Microbial Quality and Safety of Axone -Akhuni, a Fermented Soybean Food of Nagaland

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Abstract

Axone, a fermented soybean food of Nagaland, is a common side dish in North-East India and neighbouring countries. In the study 191 samples of axone collected from 7 tribes (44 Angami, 12 Ao, 36 Chakesang, 3 kuki, 62 Lotha, 2 Pochury and 32 Sema) were analyzed. The pH of matured axone (5.7 to 8.62) was significantly (pj0.001) higher than fresh (4.56 to 7.54) and dried axone (5.58 to 8.54). The pH of Sema axone (8.02) was significantly (p, 0.001) higher than average pH of axone of other tribes. Total aerobic mesophilic plate count (TAMPC) of axone ranged between 6.44 Log10 cfu/g and 12.98 Log10 cfu/g. The aerobic endospore count (AEC) in axone (5.6Log10 to 10.55 Log10 cfu/g) had strong correlation with TAMPC (r, 0.41) and was the major constituent of it. Of the twelve species of Bacillus identified in axone five most common were B. coagulans (55.5%), B. pantothenticus (12.4%), B. subtilis (11.5%), B. stearothermophilus (8.4%) and B. lentus (6.3%). About one third (31.4%) axone samples were positive for coliforms. Escherichia coli were detected in 24 samples. Other important bacteria axone growing on McConkey lactose agar (MLA) included Aeromonas eucranophila, A. hydrophila, A. salmonicida, Citrobacter spp., Enterobacter spp., Erwinia ananas, Hafnia alvei, Klebsiella pneumoniae, K. oxytoca, Morganella morganii, Providencia rettgeri, Proteus spp., Pseudomonas spp. and Salmonella enterica ser Typhimurium. Yeasts and molds were countable in 51.8% samples (2.24 Log10 to 7.41 Log10 cfu/gm). The YMC were significantly higher (p˜0.005) in fresh and dry axone than in matured axone. Enterococci were detected in 94.6% samples (2.92 Log10 to 8.26 Log10 cfu/g) and EC of dry axone (6.670.93 Log10 cfu/g) was significantly higher (p, 0.0005) than of matured axone (5.342.01 Log10 cfu/g). Of the 11 species of enterococci identified in axone, Ec. caecorum (21.9%), Ec. faecalis (17.3%) and Ec. hirae (12.6%) were the most common. All the samples tested were positive for staphylococci (5.69 Log10 to 7.56 Log10cfu/g). Commonly identified staphylococci were Staph. xylosus, Staph. epidermidis and Staph. sciuri. Out of 153 gram negative bacteria (GNB) and 160 Gram positive bacteria (GPB) isolates belonging to 40 species tested in the study 50 had multiple drug resistance (MDR). Species of bacteria and source of axone were the important determinants of antimicrobial sensitivity. The study concluded that Bacillus strains were the major bacteria present in axone and pH appeared to be an easily measurable quality indicator. Isolation of several bacteria of public health concern capable to cause foodborne illness including strains of Salmonella, Aeromonas and Klebsiella species indicated need for development of some microbial standards and standard starter culture to increase safety and quality of axone.

Keywords: Akhuni, Axone, Microbial quality, pH, Salmonella, Escherichia coli, Bacillus coagulans, Aeromonads

1. Introduction

Axone (Aakhone) is a traditional Nagamese fermented, sticky paste like product of soybean with Umami flavour (slightly alkaline in taste with ammonical smell) resembling to fermented soybean products used in other parts of North Eastern Himalayas including hawaijar of Manipur, tungrymbai of Meghalaya, bekang of Mizoram. It is quiet similar to natto of Japan, chungkukjang of Korea, thua nao of Thailand, pepok of Myanmar and sieng of Cambodia in its preparation and culinary use. It is often eaten with food as chutney (side dish) or sometimes used for making curry as kinema of Sikkim. Axone is often used as flavouring agent for preparing pork, fish and vegetable dishes in all parts of Nagaland (Singh et al., 2009). Matured axone contains about 48

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% moisture. On dry matter basis it contains about 35.8 % carbohydrate, 5.9 % ash, 34.8 % protein and 23.5 lipids (Singh and Singh, 2014 a, b; Singh et al., 2009). Axone is made from locally available soybean. Its production process generally includes cleaning, soaking, dehulling with wooden pestle and mortar, boiling for about 30 min, grinding with wooden pestle and mortar, wrapping in banana and bamboo leaves in about 50-100 g packets and putting to ferment above (1-2 mt height) kitchen-fire place for 3-7 days depending on weather. Fresh axone is considered ready after fermentation when converted in to sticky and stringy lemon coloured product. To get the axone matured, unopened packets are allowed to stay in smoke rich kitchen area for several weeks. Dried axone is made from matured axone when it got dried in situ after one or 2 months of storage, it is grounded and kept in airtight glass jars for use as curry powder (Singh et al., 2009). Except boiled and dehulled soybean paste nothing is added in it (Singh et al., 2009) while in a similar product of Sikkim, kiinema, about 1% wood ash is added before putting that for fermentation (Tamang, 2010). The quality of axone varies depending on variety of soybean used, variation in environmental temperature, kitchen conditions, and finally the tribal liking for flavour. Lotha and Ao tribes like it mildly matured, Angami mostly like strongly fermented fresh axone while Sema and Chakhesang tribes prefer to consume brown fully matured axone (Singh et al., 2009).

Consumption of axone and axone-like fermented soybean products is claimed to be associated with several health benefits despite of their very peculiar smell (Tamang, 2010). Traditionally these alkaline and smelly foods are considered good for health and enhancers of life span (Ohta, 1986). In recent studies several nutraceutical effects are noticed with fermented soy foods, viz., anticancer activity of chungkukjang (Seo et al., 2009), production and reproduction enhancement in pig, poultry and mice with axone (Singh, 2013; Singh et al., 2009; Singh and Singh, 2014a, b) and prevention of osteoporosis with natto (Yanagisawa and Sumi, 2005).

Several studies have been reported on microbiological quality of axone-like products throughout the north eastern Asian countries but little is known about axone. Of these natto (Ohta, 1986) and kinema (Taman, 2010) are well studied and microflora is characterised to standardized the production at commercial scale. Studies on limited number of axone samples of Sema (Tamang et al., 2009) and Chakesang tribes (Singh and Singh, 2014a, b) gave only a bird's eye view of one of the most widely used product. Although most of the products in this class are made with boiled soybean through aerobic fermentation, the products from different regions contain different types of bacteria. In natto, it is Bacillus natto, a variant of B. subtilis (Wei et al., 2001), in kinema it is mostly B. subtilis (Tamang, 2010), in Axone of Chakesang tribe though B. subtilis was detected but major bacteria is B. coagulans (Singh and Singh, 2014a,b), while in chungkukjang, most active bacteria identified is nontoxigenic B. cereus along with B. amyloliquefaciens and B. subtilis (Seo et al., 2009). Axone is prepared at household level without using a starter culture under different tribal practices of Nagaland. Therefore, in light of its popularity and health and production values (Singh, 2013; Singh et al., 2009, 2014a, b) this study was conducted to understand microbiological qualities of ethnic axone produced and marketed by Nagamese women of different tribes available in local vegetable markets of Nagaland.

2. Materials and Methods

2.1. Axone samples: A total of 35 fresh, 136 matured and 20 dried axone samples were collected from local markets (Dimapur, Medziphema, Phek, Shillong, Porba). Samples belonged to seven tribes of Nagas (Table. 1). Samples were brought to laboratory intact without removing their packing within 2 to 8 h of purchase depending on the distance of market from the laboratory and processed within 24 h. 2.2. Processing of the samples: From aseptically opened axone packet, 10 g of axone was weighed in to a sterile mortar. Axone was finely grounded to suspend in 90 ml of sterile distilled water and then vortexed for 2-3 min to mix properly and then allowed to stand for 5 min. Aliquot of 10 ml was taken for bacterial count in sterile tube and then pH of rest of the volume was measured with digital pH meter (PHEBO, SD Fine Chem. Ltd., Mumbai, India).

