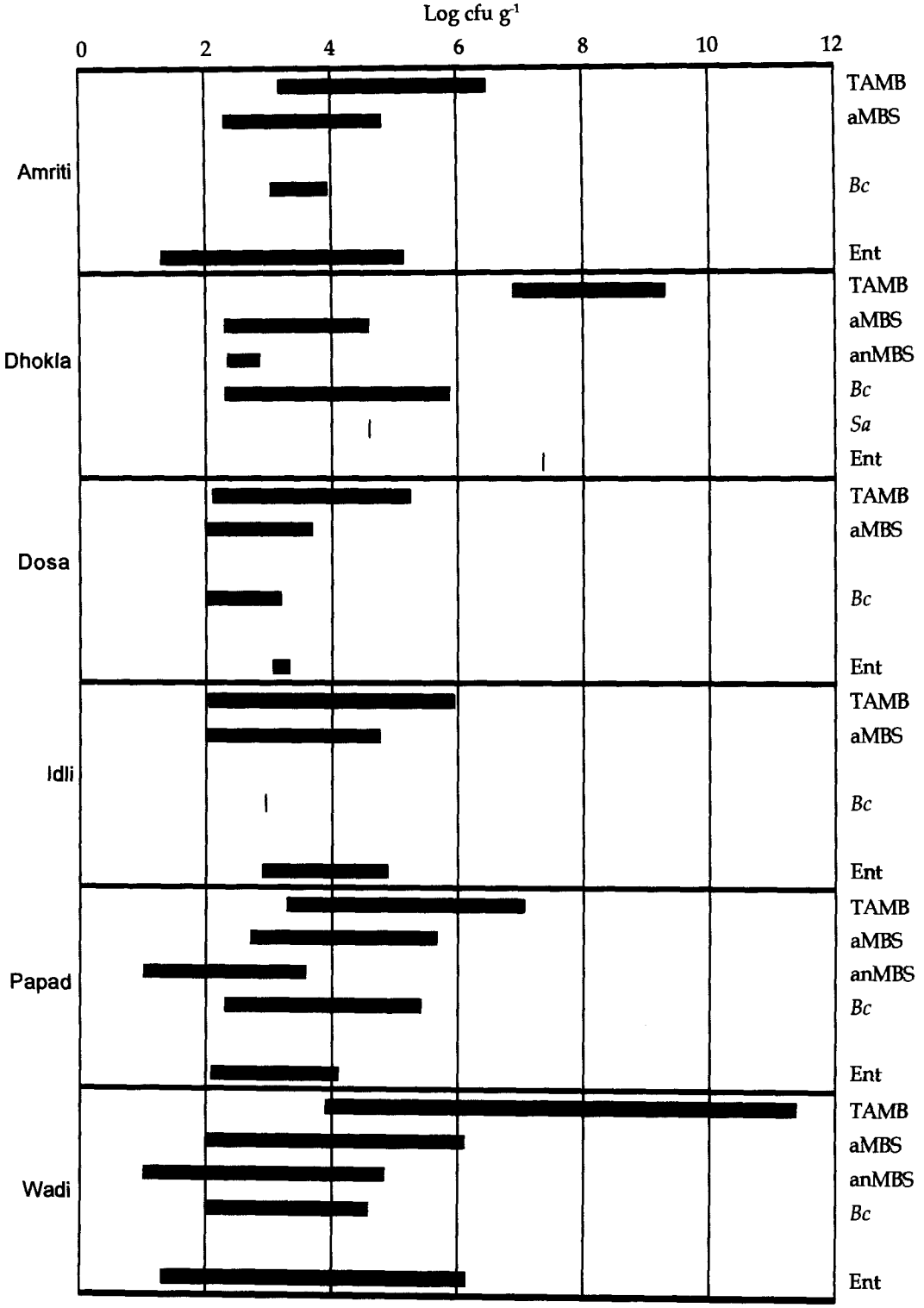


Fig. 20. Ranges of pathogenic bacterial load among the positive samples of food.
TAMB, total aerobic mesophilic bacteria; aMBS, aerobic mesophilic bacterial spores; anMBS, anaerobic mesophilic bacterial spores, *Bc*, *B. cereus*; *Sa*, *S. aureus*; Ent, Enterobacteriaceae

Table 10. Antibiogram of *the* isolates from legume-based fermented foods[a]

Mechanism of action	Antibiotics (disc^{-1})	Percent score								
		B. cereus (n = 48)			Enterobacteriaceae (n = 24)			*Salmonella* (n = 33)		
		S	I	R	S	I	R	S	I	R
Inhibition of cell wall synthesis	Ampicillin (10 µg)			100	50	33	17	9		91
	Bacitracin (10 U)		29	71	33	21	46	21	9	70
	Carbenicillin (100 µg)			100	8	21	71	42	16	42
	Cephalothin (30 µg)	2		98	58	13	29	21	3	76
	Cloxacillin (10 µg)			100	58		42	18		82
	Penicillin G (10 U)			100		8	92	15		85
	Vancomycin (10 µg)			100	4	29	67	9	6	85
Inhibition of protein synthesis	Chloramphenicol (30 µg)	96	4		75	4	21	100		
	Erythromycin (15 µg)		60	40	21	12	67			100
	Kanamycin (30 µg)	58	40	2	75	8	17	15	39	46
	Streptomycin (10 µg)	67	29	4	46	12	42	12	18	70
	Tetracycline (30 µg)	83	17		88	8	4	49	30	21
Damage to cell membrane	Polymyxin B (300 U)			100	54	42	4	45	55	
Inhibition of nucleic acid synthesis	Ciprofloxacin (10 µg)	98		2	79	4	17	79	15	6
	Nalidixic acid (30 µg)	38	58	4	54	21	25	73	18	9
	Rifampicin (15 µg)	6	25	69	12	17	71	3		97
	Metronidazole (5 µg)			100			100			100
Inhibition of folic acid synthesis	Trimethoprim (10 µg)			100	8	4	88	27		73

[a]S, sensitive; I, intermediate; R, resistant (the inhibition zone size, diameter in mm, interpretation was according to Banerjee and Sarkar 2004a).

(2-6 log cfu g^{-1}) in the counts of aerobic mesophilic bacterial spores was found in wadi. Large variation in the count of anaerobic mesophilic bacterial spores was found in papad and wadi only. Of the tested pathogenic bacteria, *B. cereus* was the most predominant one with a wide range of distribution in dhokla, papad and wadi. *S. aureus* was present in dhokla only and that at a low level. Enterobacteriaceae count was highest in dhokla. It varied widely in amriti and wadi. The results show that amriti and dosa were of better quality foods, compared to others.

4.4. Susceptibility to antimicrobials

Susceptibility to 18 antimicrobials, including β-lactams (5), benzene derivative (1), aminoglycosides (2), macrolides (2), peptides (2), glycopeptide (1), quinolones (2), nitro-imidazole (1), tetracycline and trimethoprim, are shown in Table 10. Each of the isolates of *B. cereus* showed multiple resistance. Out of the 48 isolates, 10% were resistant against 9 antibiotics, 21% against 10 antibiotics, 38% against 11 antibiotics, 29% against 12 antibiotics, and 2% against 13 antibiotics (Fig. 21). Out of the 24 Enterobacteriaceae strains, 4% each were resistant against 4, 11, 12, 13, 14 and 15 antibiotics, 25% against 5 antibiotics, 13% against 6, 7 and 9 antibiotics, and 12% against 10 antibiotics. Each of the tested 33 strains of *Salmonella* was multiple-antibiotic resistant. Six percent each were resistant against both 5 and 7 antibiotics, 3% each against both 6 and 8 antibiotics, 12% each against 9, 10 and 11 antibiotics, 28% against 12 antibiotics, and 9% each against both 13 and 14 antibiotics.

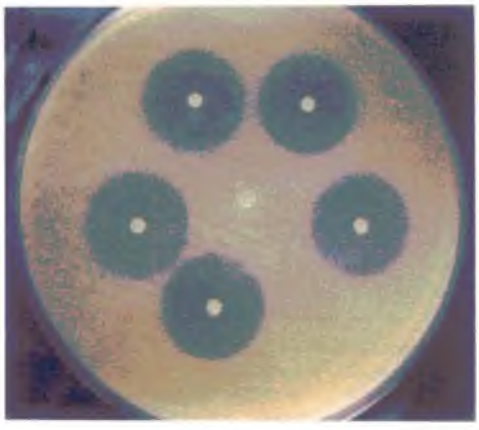


Fig. 21. Sensitivity of bacterial isolate to antimicrobial compounds

4.5. Thermal inactivation of sporeformers

The *D*-values were calculated from the regression analysis best-fit plot of the linear portion of the survivor curve (Fig. 22). In glucose-supplemented brain-heart infusion broth, the correlation coefficient (R^2) values of decimal reduction time curves for spore suspensions of 12 different isolates of *B. cereus* were at least 0.91. The mean $D_{100°C}$-values of 12 strains of *B. cereus* spores was 6.2 min (Table 11)

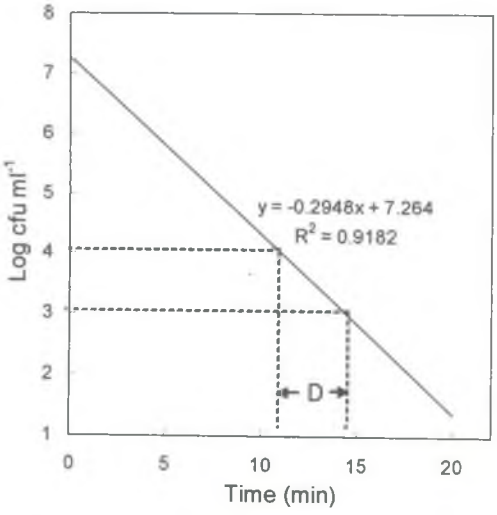


Fig. 22. Survivor curve of *B. cereus* at 100 °C

Table 11. Thermal inactivation of spores of *B. cereus* isolates from different food sources

Source	Isolate no.	$D_{100°C}$ (min)[a]
Amriti	104-B1	7.0 ± 0
	105-B1	5.2 ± 0.2
Dhokla	35-B1	5.3 ± 0.1
	37-B1	7.4 ± 0.1
Dosa	55-B1	5.6 ± 0.1
	98-B1	8.0 ± 0
Idli	94-B1	3.0 ± 0
	94-B2	4.8 ± 0.1
Papad	93-B1	9.2 ± 0.2
	113-B1	6.2 ± 0.2
Wadi	66-B1	6.0 ± 0.1
	111-B1	6.8 ± 0.2

[a]Values are mean ± SE of triplicate determinations.

4.6. Production of extracellular enzymes by *B. cereus*

The results on the production of three extracellular enzymes viz. protease, lipase and amylase are presented in Table 12. Proteolytic and amylolytic activities were found in 33% and 46%, respectively, of the isolates. However, lipolytic activity was found in only 27% of the isolates. Eleven (23%) isolates produced all the three enzymes, while 24 (50%) isolates did not produce any of these enzymes.

Table 12. Production of extracellular enzymes by *B. cereus* isolates (n = 48) from different food sources

Source	Isolate no[a]	Zone diameter (mm)[b]		
		Protease	Lipase	Amylase
Amriti	104-B3	27		30
	105-B2	26		11
	105-B3	32		33
Dhokla	34-B1	42	25	31
	37-B1			24
Dosa	55-B1		32	
Idli	94-B1			22
	94-B2			14
	94-B3	41	19	39
Papad	18-B2		22	47
	52-B2	41	36	55
	57-B2	41	26	39
	57-B3	42	30	41
	57-B5			23
	70-B1	42	28	41
	93-B2			12
	93-B3	37		44
	113-B2			12
	113-B3	40	22	35
Wadi	2-B1	40	26	35
	6-B2	45	26	38
	49-B1	32		
	66-B3	42	30	49
	111-B1	37	13	18

[a]Others had no activity.
[b]Includes diameter of the well (5 mm).

4.7. Influence of pH on growth

The effect of pH on the growth of *B. cereus* isolates, one each from the 6 different kinds of foods, is shown in Table 13. In nutrient broth, the minimum and maximum pHs permitting growth of *B. cereus* were 5.3 and 11.6, respectively. The optimum pH was 9.0-9.9.

S. aureus grew at pH range of 4.8-9.5, with an optimum being 6.1 in nutrient broth after 24 h at 35°C. The minimum and maximum pHs permitting growth of *E. coli* was 4.0 and 9.5, respectively, with an optimum of 6.1 in nutrient broth after 24 h at 35°C.

The minimum pH permitting growth of *Salmonella* was 4.3, while the maximum limit was 9.9 (Table 14), with an optimum of 7.3 in nutrient broth after 24 h at 35°C.

Table 13. Range of pH and MIC[a] of food preservatives against the growth of *B. cereus* isolates from different food sources

Source	Target strain	pH range for growth[b]	Preservative in nutrient agar[c]			
			NaCl (mg ml^{-1})	Benzoic acid (μg ml^{-1})	Sorbic acid (μg ml^{-1})	Nisin (μg ml^{-1})
Amriti	104-B1	5.3-11.6	65	400 (5.0)	500 (5.0)	175
	105-B1/B2	nd	80	650 (4.3)	500	nd
	104-B3, 105-B3	nd	85	550 (4.5)	500	nd
	104-B2	nd	85	650	500	nd
Dhokla	37-B1	5.4-11.1	50	400	500	>300
	34-B1, 35-B1	nd	85	550	500	>300
Dosa	98-B1	5.3-11.6	65	450 (4.8)	500	>300
	98-B2/B3	nd	80	650	500	nd
	16-B1, 55-B1	nd	85	600 (4.4)	500	nd
Idli	94-B1	5.3-11.6	65	450	600 (4.8)	>300
	94-B2	nd	85	450	600	nd
	94-B3	nd	85	600	500	nd
Papad	113-B1	5.3-11.6	70	400	500	>300
	57-B5	nd	80	650	500	nd
	113-B3	nd	85	550	500	nd
	18-B2/ B3/ B5, 52-B2, 57-B2/B3/B4, 70-B1/B2, 93-B1/B2/B3, 113-B2	nd	85	600	500	nd
	52-B1	nd	85	650	500	>300
Wadi	111-B1	5.3-11.6	70	450	500	175
	111-B2	nd	70	450	500	nd
	111-B3	nd	80	550	500	nd
	6-B2, 49-B2	nd	85	550	500	nd
	49-B1	nd	85	550	500	>300
	2-B3, 66-B2/B3/B4/B5	nd	85	600	500	nd
	46-B2	nd	85	650	500	>300
	66-B1	nd	85	650	500	nd
	2-B1	nd	85	700 (4.2)	500	nd

[a]MIC (minimum inhibitory concentration) signified minimum concentration of the preservative at which growth was completely inhibited.
[b]nd, not determined.
[c]Values within parentheses indicate pHs of media after the additions.