Aliquots for bacterial count were analyzed for total aerobic mesophilic plate count (TAMPC), yeasts and molds count (YMC), aerobic endosporee count (AEC), coliform count, enterococcal count (EC) and staphylococcal count (SC) after making 10 fold serial dilutions in sterile normal saline (NSS, 0.85% NaCl in distilled water) using standard bacteriological techniques (FDA, 1995; Holt et al., 1986; ICMSF, 1988; Singh, 2009). Bacterial counts were performed using direct plate count in triplicate (ICMSF, 1988) through plating (100 l on each plate in triplicate) the 10 fold serial dilutions on MacConkey lactose agar (MLA, Hi-Media, Mumbai, India) for coliforms, plate

count agar (PCA, Hi-Media) for TAMPC, yeast mold chloramphenicol agar (YMCA, Hi-Media) for YMC, Enterococcus confirmatory agar (ECA, Hi-Media) for EC and mannitol salt agar (MSA, Hi-Media) for SC. For AEC, serial dilutions in thin glass tubes were incubated in water bath at 80oC for 10 min to kill vegetative bacteria (Han et al., 2001). Thereafter, aliquots were inoculated on PCA as for TAMPC. Inoculated media plates were incubated at 37oC for 24 hr for bacteria and 72 hr for YMC. Colonies were counted on the plates and total number was determined using colony count and dilution factor (ICMSF, 1988).

2.3. Identification of bacterial isolates: Besides Bacillus spore count (AEC), identification of 4-5 colonies of Bacillus picked up from AEC plates were performed on the basis of their growth, biochemical and morphological characteristics (Holt et al., 1986) using Hi-Bacillus identification kit (Hi-Media). Similarly 3-5 colonies were picked from ECA, MSA and MLA plates for identification Enterococcus spp, Staphylococcus spp., and lactose fermenter/ non-fermeter bacteria, respectively using cultural, morphological and biochemical characteristics of isolates (Holt et al., 1986; Singh, 2009).

2.4. Antimicrobial sensitivity assay: Antimicrobial sensitivity of representative bacterial isolates from Axone samples was determined using disc diffusion method (CLSI, 2006) on Muller Hinton Agar (MHA, Hi-Media) against ampicilin (Amp, 10 mcg), ceftazidime (Caz, 30 mcg), cephotaxime (Ce, 10mcg), chloramphenicol (C, 25 mcg), ciprofloxacin (Cf, 10mcg), co-trimoxazole (Co, 25 mcg), gentamicin (G, 30mcg), tetracycline (T, 30mcg), imipenem (Imp, 10 mcg) and erythromycin (E, 15 mcg). All gram positive strains were also tested for sensitivity against clindamycin (Cd, 10 mcg). All antimicrobial discs were procured from Difco, BBL (USA). Strains showing resistance to three or more drugs were classified as multiple drug resistant (MDR) strains.

2.5. Statistical analysis: Observations were compared to determine effect of tribal origin and type of Axone using Student's T test (to compare between two tribes or two types of axone), analysis of variance (ANOVA, two compare all three types of axone and to evaluate the effect of different tribes together), Duncan's multiple range test (DMRT, to rank the parameters of axone of different tribes or of different types) and Chi-squared test (to evaluate effect of tribe on presence of different bacteria, antimicrobial sensitivity of different bacteria, and to compare sensitivity of different bacteria for individual antimicrobial substance). However, due to low number of samples of axone of Kuki (3) and Pochury (2) tribes, they were excluded from comparison for tribal effect. To determine association between different parameters of axone, correlation analysis was done with Microsoft Excel(R).

3. Results

A total of 191 samples of Axone (35 fresh, 136 matured and 20 dried) were collected from local markets in Nagaland. Samples belonged to seven tribal sources (44 Angami, 12 Ao, 36 Chakesang, 3 kuki, 62 Lotha, 2 Pochury and 32 Sema). 3.1. pH: The pH (Table. 1) of matured axone (5.7 to 8.62) was significantly (p¡0.001) higher than that of fresh (4.56 to 7.54) and dried (5.58 to 8.54) axone. The pH of matured axone was significantly higher than of fresh axone of Sema (p, 8.51E-08) and Angami (p, 0.043). Although dried axone of most of tribes (Table. 1) had lower pH than of matured axone from corresponding tribe, pH of dried Sema axone was significantly (p, 0.009) higher than that of matured axone. The pH of fresh and matured axone of different tribes differed significantly (p, 0.001). Sema axone had the highest pH while matured axone of all other tribes stands at par in second category. On comparison of average pH for all axone samples of different tribes, axone of Sema (8.02) had significantly (p, 0.001) higher average pH than average pH of axone of other tribal origin (Angami, 7.54; Ao, 7.18; Chakesang, 7.35; Lotha, 7.20).

The increase in pH of axone was positively correlated with coliform count (p, 0.05) but was negatively correlated to YMC (p, 0.05) and SC (p, 0.1). The pH had no significant correlation to TAMPC, AEC and EC.

3.2. Total aerobic mesophilic plate count (TAMPC): The total aerobic bacterial count of fresh axone was significantly higher than matured axone (p, 0.022) but not significantly differed than TAMPC in dried axone (Table. 1). On comparing TAMPC of axone of different tribes separately, TAMPC of fresh Sema axone was significantly higher than of mature (p, 0.021) and dry (p, 0.001) axone. Similarly fresh Ao (p, 0.036) and Angami (p, 0.007) axone had significantly higher counts than matured axone of corresponding tribes.

ANOVA on TAMPC of fresh axone detected significant (p, 0.001) effect of origin (tribe). TAMPC of Ao and Angami axone was much higher than that of Chakesang, Sema and Lotha axone. Lowest counts in Chakesang axone clearly ranked it

separately than axone of Sema and Lotha. Analysis of TAMPC of matured Axone indicated no significant difference among tribes except significantly (p, .01) low counts in Ao axone.

Although TAMPC had significant (p¡0.05) positive correlation with coliforms count (r, 0.17), YMC (r, 0.17), AEC (r, 0.41), EC (p, 0.18) and SC (r, 0.25), no good association could be detected with pH (r, 0.07).

3.3. Bacterial count on MLA (Coliform count): Results of coliform count indicated that of the 191 samples 60 were positive for coliforms (lactose fermenters on MLA plates) and 85 samples had non-lactose fermenting colonies on MLA plates. Escherichia coli were detected in 24 samples and 14 samples had Klebsiella pneumoniae. Other important lactose fermenters (Table. 2) detected from axone samples included strains of Citrobacter spp. (4), Enterobacter spp. (43) and Erwinia ananas (1). On MLA, non lactose fermenting colonies were identified as Aeromonas eucranophila (2), A. hydrophila (2), A. salmonicida (3), Pseudomonas spp. (55), Hafnea alvei (1) Klebsiella oxytoca (3), Morganella morganii (5), Providencia rettgeri (5), Salmonella enterica ser Typhimurium (4) and Proteus spp. (63). In several axone samples more than one type of bacteria could be detected on MLA plates simultaneously.

Table 1. Microbiological characteristics of Axone samples collected from different tribes.