4.8. Influence of food preservatives on growth

The minimum inhibitory concentrations (MICs) of different food preservatives on the growth of 48 strains of *B. cereus* isolated from the six different kinds of foods are shown in Table 13. The growth was completely inhibited at 65-85 mg sodium chloride ml^{-1}, depending on the strains. The MICs of benzoic acid and sorbic acid against the growth were 400-700 μg ml^{-1} (pH 5.0-4.2) and 500-600 μg ml^{-1} (pH 5.0-4.8), respectively. The MICs of nisin against the growth of selected 10 isolates were determined; most (80%) of the strains were resistant to 300 μg ml^{-1} nutrient agar (pH 5.0).

The MICs of sodium chloride against *S. aureus* and *E. coli* were 110 and 90 mg ml^{-1} nutrient agar, respectively. The MIC of benzoic acid for *S. aureus* and *E. coli* was 650 μg ml^{-1} (pH 4.3). Both the strains of *S. aureus* were inhibited at 800 μg of sorbic acid ml^{-1} (pH 4.6), whereas for *E. coli*, it was 600 μg ml^{-1} (pH 4.8). One of the strains of *S. aureus* was resistant to 300 μg nisin ml^{-1} nutrient agar (pH 5.0).

The MICs of sodium chloride, benzoic acid and sorbic acid against the growth of *Salmonella* isolates were 70-95 mg ml^{-1}, 450-650 μg ml^{-1} (pH 4.8-4.3) and 500-700 μg ml^{-1} nutrient agar (pH 5.0-4.7), respectively (Table 14).

Table 14. Range of pH and MIC[a] of food preservatives against the growth of *Salmonella* isolates from different food sources

Source	Target strain	pH[b] range for growth	Preservative in nutrient agar[c]		
			NaCl (mg ml^{-1})	Benzoic acid (µg ml^{-1})	Sorbic acid (µg ml^{-1})
Idli	13-S3	4.3-9.0	80	600 (4.4)	600 (4.8)
	21-S1	nd	70	500 (4.7)	500 (5.0)
	21-S2	nd	90	600	500
	21-S3	nd	90	550 (4.5)	500
Papad	23-S1	4.8-9.7	90	550	600
	23-S2, 83-S1/S2/S3	nd	90	600	500
	84-S1/S2	nd	90	650 (4.3)	600
	84-S3, 85-S1/S2/S3	nd	90	600	600
Wadi	1-S4	4.3-9.9	70	650	500
	1-S5, 17-S1	nd	75	650	500
	1-S6	nd	75	600	500
	7-S2	nd	70	550	600
	7-S3	nd	75	550	600
	7-S5	nd	70	500	700 (4.7)
	11-S2	nd	70	450 (4.8)	500
	17-S2	nd	80	600	500
	17-S3	nd	90	600	700
	43-S1/S2/S4/S5	nd	95	600	500
	43-S3, 68-S4	nd	90	600	500
	43-S6	4.3-9.0	95	600	500
	68-S1	nd	95	600	600

[a]MIC (minimum inhibitory concentration) signified minimum concentration of the preservative at which growth was completely inhibited.
[b]nd, not determined.
[c]Values within parentheses indicate pHs of media after the additions.

The effects of sodium chloride, benzoic acid and nisin on the growth of *B. cereus* 37-B1 are presented in Fig. 23. In each of these cases, the growth declined with the increase in concentration of the preservatives. These three effects along with the effect of pH were subjected to Hoke's experimental design. The selected three points were on the trend lines of growth. While the lowest limits were zero in all the cases (excepting pH), the highest limits were judiciously chosen considering the sub-inhibitory concentration levels of the preservatives along with the recommended concentrations of them (Table 15). Growth of the strain against 19 different combinations of the four types of hurdles is shown in Table 16. There was no growth in 8 sets; all of which, excepting the set A, at least one of the three preservatives was at its maximum concentration tested.

Table 15. Levels of hurdles against *B. cereus* 37-B1 used for the Hoke's experimental design

Hurdle	Hurdle levels[a] tested		
	-1	0	+1
pH	5.6	6.4	7.2
Sodium chloride (mg ml^{-1})	0	20	40
Benzoic acid (µg ml^{-1})	0	300	600
Nisin (µg ml^{-1})	0	25	50

[a]+1, highest limit; 0, mid point; and -1, lowest limit of variable taken for study.

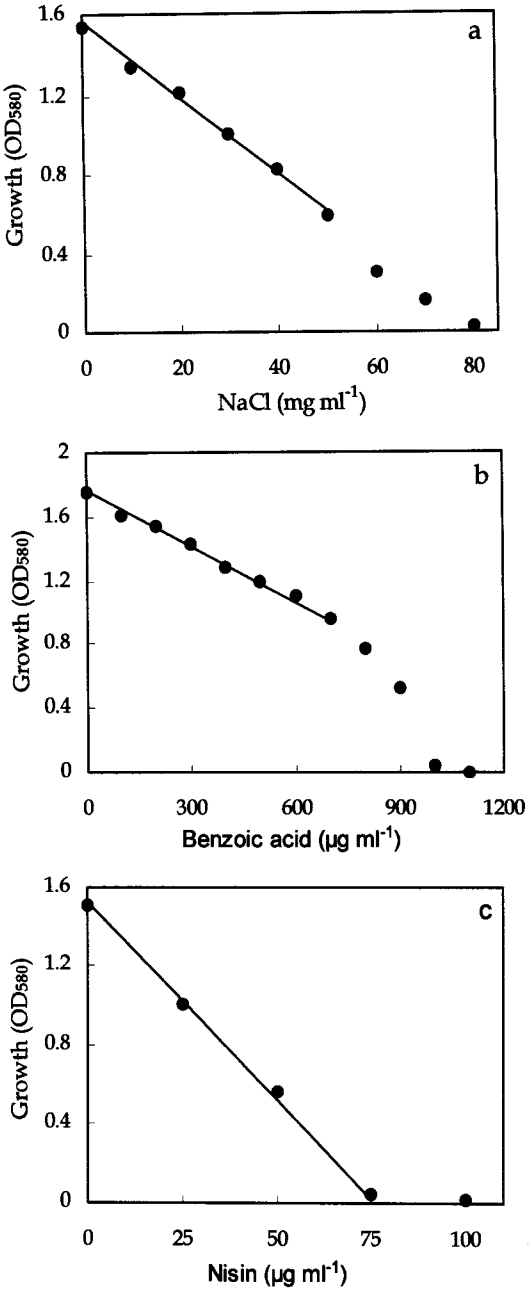


Fig. 23. The effect of sodium chloride (a), benzoic acid (b) and nisin (c) concentration on the growth of *B. cereus* 37-B1

Similarly, the effect of sodium chloride and benzoic acid on the growth of *Salmonella* 1-S4 is presented in Fig. 24. Growth of the strain against 18 different combinations of the three types of hurdles is shown in Fig. 25. There was no growth in one set only, at pH 5.4.