Tribe (n)	Type of Axone (n)	pH, Average (STD)	Average microbial counts expressed as Log_{10} values (STD)					
			TPC	Coliforms on MLA	Y&M	Aerobic Spores	Enterococci	Staphylococci
Angami (44)	Fresh (22)	7.26 (0.42)	10.14 (1.04)	7.55 (1.22)	4.37 (2.09)	8.04 (0.57)	5.94 (2.13)	6.51 (0.21)
	Matured (21)	7.73 (0.71)	9.40 (0.62)	3.34 (3.61)	2.11 (2.80)	8.20 (0.83)	4.89 (3.12)	6.32 (0.46)
	Dried (1)	NT	8.54 (0.03)	7.21 (0.04)	5.07 (0.03)	6.59 (0.04)	NT	NT
Ao (12)	Fresh (4)	NT	11.20 (1.29)	8.24 (0.13)	3.75 (2.51)	8.89 (0.64)	NT	NT
	Matured (8)	7.16 (0.21)	9.02 (0.59)	0.00	2.44 (3.09)	7.88 (1.06)	6.39 (0.32)	5.78 (0.42)
Chakhesang (36)	Fresh (4)	NT	8.14 (0.37)	3.40 (3.55)	4.92 (0.09)	7.89 (0.37)	NT	NT
	Matured (30)	7.42 (0.67)	9.47 (0.64)	2.99 (2.18)	2.57 (2.37)	8.02 (0.81)	4.40 (1.11)	NT
	Dried (2)	6.35 (0.07)	10.19 (1.41)	2.85 (1.13)	0	8.70 (2.02)	6.44 (1.11)	6.94 (0.13)
Kuki (3)	Fresh (1)	4.56 (0.01)	9.83 (0.01)	0	0	7.94 (0.02)	3.43 (0.02)	0
	Matured (1)	8.25 (0.01)	9.35 (0.02)	2.72 (0.02)	0	8.99 (0.06)	7.25 (0.33)	0
	Dried (1)	6.66 (0.01)	9.31 (0.02)	0	0	8.06 (0.03)	6.44 (0.04)	0
Lotha (62)	Fresh (2)	NT	9.30 (0.23)	5.85 (2.53)	2.46 (0.13)	8.48 (0.19)	NT	NT
	Matured (47)	7.41 (0.56)	9.39 (0.70)	1.50 (2.38)	2.00 (2.31)	8.00 (0.69)	6.89 (0.91)	7.01 (0.01
	Dried (13)	6.72 (0.60)	9.36 (0.70)	0	5.71 (2.65)	8.11 (0.64)	6.89 (0.88)	7.01 (0.54)
Pochury (2)	Matured (1)	8.48 (0.01)	9.57 (0.08)	4.85 (0.02)	NT	9.08-9.12	7.33 (0.02)	NT
	Dried (1)	NT	9.65 (0.06)	6.94 (0.01)	4.98 (0.02)	7.41 (0.03)	NT	NT
Sema (32)	Fresh (2)	6.87 (0.03)	8.43 (0.58)	5.66 (0.10)	1.44 (0.02)	7.91 (0.33)	3.45 (0.06)	NT
	Matured (28)	8.04 (0.59)	9.21 (0.73)	1.65 (2.91)	1.50 (2.26)	8.09 (0.83)	4.51 (1.89)	6.91 (0.30)
	Dried (2)	8.48 (0.08)	9.99 (0.03)	2.61 (0.03)	0	8.29 (0.09)	5.43 (0.61)	7.18 (0.11)
Total (191)	Fresh (35)	7.03 (0.84)	9.88 (1.26)	6.73 (2.46)	3.96 (2.22)	8.14 (0.59)	5.56 (2.16)	6.51 (0.21)
	Matured (136)	7.62 (0.67)	9.35 (0.67)	2.10 (2.74)	2.03 (2.42)	8.06 (0.79)	5.31 (2.02)	6.69 (0.49)
	Dried (20)	6.88 (0.81)	9.44 (0.67)	1.03 (2.29)	4.43 (3.20)	8.04 (0.67)	6.67 (0.93)	7.03 (0.47)

TPC, total plate count; MLA, counts on MacConkey lactose agar; Y & M, yeast and mold count

Fig. 1: Table 1

Average coliform count was significantly higher in fresh axone (Table. 1) than in matured (p, 1.02E-13) and dried (p, 1.94E-10) axone. Though coliform count was significantly (p, 0.075) higher in matured axone than dried Axone, DMRT analysis put both of them together, much lower than counts in fresh axone, irrespective of tribe.

ANOVA on coliform counts in fresh axone indicated significant variation (p, 0.001) with reference to their tribal origin. DMRT classified Chakesang , Sema and Lotha axone together with low counts while axone of Ao and Angami tribes had significantly high counts. In matured axone, though coliform counts were low in Lotha and Sema Axone, difference was insignificant from counts in axone of others tribes.

Higher coliform count in axone had significant (p¡ 0.05) positive correlation with pH (r, 0.17), TAMPC (r, 0.32) but had negative correlation with SC (r, -0.32).

Table. 2. Frequency of isolation of bacteria of different species from Axone samples of different tribes.

Bacteria isolated from Axone samples	No. of samples positive	Number of samples positive for the bacteria collected from different tribes							X^2 test probability for source effect
		Angami n=44	Ao n=12	Chakhesang n=36	Pochury n=2	Lotha n= 62	Sema n= 32	Kuki n= 3	
Aeromonas eucranophila	2	0	0	0	0	2	0	0	0.749
Aeromonas hydrophila	2	0	0	0	0	2	0	0	0.749
Aeromonas salmonicida ssp. *salmonicida*	3	0	0	0	0	3	0	0	0.495
Bacillus anthracoides	6	0	2	0	0	4	0	0	0.066
Bacillus badius	7	0	1	0	0	5	1	0	0.388
Bacillus brevis	3	0	0	0	0	3	0	0	0.495
Bacillus circulans	6	0	1	0	0	5	0	0	0.200
Bacillus coagulans	106	36	0	14	1	22	30	3	2.55E-11
Bacillus laterosporus	2	1	0	0	0	0	0	1	6.02E-160
Bacillus lentus	12	0	3	0	0	6	3	0	0.042
Bacillus marcescens	5	2	0	0	0	3	0	0	0.763
Bacillus mycoides	2	1	1	0	0	0	0	0	0.309
Bacillus pantothenticus	23	1	3	0	1	18	0	0	1.28E-06
Bacillus stearothermophilus	16	2	1	8	0	0	5	0	0.012
Bacillus subtilis	22	1	1	18	1	0	0	1	8.73E-14
Citrobacter amalonaticus	3	2	1	0	0	0	0	0	0.31
Citrobacter diversus	1	1	0	0	0	0	0	0	0.843
Enterobacter agglomerans	29	13	2	0	0	13	1	0	0.006
Enterobacter amnigenus biogroup I	1	0	0	1	0	0	0	0	0.733
Enterobacter cloacae	5	5	0	0	0	0	0	0	0.016
Enterobacter gregoviae	8	8	0	0	0	0	0	0	2.29E-04
Enterococcus azaci hrofyticus	3	3	0	0	0	0	0	0	0.176
Enterococcus avium	5	0	2	3	0	0	0	0	0.013
Enterococcus casseliflavus	42	0	2	0	1	24	12	3	2.82E-09
Enterococcus cecorum	7	4	0	0	0	3	0	0	0.414
Enterococcus dispar	8	0	0	0	1	7	0	0	6.55E-06
Enterococcus faecalis	33	0	0	30	0	3	0	0	2.62E-26
Enterococcus gallinarum	11	5	0	0	0	6	0	0	0.221
Enterococcus hirae	24	16	0	2	0	3	2	1	1.34E-05
Enterococcus malodoratus	6	2	0	0	1	2	1	0	2.90E-05
Enterococcus mundtii	8	0	0	5	1	2	0	0	9.20E-06
Enterococcus raffinosus	10	2	0	0	0	0	8	0	4.83E-05
Erwinia ananas	1	1	0	0	0	0	0	0	0.843
Escherichia coli	24	5	0	2	0	12	5	0	0.438
Hafnia alvei	1	1	0	0	0	0	0	0	0.843
Klebsiella oxytoca	3	2	1	0	0	0	0	0	0.310
Klebsiella pneumoniae	14	2	2	1		6	3	0	0.759
Lactobacillus acidophilus	1	0	0	0	0	1	0	0	0.950
Lactobacillus fermentum	3	0	0	0	0	3	0	0	0.495
Micrococcus spp.	3	0	0	0	0	0	3	0	0.034
Morganella morganii	5	5	0	0	0	0	0	0	0.016
Proteus mirabilis	39	23	2	1	0	5	3	0	3.37E-08
Proteus myxofaciens	2	2	0	0	0	0	0	0	0.449
Proteus penneri	8	7	0	0	0	0	1	0	0.005
Proteus vulgaris	14	14	0	0	0	0	0	0	1.15E-08
Providencia rettgeri	5	4	0	0	0	0	1	0	0.16E
Pseudomonas spp.	55	32	2	0	1	15	5	0	1.44E-11
Salmonella enterica serovar Typhimurium	4	4	0	0	0	0	0	0	0.057
Staphylococcus aureus	17	0	0	0	0	11	6	0	0.007
Staphylococcus xylosus	5	0	0	0	0	0	5	0	6.07E-04
Staphylococcus epidermidis	12	10	1	1	0	0	0	0	2.53E-04

For enterococci a total of 123 samples were positive, 34 for staphylococci, all 191 samples had one or more bacillus strains, 60 samples were positive for lactose fermenting and 85 for non-lactose fermenting bacteria on MacConkey agar. Out of 86 Samples tested for anaerobic spores 74 were positive after anaerobic enrichment; E in value indicate exponent viz, 7.95E-06= 0.00000795.

Fig. 2: Table 2

3.4. Yeasts and molds count (YMC): Out of 191 samples of axone, yeasts and molds were countable in 99 (51.8%) samples. YMC ranged from 0 to 7.41 Log10 cfu/ gm of axone. YMC in fresh

and dry axone were significantly higher (p¡0.005) than in matured axone (Table. 1). Though YMC in dried Axone were the highest but not significantly (p, 0.569) different from fresh axone, irrespective of tribe. The ANOVA revealed significant effect of tribes on YMC of fresh axone (p, 0.001) and matured axone (p, 0.05). It was lowest in Sema axone while highest in Chakesang axone. The YMC had significant (p, ¡0.05) positive association with TAMPC and EC but negative correlation with pH (r, -0.17) and SC (r, -0.23).

Table. 3. Antimicrobial drug resistance in bacteria of different species isolated from Axone samples.

Bacteria (Number of isolates tested)	Percent resistance to antimicrobials											MDR
	Amp	Caz	Ce	C	Cf	Co	G	T	Imp	E	Cd	
Aeromonas eucrenophila (2)	100.00	100.00	50.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	NT	100.00
Aeromonas hydrophila (2)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	NT	0.00
Aeromonas salmonicida ssp. salmonicida (3)	33.33	0.00	66.67	0.00	0.00	33.33	0.00	0.00	0.00	33.33	NT	0.00
Bacillus amiloliquefaciens (5)	40.00	0.00	20.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacillus badius (7)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	14.29	0.00	0.00	0.00	0.00
Bacillus brevis (3)	33.33	0.00	0.00	66.67	0.00	33.33	0.00	0.00	0.00	0.00	0.00	33.33
Bacillus circulans (6)	16.67	16.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	16.67	0.00	0.00
Bacillus coagulans (39)	54.17	18.18	16.67	41.03	3.03	33.33	0.00	23.08	0.00	27.27	12.12	41.03
Bacillus lentus (9)	22.22	11.11	0.00	11.11	0.00	11.11	0.00	0.00	0.00	0.00	11.11	11.11
Bacillus marcescens (5)	40.00	20.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	20.00	0.00	20.00
Bacillus pantothenticus (19)	15.79	10.53	10.53	5.26	0.00	5.26	0.00	5.26	0.00	5.26	0.00	5.26
Citrobacter amalonaticus (3)	66.67	33.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	NT	0.00
Citrobacter diversus (1)	NT	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	NT	0.00
Enterobacter agglomerans (16)	66.67	14.29	11.11	0.00	0.00	9.09	0.00	12.50	0.00	37.50	NT	6.25
Enterobacter amnigenus biogroup I (1)	NT	0.00		0.00	0.00	0.00	0.00	0.00	0.00	100.00	NT	0.00
Enterobacter cloacae (5)	80.00	0.00	20.00	0.00	0.00	20.00	0.00	0.00	0.00		NT	0.00
Enterobacter gregoriae (6)	100.00	16.67	0.00	0.00	0.00	16.67	0.00	16.67	0.00	66.67	NT	0.00
Enterococcus casseliflavus (1)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Enterococcus caecorum (24)	29.17	37.50	12.50	12.50	0.00	66.67	4.17	0.00	0.00	0.00	5.56	16.67
Enterococcus casseliflavus (3)	0.00	33.33	33.33	0.00	33.33	0.00	0.00	0.00	0.00	0.00	0.00	33.33
Enterococcus dispar (6)	16.67	0.00	16.67	16.67	0.00	83.33	16.67	33.33	0.00	0.00	0.00	0.00
Enterococcus gallinarum (2)	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	50.00	0.00	0.00
Enterococcus hirae (7)	0.00	0.00	0.00	0.00	0.00	71.43	0.00	0.00	0.00	0.00	28.57	0.00
Enterococcus malodoratus (3)	0.00	0.00	50.00	33.33	0.00	66.67	33.33	0.00	0.00	0.00	33.33	0.00
Enterococcus raffinosus (5)	100.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	20.00	0.00
Escherichia coli (6)	0.00	0.00	0.00	0.00	0.00	33.33	0.00	50.00	0.00	50.00	NT	0.00
Hafnia alvei (1)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	NT	0.00
Klebsiella oxytoca (3)	100.00	66.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	NT	0.00
Klebsiella pneumoniae (6)	0.00	33.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	66.67	NT	0.00
Lactobacillus acidophilus (1)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Lactobacillus fermentum (3)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Morganella morganii (4)	100.00	25.00	0.00	50.00	25.00	0.00	0.00	75.00	0.00	100.00	NT	25.00
Proteus mirabilis (27)	57.89	33.33	21.05	25.93	0.00	29.17	0.00	81.48	3.70	100.00	NT	37.04
Proteus myxofaciens (1)	NT	0.00	NT	100.00	0.00	100.00	0.00	100.00	0.00	100.00	NT	100.00
Proteus penneri (6)	NT	0.00	NT	33.33	0.00	16.67	0.00	83.33	0.00	83.33	NT	16.67
Proteus vulgaris (13)	80.00	30.77	7.69	38.46	7.69	23.08	7.69	53.85	0.00	100.00	NT	46.15
Providencia rettgeri (5)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	NT	0.00
Pseudomonas spp. (37)	12.50	25.71	8.25	5.41	3.85	0.00	3.03	5.41	5.41	13.33	NT	8.11
Salmonella enterica ser. Typhimurium (5)	0.00	20.00	0.00	0.00	0.00	20.00	0.00	0.00	0.00	0.00	NT	0.00
Staphylococcus aureus (10)	10.00	0.00	0.00	10.00	0.00	0.00	20.00	0.00	0.00	0.00	0.00	0.00
Total Gram negative (153)	46.32	22.15	12.63	13.73	2.14	17.19	1.38	27.45	1.96	51.85	NT	15.69
Total Gram positive (160)	29.77	13.64	8.80	16.88	0.65	27.81	3.13	11.25	0.00	8.44	6.90	15.63
Total (313)	36.73	17.82	10.45	15.34	1.36	22.94	2.30	19.17	0.96	23.40	6.90	15.97
X^2 probability value for effect of bacterial species on antimicrobial drug sensitivity	0.008	4.38E-30	0.441	0.001	0.414	2.56E-07	0.397	6.13E-15	1.000	1.14E-08	0.999	4.35E-04
X^2 probability value for effect of Gram reaction of Bacteria on antimicrobial drug sensitivity	0.090	0.290	0.838	0.897	0.748	0.219	0.793	0.004	0.366	4.64E-12	NA	0.9994

Amp, ampicillin; Caz, ceftazidime; Ce, cefotaxime; C, chloramphenicol; Cf, ciprofloxacin; Co, co-trimoxazole; G, gentamicin; T, tetracycline; Imp, imipenem; E, erythromycin; Cd, clindamycin, MDR, multiple drug resistance, resistant to three or more drugs; NT, not tested; NA, not applicable.