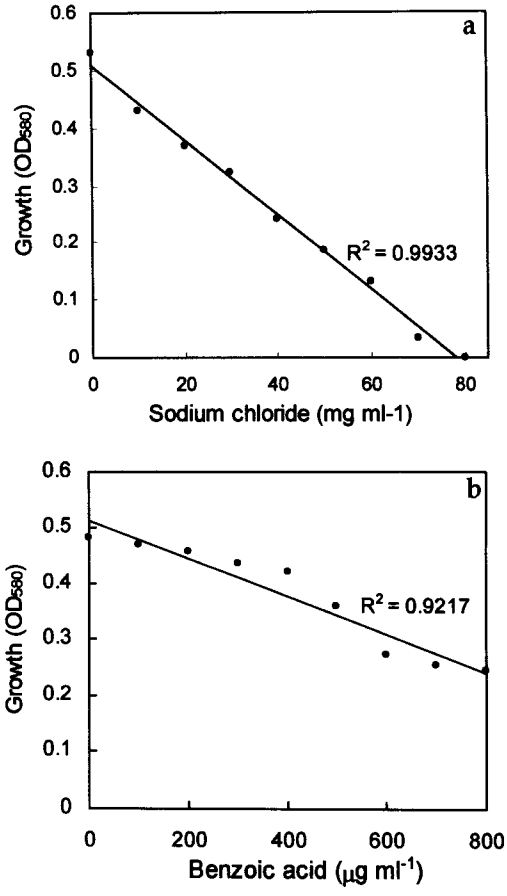


Fig. 24. The effect of sodium chloride (a) and benzoic acid (b) concentration on the growth of *Salmonella* 1-S4

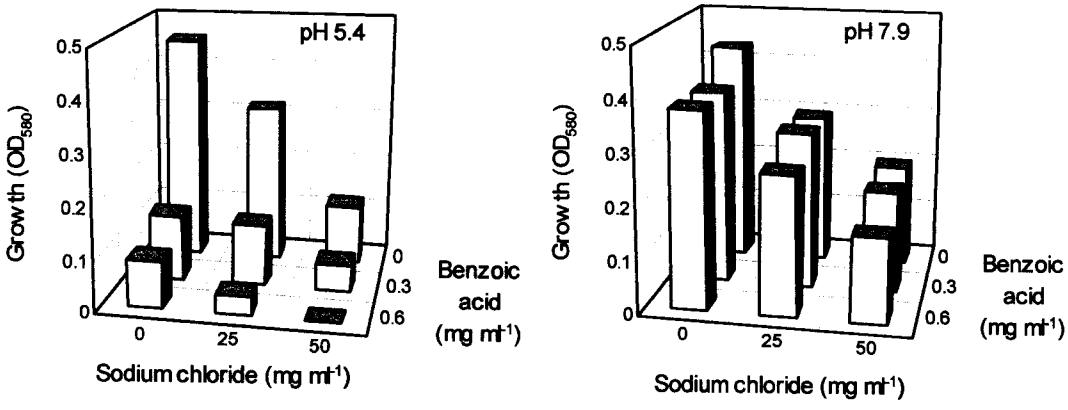


Fig. 25. Combined effect of sodium chloride and benzoic acid at two different levels of pH on the growth of *Salmonella* 1-S4 in nutrient broth at 35°C for 24 h

Table 16. Growth of *B. cereus* 37-B1 in nutrient broth as influenced by a combination of four independent variables (hurdles) following Hoke's response surface design

Hurdle combination no.	Independent variables				Growth (OD_{580}) Mean±SE[a]
	pH	Sodium chloride (mg ml^{-1})	Benzoic acid (µg ml^{-1})	Nisin (µg ml^{-1})	
A	5.6	20	300	25	0
B	6.4	0	300	25	0.32b ± 0.03
C	6.4	20	0	25	0.18d ± 0.02
D	6.4	20	300	0	0.43a ± 0.02
E	5.6	0	0	0	0.46a ± 0.04
F	5.6	40	600	50	0
G	7.2	0	600	50	0.18d± 0.01
H	7.2	40	0	50	0
I	7.2	40	600	0	0.25c ± 0.02
J	7.2	40	0	0	0.26c ± 0.01
K	7.2	0	600	0	0.30b± 0.02
L	7.2	0	0	50	0.24c ± 0.03
M	5.6	40	600	0	0.21d± 0.01
N	5.6	40	0	50	0
O	5.6	0	600	50	0.26c ± 0.02
P	6.4	40	600	50	0
Q	7.2	40	600	50	0
R	7.2	40	300	50	0
S	7.2	40	600	25	0

[a]Values with standard error (SE) were obtained from three replicates. Means within a column sharing a common letter are not significantly different ($P < 0.05$).