Fig. 3: Table 3

3.5. Aerobic endospore count (AEC): Aerobic spores of Bacillus spp. were present (Table. 1) in high numbers (5.6 Log10 to 10.55 Log10 cfu/g) in all 191 samples. The effect of maturation or drying was insignificant on AEC (p, ¿0.54) on axone of most of the tribes. However, AEC of dry Sema axone was significantly (p, 0.034) higher than matured axone of Sema. On the other hand, fresh Lotha axone had higher AEC than matured (p, 0.0008) and dry (p, 0.076) axone, but difference was insignificant (p, 0.597) in AEC of dry and matured Lotha axone. Similar to Lotha axone, fresh Ao axone had more AEC than matured axone (p, 0.08). AEC of fresh and matured axone of Angami tribe was about a log lower than of dried axone.

Although ANOVA of AEC of fresh and matured axone indicated significant (p, 0.001) effect of tribe, could not be ranked separately with DMRT. Axone of Angami and Ao had the highest and the lowest AEC, respectively. The AEC had strong positive association (p, 0.001) with TAMPC (r, 0.41).

Characterization of representative colonies from AEC plates lead to identification of 12 species of Bacillus from axone samples. Bacillus coagulans being the most common could be detected in 106 (55.5%) samples followed by B. pentothenticus, B. subtilis, B. stearothermophilus and B. lentus isolated from 23 (12.0%), 22 (11.5%), 16 (8.4%) and 12 (6.3%) samples, respectively. Strains of other Bacillus species (Table. 2) were detected in only few (¡3.5%) samples. Isolation rate of different Bacillus species varied significantly among axone of different tribes (Table. 2). Though strains of all common Bacillus spp. were detected in axone of several tribes, none of the Bacillus species was present in axone of all tribes. Bacillus coagulans, the most prevalent type of Bacillus in axone was absent in Ao axone, similarly B. pentothenticus could not be detected in Sema, Kuki and and Chakesang axone. Lotha axone contained the widest variety of Bacillus species and out of 12; strains of eight Bacillus species were detected in Lotha axone. On the other hand strains of only three species could be detected in Chakesang axone.

3.6. Enterococcus count (EC): Of the 130 samples for which EC was determined (Table. 1), 123 (94.6%) were positive with count ranging from 2.92 Log10 to 8.26 Log10 cfu/g. There was only little difference in EC of fresh and matured (p, 0.703), fresh and dry (p, 0.103) axone but EC of dry axone (6.670.93 Log10 cfu/g) was significantly higher (p, 0.0005) than that of matured axone (5.34 2.01 Log10 cfu/g). The difference in EC of axone of different tribes was evident (p, 0.001) and matured axone of Lotha and Chakesang tribe had significantly higher EC than axone of all other tribes. Lowest EC were recorded in Sema Axone (Table. 1). Matured Sema axone (p, 0.025) had higher EC than EC of fresh axone but such difference was insignificant in axone of other tribes.

Enterococci from axone could be classified in to 11 species (Table. 2). Enterococcus caecorum was identified from 42 samples of axone but could not be detected in axone of Angami and Chakesang tribes. The second most commonly identified eneterococci was E. facecalis, detected in 30 (83.3%) of Chakesang and three of Lotha (4.8%) axone samples. The third most commonly identified Enterococcus was E. hirae, isolated from 24 samples of Axone of 5 tribes; it was present in 36.4% of Angami axone. Origin of axone had significant (p, 0.001) effect on EC (Table. 2). EC was positively (p, 0.05) correlated with TAMPC (r, 0.18), YMC (r, 0.18) and AEC (r, 0.16) but

not with pH, coliform and SC.

3.7. Staphylococcus count (SC): Only 34 samples were processed for SC and all were positive containing 5.69 Log10 to 7.56 Log10 cfu/g. The SC differed significantly (p, 0.001) in three different types of axone. Staphylococcal count of matured axone was slightly higher (p, 0.253) than that of fresh axone (Table. 1) but dried axone had significantly higher SC than fresh (p, 0.004) and matured (p, 0.069) axone. However, SC of fresh Angami axone (6.510.21 Log10 cfu/ g), was slightly higher than in matured axone (6.320.46 Log10 cfu/ g). SC in axone of different tribes differed significantly (p, 0.001). Although SCs were higher in matured Sema and Lotha axone than Angami axone, DMRT revealed it as insignificant.

Similar to SC, there was also variation in distribution of Staphylococcus spp. in axone of different tribes. Staphylococcus xylosus was detected in Sema axone only, while S. sciuri was identified in Sema and Lotha axone. On the other hand, S. epidermidis were present only in Angami, Ao and Chakhesang axone samples.

3.8. Antimicrobial drug sensitivity: Of the 11 antimicrobials tested for their effectiveness on 153 gram negative bacteria (GNB) and 160 Gram positive bacteria (GPB) isolates belonging to 40 species (Table. 3), none of the drug was effective against all strains. Species of bacteria was detected as one of the important determinant of sensitivity to ampicillin (p, 0.008), ceftazidime (p, 4.38E-30), chloramphenicol (p, 0.001), cotrimoxazole (p, 2.56E-07), tetracycline (p, 6.13E-15) and erythromycin (p, 1.14E-08). Association of Gram reaction of bacteria with antimicrobial drug sensitivity was evident for tetracycline (p, 0.004) and erythromycin (p, 4.64E-12); resistance to these two drugs was more common in Gram negative bacteria.

Effect of source of axone from which bacteria were isolated on antimicrobial sensitivity could be evaluated for only few bacteria because of the numbers of strains tested (Table. 4, 5). On comparing all isolates and GPB, source had significant effect on sensitivity of bacteria to ampicillin, ceftazidime, cefotaxime, cotrimoxazole, gentamicin, tetracycline and erythromycin. Majority of bacteria isolated from Ao (60.8%), Sema (57.7%) and Angami (50.8%) axone while only a few strains from axone of other tribes (˜20%) were resistant to ampicillin. Cefotaxime resistant strains were detected in axone of all but Sema tribe. Ceftazidime resistant strains were limited to isolates from Lotha (23.7%), Ao (21.7%) and Angami (17.5%) axone. Imipenem resistance was only detected in isolates from Lotha (2.6%) axone and ciprofloxacin resistant strains from Lotha (0.9%) and Angami (2.5%) axone samples (Table. 4). Among GNB, source was an important determinant for sensitivity to ampicillin, tetracycline, imipenem and erythromycin. Most of GNB from Ao axone were resistant but those from Lotha axone were sensitive to ampicillin and tetracycline. All GNB isolates from Chakesang and Sema axone and majority from Angami (60.9%) axone were resistant but 80.2% from Lotha axone were sensitive to erythromycin.

On comparing 95 Bacillus species strains and 39 strains of B. coagulans of different origin for their sensitivity pattern, source affected their sensitivity for ampicillin, chloramphenicol, cotrimoxazole and tetracycline (Table. 4, 5). Sensitivity of Enterococcus isolates from axone to ampicillin, ceftazidime, chloramphenicol, tetracycline and clindamycin was also modulated by the source of axone. However, with specific reference to the most common Enterococcus in axone (E. caecorum), source had effect only on sensitivity to ampicillin, ceftazidime and cephotaxime. Antimicrobial sensitivity of Proteus strains not varied significantly with the source of axone. Among pseudomonads sensitivity to cephotaxime was source dependent but no effect of source was detectable on sensitivity to other antimicrobials.

Table. 4. Effect of source (tribe) on antimicrobial drug resistance of bacteria isolated from Axone samples of different tribes.