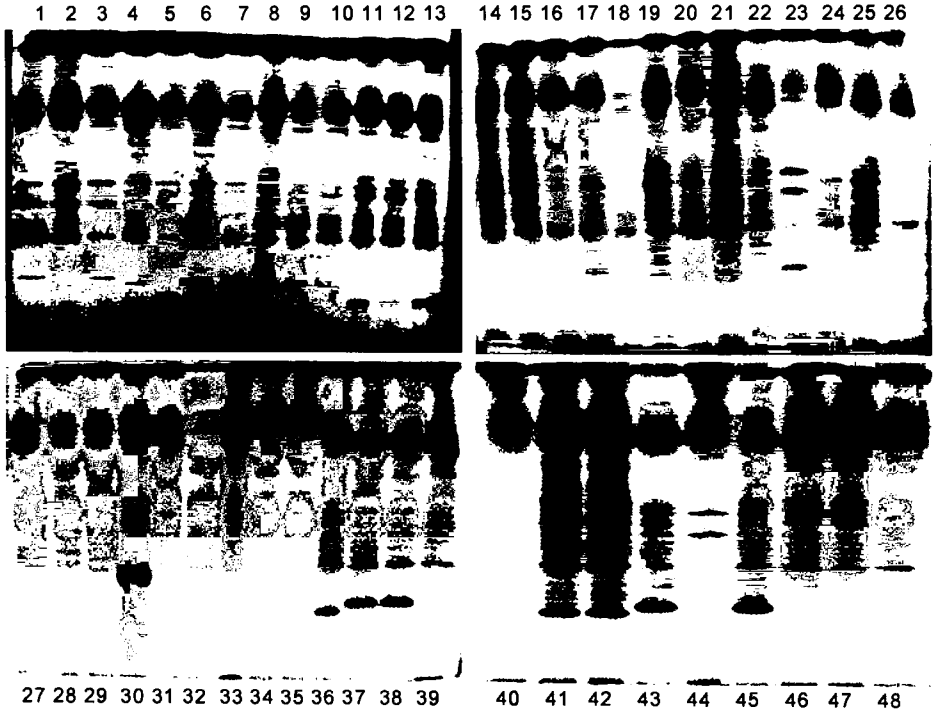


Fig. 26. SDS-PAGE profiles of whole-cell *B. cereus* strains. Lanes: 1, 111-B3 (A11); 2, 111-B2 (A11); 3, 111-B1 (A11); 4, 66-B4 (A9); 5, 66-B3 (C2); 6, 66-B2 (A9); 7, 66-B1 (C1); 8, 49-B2 (A5); 9, 49-B1 (A4); 10, 46-B2 (A4); 11, 6-B2 (A2); 12, 2-B3 (A3); 13, 2-B1 (A1); 14, 18-B2 (B1); 15, 18-B3 (B2); 16, 18-B5 (B3); 17, 52-B1 (B5); 18, 52-B2 (B14); 19, 57-B2 (B6); 20, 57-B3 (B6); 21, 57-B4 (B6); 22, 57-B5 (B15); 23, 70-B1 (B17); 24, 70-B2 (B7); 25, 93-B1 (B16); 26, 93-B2 (B16); 27, 93-B3 (B10); 28, 113-B1 (A12); 29, 113-B2 (B11); 30, 113-B3 (B4); 31, 34-B1 (B11); 32, 35-B1 (C3); 33, 37-B1 (B12); 34, 16-B1 (D); 35, 55-B1 (D); 36, 98-B1 (A7); 37, 98-B2 (A10); 38, 98-B3 (A10); 39, 66-B5 (A8); 40, 104-B1 (B8); 41, 104-B2 (B8); 42, 104-B3 (B8); 43, 105-B1 (A6); 44, 105-B2 (A13); 45, 105-B3 (A5); 46, 94-B1 (B9); 47, 94-B2 (B9); 48, 94-B3 (B13). Cluster/subcluster numbers are shown within parentheses (cf. Fig. 27).

4.9. Whole-cell protein fingerprinting

The whole-cell protein fingerprinting (WCPF) of the 48 isolates of *B. cereus* (Fig. 26) and 33 isolates of *Salmonella* (Fig. 28) yielded distinctly different band patterns. Majority of the strains isolated from the same kind of food were distinguished by their WCPF patterns. Fig. 27 and 29 show a simplified version of the dendrograms obtained from the strains of *B. cereus* and *Salmonella*, respectively. Basically, the WCPF profiles of *B. cereus* could be grouped into four major clusters emerging at a similarity level of 60%. These clusters, designated A through D, represented 40%, 50%, 6% and 2%, respectively, of the total strains. On the other hand, the WCPF profiles of *Salmonella* could be grouped into six major clusters emerging at a similarity level of 80%. These clusters, designated A through F, represented 12%, 15%, 55%, 12%, 3% and 3%, respectively, of the total strains.

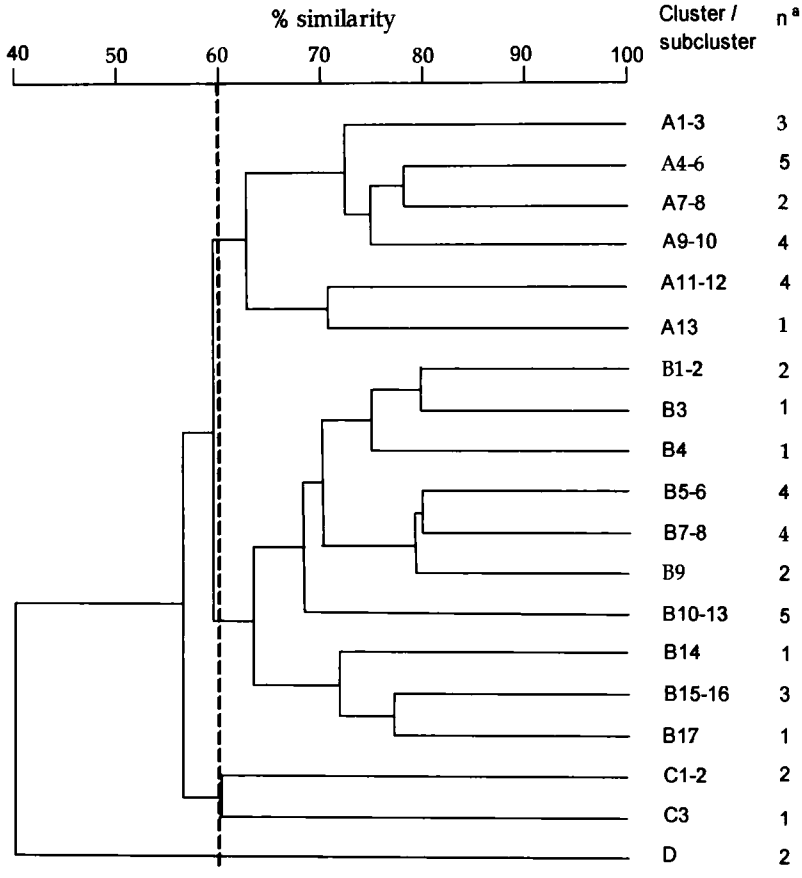


Fig. 27. Simplified dendrogram based on the UPGMA clustering of similarity coefficients (S_D) of whole-cell protein profiles of the 48 strains of *B. cereus* (as shown in Fig. 26). The fingerprint patterns were grouped into four major clusters, designated A through D, on the basis of 60% similarity (arbitrarily chosen) among the strains used.
[a] n, number of strains in cluster/ subcluster.

4.10. Antagonistic activity of lactic acid bacteria against food pathogens

A total of 84 strains of lactic acid bacteria isolated from the batter of dhokla, dosa and idli, and of dried wadi were tested for their antibacterial activity. No antagonistic activity against the *B. cereus*