Type of bacteria	Axone of Tribe	Strains tested	% resistant bacterial strains to										
			Amp	Caz	Ce	C	Cip	Co	T	Imp	E	Cd	MDR
All strains	Angami	130	50.79	17.46	7.94	16.92	2.54	23.68	1.54	28.46	0.00	43.75	14.71	17.69
313	Ao	23	60.87	21.74	13.04	13.04	0.00	4.55	0.00	34.78	0.00	0.00	0.00	17.39
	Chakhesang	1	NT	0.00	NT	0.00	0.00	0.00	0.00	0.00	0.00	100.00	NT	0.00
	Lotha	114	20.19	23.68	10.58	10.53	0.88	19.81	0.94	5.26	2.63	10.48	5.06	13.16
	Pochury	10	10.00	0.00	40.00	30.00	0.00	70.00	20.00	20.00	0.00	0.00	0.00	10.00
	Sema	35	57.69	0.00	0.00	22.86	0.00	30.77	5.71	20.00	0.00	27.59	8.33	20.00
Gram Positive	Angami	34	57.14	14.71	14.29	17.65	2.94	32.35	0.00	14.71	0.00	20.59	14.71	20.59
160	Ao	10	40.00	0.00	0.00	10.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Lotha	79	19.23	20.25	7.69	12.66	0.00	21.52	1.27	2.53	0.00	7.59	5.06	13.92
	Pochury	10	10.00	0.00	40.00	30.00	0.00	70.00	20.00	20.00	0.00	0.00	0.00	10.00
	Sema	27	57.69	0.00	0.00	25.93	0.00	38.89	7.41	14.81	0.00	0.00	8.33	22.22
Gram negative	Angami	96	50.00	18.48	7.14	16.67	2.38	20.00	2.08	33.33	0.00	60.87	NT	16.67
153	Ao	13	76.92	38.46	23.08	15.38	0.00	8.33	0.00	61.54	0.00	NT	NT	30.77
	Chakhesang	1	NT	0.00	NT	0.00	0.00	0.00	0.00	0.00	0.00	100.00	NT	0.00
	Lotha	35	23.08	31.43	19.23	5.71	2.86	14.81	0.00	11.43	8.57	19.23	NT	11.43
	Sema	8	NT	0.00	NT	12.50	0.00	12.50	0.00	37.50	0.00	100.00	NT	12.50
Bacillus spp.	Angami	22	57.14	22.73	14.29	27.27	4.55	13.64	0.00	22.73	0.00	27.27	13.64	31.82
95	Ao	10	40.00	0.00	0.00	10.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Lotha	56	23.21	10.71	7.14	14.29	0.00	7.14	0.00	3.57	0.00	10.71	3.57	12.50
	Sema	7	85.71	0.00	0.00	85.71	0.00	85.71	0.00	57.14	0.00	0.00	0.00	85.71
Enterococcus	Angami	12	NT	0.00	NT	0.00	0.00	66.67	0.00	0.00	0.00	8.33	16.67	0.00
spp. 51	Lotha	19	11.11	52.63	11.11	10.53	0.00	68.42	5.26	0.00	0.00	0.00	10.53	21.05
	Pochury	10	10.00	0.00	40.00	30.00	0.00	70.00	20.00	20.00	0.00	0.00	0.00	10.00
	Sema	10	88.89	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	100.00	0.00
Proteus spp.	Angami	33	66.67	27.27	14.29	36.36	3.03	30.30	3.03	69.70	0.00	93.33	NT	42.42
47	Ao	7	71.43	28.57	28.57	28.57	0.00	14.29	0.00	100.00	0.00	NT	NT	42.86
	Lotha	4	0.00	50.00	0.00	0.00	0.00	0.00	0.00	75.00	25.00	100.00	NT	0.00
	Sema	3	NT	0.00	NT	33.33	0.00	33.33	0.00	33.33	0.00	100.00	NT	33.33
Pseudomonas	Angami	15	6.67	30.77	0.00	0.00	0.00	0.00	6.67	6.67	0.00	NT	NT	6.67
spp. 37	Ao	2	50.00	50.00	50.00	0.00	0.00	0.00	0.00	0.00	0.00	NT	NT	50.00
	Lotha	20	13.33	20.00	6.67	10.00	5.00	0.00	0.00	5.00	10.00	0.00	NT	5.00
B. coagulans	Angami	19	25.00	21.05	25.00	31.58	5.26	15.79	0.00	26.32	0.00	26.32	15.79	31.58
39	Lotha	14	57.14	14.29	14.29	28.57	0.00	7.14	0.00	0.00	0.00	28.57	7.14	28.57
	Sema	6	100.00	NT	NT	100.00	NT	100.00	0.00	66.67	0.00	NT	NT	100.00
Enterococcus	Lotha	16	12.50	56.25	6.25	12.50	0.00	68.75	6.25	0.00	0.00	0.00	6.25	18.75
caecorum 24	Pochury	2	0.00	0.00	100.00	50.00	0.00	50.00	0.00	0.00	0.00	0.00	0.00	50.00
	Sema	6	83.33	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	NT	0.00

Amp, ampicillin; Caz, ceftazidime; Ce, cefoxaxime; C, chloramphenicol; Cf, ciprofloxacin; Co, co-trimoxazole; G, gentamicin; T, tetracycline; Img, imipenem; E, erythromycin; Cd, clindamycin, MDR, multiple drug resistance, resistant to three or more drugs; NT, not tested.

Fig. 4: Table 4

MDR strains were equally distributed among GNB and GPB (p, 0.999) but they belonged to only few species of bacteria (p, 4.35E-04). Fifty MDR strains isolated from axone (Table. 3) belonged to B. coagulans (16), B. pantothenticus (1), B. marcescens (1), B. lentus (1), B. brevis (1); A. eucranophila (2), En. agglomerans (1), Ec. casseliflavus (1), Ec. caecorum (4), P. mirabilis

(10), P. vulgaris (6), P. penneri (1), P. myxofaciens (1), M. morganii (1) and Pseudomonas spp. (3) indicting bacterial species as an impotent (p, 4.35E-04) determinant of MDR. Except in Bacillus strains (p, 2.2E-05) and pseudomonads (p, 0.082) source had no significant association with occurrence of MDR in isolates. Majority of Bacillus strains from Sema (85.7%) but none from Ao axone had MDR while probability of MDR pseudomonads was more in isolates from Ao axone (50%).

Table. 5. Effect of source (tribe) on antimicrobial drug sensitivity pattern of bacteria isolated from Axone samples (significance determined with Chi square X^2 test with the hypothesis that there is no effect of source on antimicrobial drug sensitivity of bacteria).

Bacteria from Axone	X^2 probability for effect of source on bacterial sensitivity to											
	Amp	Caz	Ce	C	Cip	Co	G	T	Imp	E	Cd	MDR
All bacteria (313)	7.95E-06	0.041	0.031	0.332	0.809	0.002	0.003	0.0001	0.382	3.90E-07	0.396	0.866
G+ve bacteria (160)	0.002	0.093	0.009	0.527	0.615	0.008	0.020	0.085	1.000	0.066	0.396	0.579
G-ve bacteria (153)	0.054	0.385	0.573	0.731	0.989	0.908	0.595	0.025	0.067	0.008	NA	0.717
All *Bacillus* spp. (95)	0.004	0.275	0.696	2.00E-04	0.379	1.70E-07	1.000	7.60E-05	1.000	0.129	0.288	2.20E-05
Bacillus coagulans (39)	0.046	0.883	0.879	0.006	0.684	2.9E-05	1.000	0.005	1.000	0.989	0.754	0.006
All *Enterococcus* spp. (51)	1.80E-04	1.10E-04	0.100	0.071	1.000	0.922	0.173	0.036	1.000	0.346	0.029	0.164
Enterococcus casseroum (24)	0.003	0.028	4.5E-04	0.180	1.000	0.869	0.770	1.000	1.000	1.000	0.936	0.241
Proteus spp. (47)	0.568	0.542	0.809	0.529	0.933	0.758	0.952	0.166	1.000	0.963	NA	0.421
Pseudomonas spp. (37)	0.218	0.567	0.023	0.407	0.856	1.000	0.557	0.919	1.000	NA	NA	0.082

Amp, ampicilin; Caz, ceftazidime; Ce, cefotaxime; C, chloramphenicol; Cf, ciprofloxacin; Co, co-trimoxazole; G, gentamicin; T, tetracycline; Imp, Imipenem; E, erythromycin; Cd, clindamycin, MDR, multiple drug resistance, resistant to three or more drugs; E in value indicate exponent viz, 7.95E-06= 0.00000795.