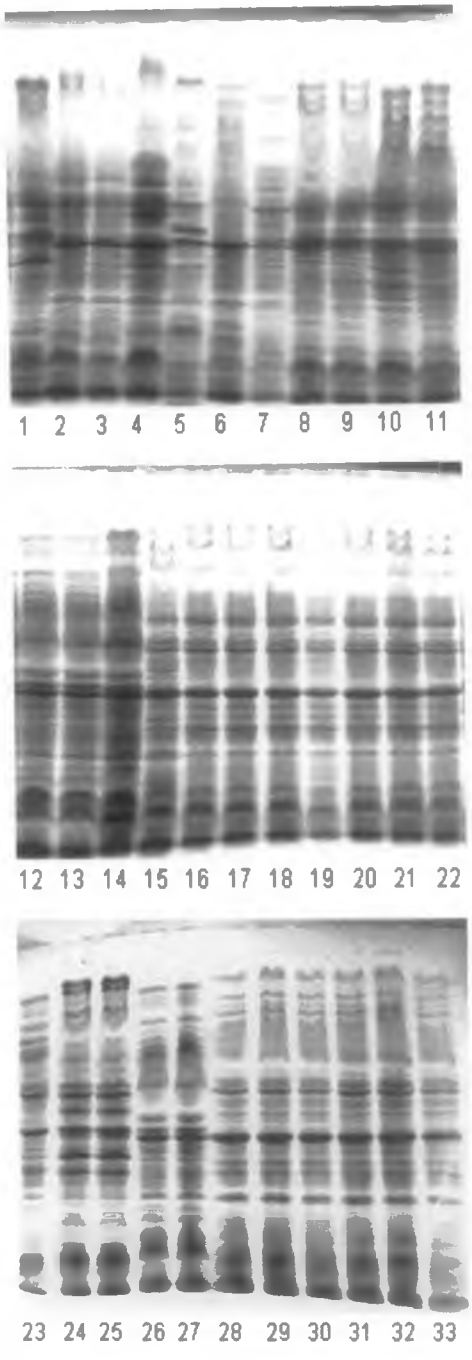


Fig. 28. SDS-PAGE profiles of whole-cell *Salmonella* strains. Lanes 1, 21-S1 (B4); 2, 17-S3 (B3); 3, 17-S2 (B2); 4, 17-S1 (B2); 5, 13-S3 (B1); 6, 11-S2 (F); 7, 7-S5 (E); 8, 7-S3 (C2); 9, 7-S2 (C6); 10, 1-S6 (C1); 11, 1-S5 (A1); 12, 83-S1 (D1); 13, 68-S4 (D1); 14, 68-S1 (C3); 15, 43-S6 (C3); 16, 43-S5 (C3); 17, 43-S4 (C3); 18, 43-S3 (C3); 19, 43-S2 (C3); 20, 43-S1 (C3); 21, 23-S2 (C3); 22, 23-S1 (C3); 23, 1-S4 (A3); 24, 21-S2 (A2); 25, 21-S3 (A2); 26, 83-S2 (D2); 27, 83-S3 (D2); 28, 84-S1 (C4); 29, 84-S2 (C4); 30, 84-S3 (C4); 31, 85-S1 (C4); 32, 85-S2 (C4); 33, 85-S3 (C5). Cluster/ subcluster numbers are shown within parentheses (cf. Fig. 29).

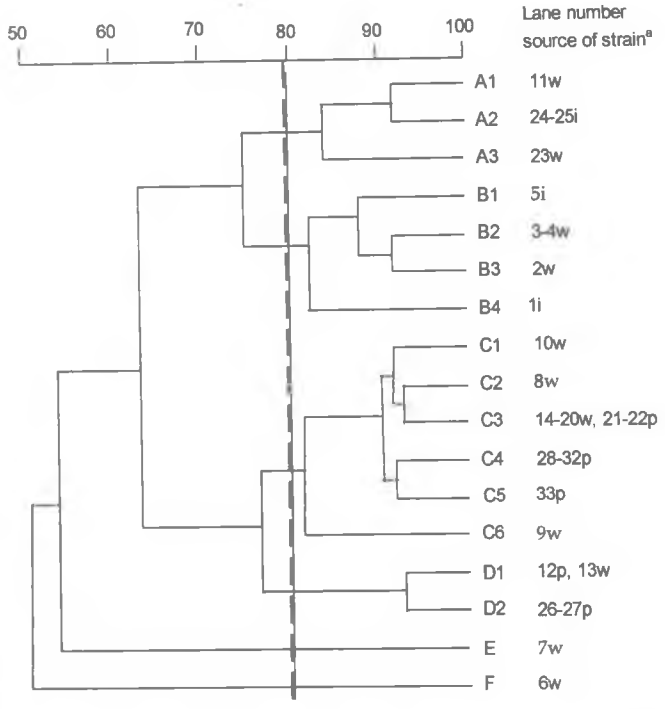


Fig. 29. Simplified dendrogram based on the UPGMA clustering of similarity coefficients (S_D) of whole-cell protein fingerprint profiles of the 33 strains of *Salmonella* (as shown in Fig. 28). The fingerprint patterns were grouped into six major clusters, designated A through F, on the basis of 80% similarity (arbitrarily chosen) among the strains used.
[a]Source: i, idli; p, papad; w, wadi.

isolated from wadi (strain S2-B1) or idli (strain S94-B1) could be found. The agar-spot assay revealed that none of the tested 15 strains of lactic acid bacteria isolated from laboratory-made fermenting batter of idli inhibited the growth of *B. cereus* 94-B1, *E. coli* 61-E2 and *S. aureus* 34-S1.

4.11. Microbial challenge testing

The quantitative changes in microbiota along with pH and volume of dhokla batter during natural fermentation are presented in Fig. 30. The count of total aerobic mesophilic bacteria occurring in the batter increased ($P < 0.05$) from initial 6.5 log cfu g^{-1} batter to 10.5 log cfu g^{-1} after 15 h of fermentation. The lactic acid bacterial count increased ($P < 0.05$) from initial 5.2 log cfu g^{-1} to 8.5 log cfu g^{-1} at the end. From an initial level of 3.6 log cfu g^{-1}, the yeast population reached 7.1 log cfu g^{-1} after 15 h of fermentation. When freshly prepared batter was intentionally inoculated

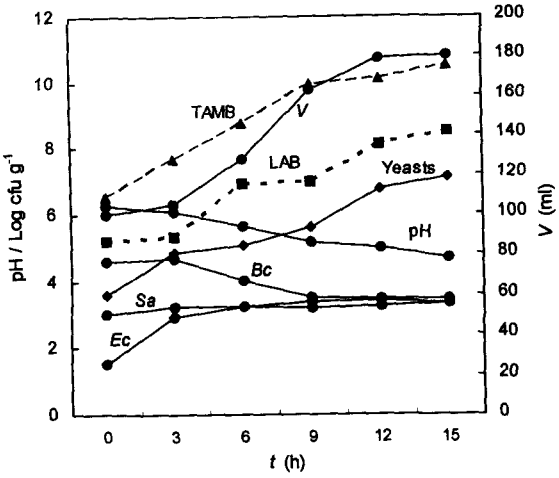


Fig. 30. Changes in pH, volume (*V*) and microbial cell count of dhokla batter during natural fermentation. Values are the means of nine batches of fermentations.
Abbreviations: TAMB, total aerobic mesophilic bacteria; LAB, lactic acid bacteria; *Bc, B. cereus; Sa, S. aureus; Ec, E. coli.*

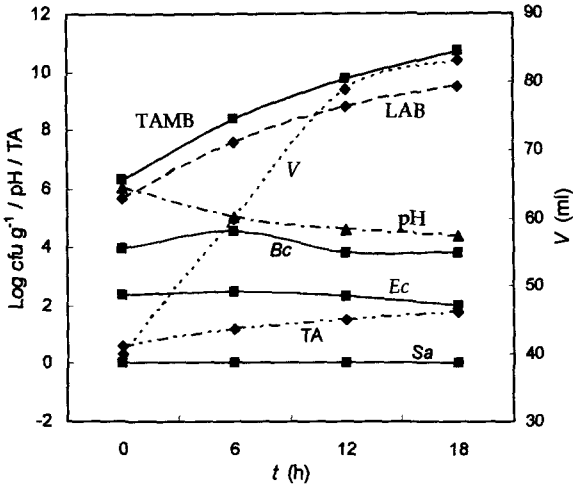


Fig. 32. Changes in pH, volume (*V*), titratable acidity (TA) and bacterial cell count of idli batter during natural fermentation. Values are the means of nine batches of fermentation.
Abbreviations: TAMB, total aerobic mesophilic bacteria; LAB, lactic acid bacteria; *Bc, B. cereus; Sa, S. aureus; Ec, E. coli.*