Fig. 5: Table 5

4. Discussion

Though fermentation of foods often leads to biological enrichment, the exact effect depends on substrate-food quality, microbes involved in fermentation, mode of fermentation, temperature of fermentation, additives and also on several less understood factors (Chukeakirote et al., 2010; Joo et al., 2007; Lee et al., 2005; Ohta, 1986). In fermentation of protein rich foods including soybean hydrolytic enzymes of bacteria cause proteolysis, lipolysis and sacchrolysis leading to production of easily digestible peptides, amino acids, free sugars and fatty acids, respectively (Ashiuchi et al., 2001; Lee and Kim, 2004; Tamang and Nikkuni 1998). Fermented soybean foods besides being easily digestible have also been claimed to prevent diseases and confer good health and long life (Ohta, 1986; Singh et al., 2009; Tamang, 2010) due to their numerous functional properties including antimicrobial, fibrinolytic, immunohistochemical, hypocholestremic and antioxidant effects (Dajanta et al., 2012; Joo et al., 2007; Kim et al., 2003, 2004; Ko et al., 2004; Lee et al., 2005). There are several studies on microbiological quality of fermented soybean foods available in different parts of world (Ashraf et al., 1999; Nout et al., 1998; Pao, 1989; Rehberger et al., 1984; Samson et al., 1987; Seo et al., 2009; van Kooij and de Boer, 1985) but only little is known about axone. The present study explored the microbiological quality of axone prepared by different tribes of Nagaland. There are about 66 Naga tribes of which 17 reside in Nagaland (Shimray, 2007; Tohring, 2010). Although different Naga tribes have their very own socio-cultural traditions and food habits and make their own fermented soybean-axone, a side dish, still consider Sema axone as the best one in taste and flavour (Singh et al., 2009; Tamang, 2010). The taste of axone depend on several factors but typical umami taste is mainly associated with alkaline fermentation of soybean (Tamang, 2010) therefore pH of axone may be an important indicator of its quality and flavour. Ammonia is an important outcome of soybean fermentation which imparts alkaline pH to axone and other similar preparations (Ohta, 1986, van Kooij and de Boer, 1985; Tamang, 2010). Among all types, Sema axone had the highest pH; an indicator of better umami taste (Tamang, 2010) further indicated the reason for liking of Sema axone by all people of Nagaland. Lower pH in fresh axone might be due to incomplete fermentation while in dried axone it might be due to evaporation of ammonia in process of drying. However, it was unexplainable why pH of dried Sema axone of was higher than fresh and matured axone.

The pH of axone was positively correlated with coliform count and negatively correlated to YMC and SC. Though coliforms are considered as one of the important indicator of microbiological quality and safety of foods (FDA, 1995; ICMSF, 1988), probably in axone this association might be not true and needs further studies. Negative association of pH with YMC and SC was very much expected because these organism may produce acid on fermentation of available polysaccharides which may neutralize ammonia produced during soybean fermentation (Samson et al., 1987; van Kooij anf Boer, 1985).

TAMPC and AEC in axone had strong correlation (r, 0.41) and there was not a single sample where AEC was less than 3.6x10*5 cfu/g indicating that the aerobically growing spore forming bacteria (Bacillus) were the major constituents of microbiota in axone. Identification of representative colonies from AEC plates confirmed that all colonies were of Bacillus strains indicating that Bacillus strains are the most important bacteria which might be responsible for soybean fermen-

tation to form axone. Our findings are in concurrence to earlier studies on similar fermented soybean foods (Nout et al., 1998; Ohta, 1986; Samson et al., 1987; Seo et al., 2009; Singh et al., 2009; Tamang, 2003; Tamang, 2010, Tamang and Kailasapathy, 2010; Tamang et al., 2009). Characterization of Bacillus strains revealed the absence of B. cereus in axone, a putative toxigenic bacteria often reported in fermented soybean foods including kinema (Nout et al., 1998), sufu (Han et al., 2001) and other similar foods (Tamang, 2010). Thus it can be deduced from the Bacillus characterization that axone is comparative more safe than other similar foods specifically in terms of foodborne illness caused by B. cereus.

Bacillus spp. are reported to be the major bacteria responsible for production of axone and axone-like products (Ohta, 1986; Seo et al., 2009; Singh et al., 2009; Tamang et al., 2009). In the study Bacillus strains of 12 species were detected in axone, B. coagulans (55.5%), B. pentothenticus (12.0%), B. subtilis (11.5%), B. steariothermophilus (8.4%) and B. lentus (6.3%) were the most common types. In earlier studies on a few samples of axone either B. subtilis (Tamang, 2010) or B. coagulans (Singh et al., 2009) were reported as the major fermenting organisms. The present study also confirms the earlier finding (Singh et al., 2009; Tamang, 2010) but also opened a plethora of 10 other species of Bacillus present in axone. Observations are concurrence to earlier studies on other ethnically produced similar type of fermented soybean food viz., in chungkukjang (Joo et al., 2007) and thua nao (Chukeatirote et al., 2010) many different types of Bacillus species strains have been reported. The multiplicity of Bacillus strains as well as dominance of B. cogaulans, a known probiotic organism, in axone might be responsible for pronounced biological effects on health and production parameters reported earlier (Singh, 2013; Singh et al, 2009, 2014a, b).

Isolation rate of different Bacillus species varied significantly among axone of different origin (Table. 2) that variation might be due to either difference in soybean varieties used or due to minor variations in process of making axone (Chukeakirote et al., 2010; Joo et al., 2007; Lee et al., 2005; Ohta, 1986).

Presence of countable number of lactose fermenting bacteria (LFB) or putative coliforms in 31.4% axone samples and E. coli in 12.6% samples indicated that during preparation of axone hygienic handling might be an important lacunae (ICMSF, 1988). The way axone is prepared and handled (Singh et al., 2009; Tamang, 2010), there are lot many chances of entry of coliforms from hands, from packing material and environmental sources. The study revealed the highest coliform count in fresh axone and the lowest in dried axone. It might be due to death of coliforms on maturation and dying under reducing water activity and also probably due to antimicrobial substances produced during fermentation (Chukeatirote et al., 2010). In earlier studies on fermented soybean products isolation of members of Enterobacteriacea including E. coli has been frequently reported in tempeh (Samson et al., 1987), kinema (Nout et al., 1998) and natto (Ohta, 1986). To solve the problem of coliforms and E. coli in soybean fermented products use of pure starter culture and production under controlled conditions has been attempted for kinema (Sarkar and Tamang, 1995; Tamag and Nikkuni, 1996), soy-dad-dawa (Terlabie et al., 2006), chungkukjang (Lee et al., 2005a) and natto (Omafuvbe et al., 2002). However, there is very scanty information on development of a starter culture for axone (Singh et al., 2009, 2014). Further, to convince different Naga tribes to commercialize production of specific axone instead of their very own home-made ethnic products might be a big issue.

Although E. coli is the most commonly reported LFB growing on MLA (coliform) in foods (ICMSF, 1988), it was isolated from only 40% of the axone positive for LFBs. In axone the most common LFBs included Enterobacter species detected in 70% of LFB positive and 22.5% of total axone samples. Other LFBs including K. pneumoniae (7.3%), Citrobacter spp. (2.1%) and Erwinia ananas (0.5%) were also detected in some of the axone samples. In earlier studies on ethnic fermented foods, members of Enterobacteriaceae (Nout et al., 1998; Samson et al., 1987; Tamang, 2003), K. pneumoniae, K. pneumoniae subsp. ozaenae, En. cloacae (Tamang and Kailasapathy, 2010) are reported commonly. Although E. coli and other LFBs isolated from axone might have importance as indicator organism for microbial safety of foods, may often be associated with acute and long lasting chronic diarrhoea (Lindsay, 1997). In earlier studies too enteropathogenic, enterotoxigenic (Singh and Kulshreshtha, 1992; Singh and Roy, 2012; Singh et al., 1999) and enteroinvasive (Sabota et al., 1998) klebsiellae has frequently been detected in foods as an important health threats to consumers. Once consumed klebsiellae may persist in intestines for long which may be associated with ankylosing spondylitis (Burning et al., 1997), therefore isolation of potentially harmful microbes in large number of ax-

one samples indicate a need for quality control in axone.

In addition to LFBs, non-lactose fermenting (NLFBs) members of Enterobacteriaceae isolated (Table. 2) from 85 (44.5%) axone samples included H. alvei (1), K. oxytoca (3), M. morganii (5), Providencia rettgeri (5), S. Typhimurium (4) and Proteus spp. (63). Members of Enterobacteriaceae (though not identified) have been reported frequently in other ethnic soybean food (Nout et al., 1998; Samson et al., 1987; Tamang, 2003). Salmonella Typhimurium isolated from Angami axone might be of public health concern being the second most common serotype of Salmonella causing foodborne illness (CDC, 2009, 2014). However, it appears to be the first report of isolation of S. Typhimurium from axone, and is rarely reported in fermented soybean products (Samson et al., 1989, Tamang, 2010), a recent outbreak of Salmonella Paratyphi B var Java has been attributed to consumption of contaminated tempeh, an axone like product, in Carolina state (Greise et al., 2013). Proteeae (Proteus, Morganella and Providencia) were commonly isolated from axone, as reported from other foods (O'hara et al., 2000; Oni et al., 2009), are rarely associated with foodborne illness but are often associated with decaying organic matter, food, faeces and contaminated wounds (O'hara et al., 2000). Though associated with several types of clinical infections, major importance of Proteeae is their transferable MDR (Stamm, 1999). In the study, out of 25 MDR strain of GNB isolated from axone 19 belonged to Proteeae.

Besides members of Enterobacteriaceae, some other important potentially pathogenic NLFBs growing on MLA plates included aeromonads and pseudomonads isolated from 7 (3.7%) and 55(28.8%) samples of axone, respectively. Although pseudomonads have often been reported from contaminated fermented soybean food (Nout et al., 1998; Ohta, 1986; Samson et al., 1987; Tamang, 2003), aeromonads have rarely been reported earlier from axone or other similar fermented soybean foods. Isolation of aeromonads belonging to three potentially pathogenic species (A. eucranophila, A. hydrophila and A. salmonicida) is of public health concern because of their emerging role in diarrhoeic and septicaemic diseases in humans (Singh and Roy, 2012; Singh et al., 1997). All axone samples containing aeromonads belonged to Lotha tribe that might be associated with backyard fishery practices of Lothas. Aeromonads are often been reported to be prevalent in fish in India (Singh et al., 1997). Besides fish, other foods may also be associated with Aeromonas related foodborne illness and other infections in humans (Janda and Abbott, 2010).

More than 50% samples of axone, examined for yeasts molds, had countable numbers; the counts were the highest in dried axone (4.43 3.2 Log10 cfu/g) followed by fresh (3.95 2.22 Log10 cfu/g) and matured (2.03 2.42 Log10 cfu/g) axone. Yeasts and molds has an important role in production of many commercial and traditional foods and beverages throughout the world (Aidoo, et al., 2006), but they are not the choice for fermentation of soybean except mold fermented tempeh (Tamang, 2010). Presence of yeasts and molds in axone seems to be normal and in concurrence to earlier observations on maseura (up-to 10*4 cfu/g), fermented black gram (Chettri and Tamang, 2008), sufu (10*2 to 10*6 cfu/g). Increase in YMC on drying of axone appears to be in contrast to earlier observations on aged sufu (Pao, 1994). It might be due to variation of environment and processing practices.

Enterococci are time tested very good indicator of faecal contamination even in processed foods due to their comparatively high resistance to ambient temperature and for commonly used decontaminants (FDA, 1995; ICMSF, 1988; Singh, 2009). Though boiling, an integral in process of axone preparation, kills enterococci, they could be detected in 94.6% of axone samples in high numbers (2.92 Log10 to 8.26 Log10 cfu/g) without much difference between fresh and matured axone (p, 0.703). Enterococci might have gained entry to axone in manual processing of axone after boiling step. Significantly high EC in dry axone might be due to better survival of enterococci in harsh environment (FDA, 1995; ICMSF, 1988). The lowest EC in Sema axone indicated its quality and might be the reason behind better flavour of Sema axone liked by most of the Nagas (Singh et al., 2009; Tamang, 2010). Detection of enterococci in large numbers in axone was in concurrence to earlier observations on kinema in Sikkim (Nout et al., 1988).

Of the 11 species of Enterococcus detected in axone (Table. 2), E. caecorum was the most common (42) followed by E. facecalis (33), E. hirae (24), E. gallinarum (11), E. raffinosus (10), E. mundtii (8), E. malodoratus (6), E. dispar (8), E. casseliflavus (7), E. avium (5) and E. asacchrolyticus (3). However, in earlier studies on similar food, kinema, E. faecalis dominated the count after Bacillus strains (Nout et al., 1998). In no earlier report this much variation in Enterococcus population is reported, it might be attributed to the fact that axone of 7 distinct tribes

was analyzed in the study. Though E. caecorum was detected in axone of five tribes, none of Enterococcus spp. was detected in axone of all tribes indicating that tribe has significant effect on Enterococcus species in their axone (Table. 2).

Entecoccus count could be correlated well with TAMPC but contributed little (¡1%) for the total bacterial load in axone which is in contrast to earlier observation on kinema (Nout et al., 1998). Nout and co-workers (1998) suggested that dominance of EC in TAMPC might be due to proliferation of enterococci in kinema when Bacillus stopped proliferation after fermentation was complete. Though in dried axone EC was higher than in fresh or matured axone; it never constituted a major part of total microbiota in axone. Even in the samples where EC exceeded 108cfu/g it was not more than 8% of TAMPC. It may be due to variation in processing of the two foods. Although enterococci rarely caused foodborne infection/ intoxications, there detection in food is important because of their indicator value for faecal contamination and transferable drug resistance (FDA, 1995; Singh, 2009).

Detection of staphylococci in all examined axone samples indicated its ubiquitous presence in axone. Similar results have been reported from studies on other home fermented soybean foods (Chukeakirote et al., 2010; Joo et al., 2007; Lee et al., 2005; Nout et al., 1998; Ohta, 1986). From axone staphylococci belonging to three species (S. xylosus, S. sciuri and S. epidermidis) could be detected and their distribution varied significantly among axone of different tribes (p, ¡0.001). This variation might be due to the fact that staphylococci are residents on human skin and their population in food may vary with food handler. Though isolation of potentially enterotoxigenic S. aureus strains have been reported earlier from fermented soybean products (Omafuvbe et al., 2000; Samson et al., 1987; van Kooij and de Boer, 1985), in none of the axone samples S. aureus were detected. Our observations are in concurrence to earlier studies on Kinema (Nout et al., 1998) and sufu (Han et al., 2001).

Variation in antimicrobial drug sensitivity of bacteria isolated from axone was dependent both on type of bacteria and source of axone. In the study, all strains of klebsiellae and majority of aeromonads were resistant to ampicillin, it might be due to their inherent resistance to ampicillin (Janda and Abbott, 2010; Singh and Sharma, 2001; Singh et al., 2000). Isolation of carbapenem (imipenem) resistant P. mirabilis and Pseudomonas strain from axone indicated that resistance to newer drugs has already reached to Nagaland, an organic state of India. Isolation of carbapenemase producing Pseudomonads is not uncommon from different sources including foods due to their carriage on human hands and biofilms (Kampf and Kramer, 2004). Multiple drug resistant pseudomonads and other bacteria have recently been reported to be associated with clinical cases and also in environment in Nagaland (Singh, 2012; Singh et al., 2012). MDR was detected in about 15.5% bacterial strains isolated from axone, though alarming is not novel in light of the fact that MDR strains have also been frequently reported from Nagaland in healthy as well as sick persons, birds and lizards (Singh, 2012; Singh et al., 2012, 2013).

The study concluded that Bacillus strains of many different species are the major bacteria present in axone. The pH appeared to be an easily measureable quality indicator for axone. Though coliforms were detected in many axone samples enterococci might be more appropriate indicators of contamination. Isolation of several bacteria of public health concern capable to cause foodborne illness including strains of Salmonella, Aeromonas and Klebsiella species indicated need for development of some microbiological standards at least for marketed axone and standard starter culture to increase safety and quality of axone.

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