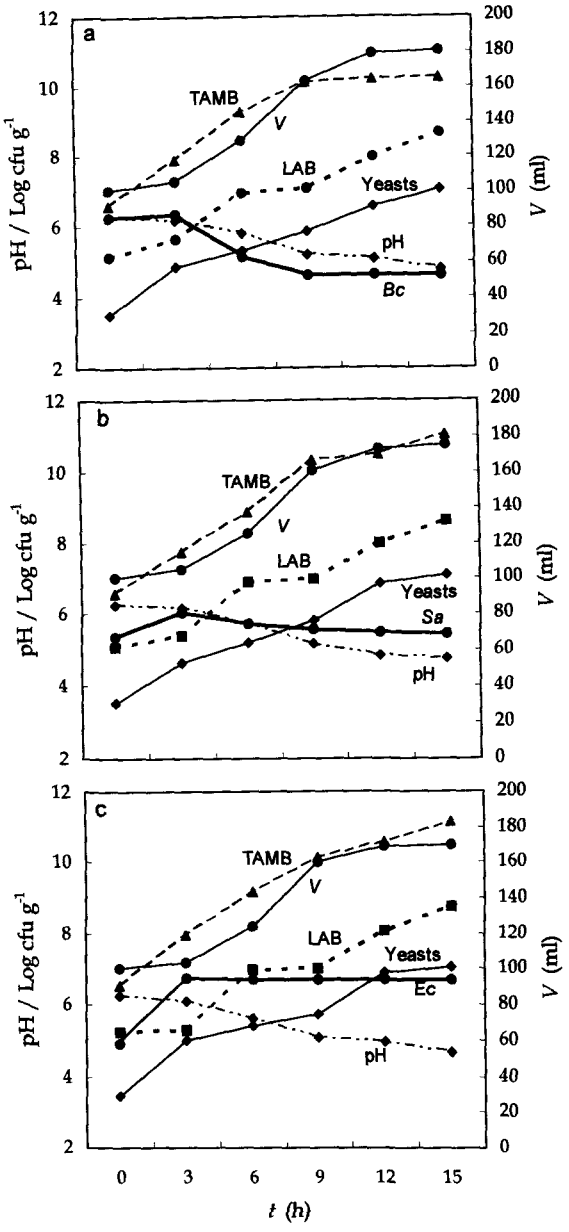


Fig. 31. Changes in pH, volume (*V*) and microbial cell count during fermentation of dhokla batter which was intentionally inoculated at the start with *Bc (a), Sa (b)* or *Ec (c)*. Values are the means of nine batches of fermentations.
Abbreviations: TAMB, total aerobic mesophilic bacteria; LAB, lactic acid bacteria; *Bc, B. cereus; Sa, S. aureus; Ec, E. coli.*

with *B. cereus* at a level of 6.2 log cfu g^{-1}, the pathogen remained unaffected ($P < 0.05$) during the first 3 h of fermentation, however the count decreased ($P < 0.05$) after 6 h and then again after 9 h of fermentation (Fig. 31a). *S. aureus*, although increased 1 log-cycle after 3 h, returned to the original level after 6 h and then remained unchanged ($P < 0.05$) till the end of fermentation (Fig. 31b). *E. coli*, on the other hand, exhibited a better ($P < 0.05$) growth during the fermentation (Fig. 31c). After steaming the uninoculated but fully fermented batter for 15 min in a pan, the load of *B. cereus, S. aureus* and *E. coli* in dhokla cakes reached below their detection limit (Table 17). However, in intentionally inoculated batter, *B. cereus*

Table 17. Viable count of indicator organisms in fermented batters and cakes of dhokla and idli, prepared following steaming for 15 min

Organism	Intentional inocualtion	log cfu g^{-1a} fresh wt			
		Dhokla		Idli	
		Before steaming	After steaming	Before steaming	After steaming
B. cereus	-	3.4	<dl	3.8	<dl
	+	4.6	2.6	4.1	2.0
S. aureus	-	3.3	<dl	<dl	<dl
	+	4.5	<dl	4.8	<dl
E. coli	-	3.3	<dl	2.0	<dl
	+	4.7	<dl	4.4	<dl

[a]dl, detection limit (2.0 log cfu g^{-1}).

reduced (P <0.05) but survived steaming at a level of 2.6 log cfu g^{-1} dhokla cakes. *S. aureus* and *E. coli* could not be detected in the cakes prepared from intentionally inoculated batter.

The quantitative changes in microbiota along with pH, titratable acidity and volume of idli batter during natural fermentation are presented in Fig. 32. When freshly prepared batter was intentionally inoculated with *B. cereus* at a level of 5.6 log cfu g^{-1}, the pathogen not only survived but also grew positively (P <0.05) during the first 6 h of fermentation, however the cell count reduced (P <0.05) by more than 1 log cycle after 12 h and remained unchanged (P <0.05) thereafter (Fig. 33a). A similar trend was noticed in cases of *S. aureus* (Fig. 33b) and *E. coli* (Fig. 33c). After steaming the naturally fermented batter for 15 min in an idli pan, the load of *B. cereus* and *E. coli* in idli cakes reached below their detection limit (Table 17). However, in intentionally inoculated batter at a much higher load, *B. cereus* reduced quantitatively but survived steaming at a level of 2.0 log cfu g^{-1} idli cakes. *S. aureus* and *E. coli* could not be detected in idli cakes prepared from intentionally inoculated fermented batter.

The quantitative changes in microbiota along with pH and moisture content of wadi dough during natural fermentation are presented in Fig. 34. The count (3 log cfu g^{-1}) of *B. cereus* decreased (P <0.05) during the first 10 h of fermentation, and again further during the first 12 h of drying; after 24 h of drying the count went below the limit of detection. *S. aureus* and *E. coli* could not be detected at any stage of fermentation and drying. Intentional inoculation of wadi dough at the onset of fermentation with *B. cereus*,

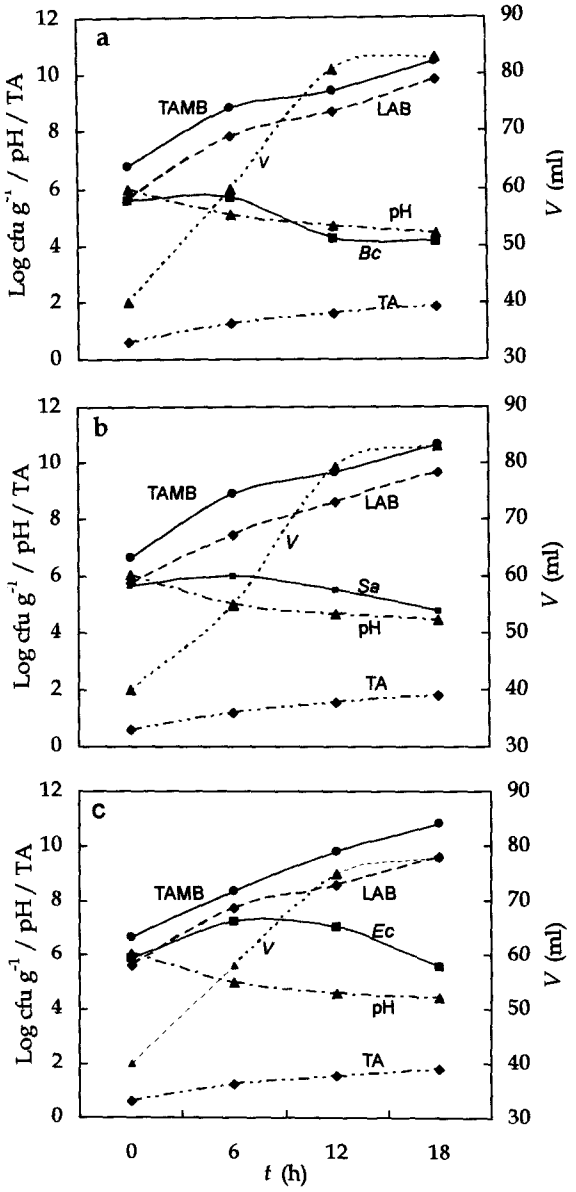


Fig. 33. Changes in pH, volume (*V*), titratable acidity and bacterial cell count during fermentation of idli batter which was intentionally inoculated at the start with *Bc* (a), *Sa* (b) or *Ec* (c). Values are the means of nine batches of fermentation.
Abbreviations: TAMB, total aerobic mesophilic bacteria; LAB, lactic acid bacteria; *Bc*, *B. cereus*; *Sa*, *S. aureus*; *Ec*, *E. coli*; TA, titratable acid (as % lactic acid).

S. aureus or *E. coli* cells had no apparent influence on the growth of inherent lactic acid bacteria, yeasts and total aerobic mesophilic bacteria, and changes in pH and dough volume, which are the cause and consequence of this autofermentation (Fig. 35). None of the pathogenic bacteria, either inherent or introduced to the dough at the start of fermentation, survived after 36 h of drying (Figs. 34 and 35). When freshly prepared dough was intentionally inoculated at a level of 5.2 log cfu g^{-1} (Fig. 35a), *B. cereus* could survive only for a while; the count reduced by 1 log cycle after 10 h of fermentation, and after 24 h of drying it could not be detected. After inoculation of dough with *S. aureus* at a level of 5.4 log cfu g^{-1} (Fig. 35b), the count remained unchanged ($P < 0.05$) during the first 10 h of fermentation, but decreased ($P < 0.05$) at every 12 h interval of drying, and went below the detection limit after 36 h. In contrast to the earlier two, the count of *E. coli* increased ($P < 0.05$) from 5.1 log cfu g^{-1} dough to 5.5 log cfu g^{-1} during the first 10 h of fermentation (Fig. 35c). The count then remained unchanged ($P < 0.05$) till 24 h of drying, however, *E. coli* cells could not be detected after 36 h.

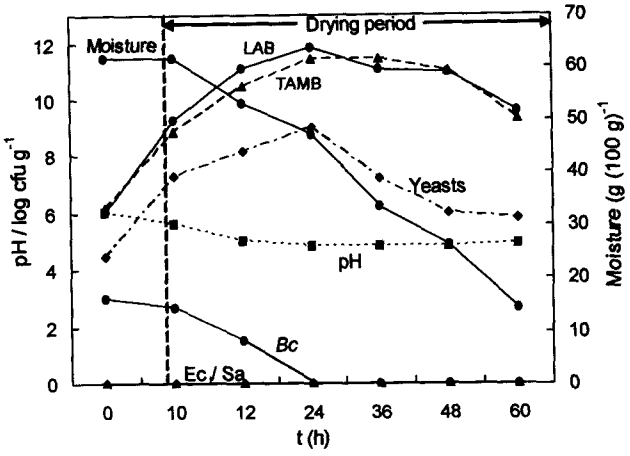


Fig. 34. Changes in pH, moisture and cell count of wadi dough during natural fermentation and drying. Values are the means of nine batches of fermentations.
Abbreviations: TAMB, total aerobic mesophilic bacteria; LAB, lactic acid bacteria; *Bc*, *B. cereus*; *Sa*, *S. aureus*; *Ec*, *E. coli*.

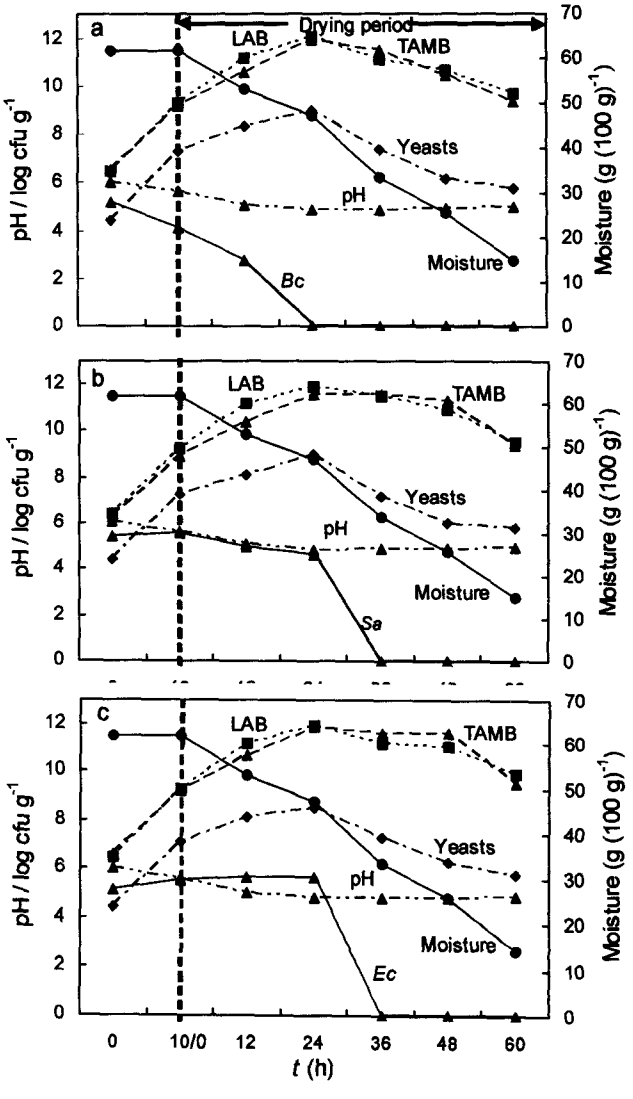


Fig. 35. Changes in pH, moisture and cell count during fermentation and drying of wadi dough which was intentionally inoculated at the start with *Bc* (a), *Sa* (b) or *Ec* (c). Values are the means of nine batches of fermentations.
Abbreviations: TAMB, total aerobic mesophilic bacteria; LAB, lactic acid bacteria; *Bc*, *B. cereus*; *Sa*, *S. aureus*; *Ec*, *E. coli*.