Ethno-microbiology to Next Generation Sequencing in Some Fermented Foods of World

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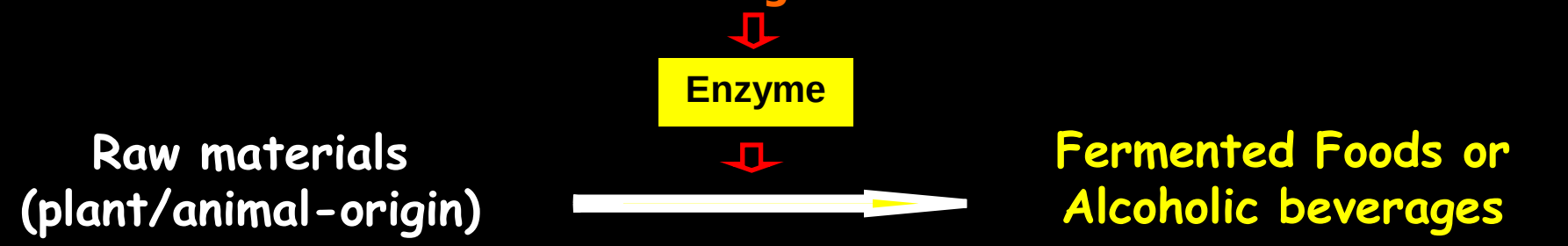
What is a Food?

Food is any substance, composed of carbohydrates, fats, proteins, moisture, minerals, vitamins and bio-active compounds that can be eaten or drunk by human, for nutrition or pleasure. Food is categorized into two parts: Fermented Foods and Non-fermented Foods.

What is a Fermented Food ?

Fermented foods are defined as foods produced by the people using their native knowledge of food fermentation from locally available raw materials of plant or animal sources either naturally or by adding starter culture(s) containing functional microorganisms which modify the substrates biochemically and organoleptically into edible products that are culturally and socially acceptable to the consumers (Tamang, 2010)



Global Food Culture

Global food culture has 3 major traditional food habits based on staple cereal-based diets:

1. cooked rice of the Eastern food culture,
2. wheat/barley-based breads/loaves of the Western and Australian food culture,
3. sorghum/maize porridges of Africa and South America food culture.

Global Food Culture (Tamang,2010)

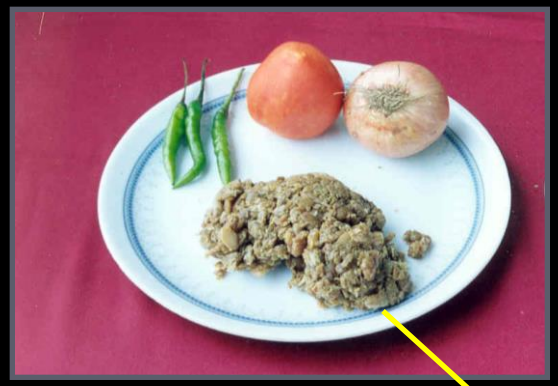
Ref: Tamang (2010). *Fermented Foods and Beverages of the World* , CRC Press, New York.

- **Indian** foods are spicy, and salt is added directly while cooking; seasonings such as soy sauce and monosodium glutamate (**MSG**) are never used.
- Chinese, Korean and Japanese foods are not spicy and use soy sauce as seasoning and other taste-maker such as **MS**G.
- European and American food is grilled, fried, roasted and baked.
- African food is also grilled, steamed and hot

- **Drinking of animal milk is not a food culture of ethnic Chinese, Koreans, Japanese, and many Mongolian-origin races despite of an abundance of cow in their possession (Tamang and Samuel, 2010).**

- **Soybean is processed to make soy milk, *tofu*, and fermented soybean products such as *miso, shoyu*, *natto, thua nau, douchi, chungkokjang, tempe* and *sufu*.**

- **Whereas, the Indians, Europeans, Semites and the nomadic tribesmen of North Central Asia are traditionally animal milk drinkers.**



ETHNIC INDIAN MEAL

European/

American/

Australian Meal

Chinese Meal

Japanese Meal

Korean Meal

Soybean [*Glycine max* (L.) Merrill], [大豆 (*Dàdòu*) in Chinese] "भटमास" in Nepali, was probably introduced to India, Nepal and Bhutan from China probably from Yunnan province and to other Asian countries (Tamang and Samuel 2010; Shurtleff and Aoyagi 2010).

- The Mekong river-basin (Mekong is a trans-boundary river in Southeast Asia and runs from the Tibetan Plateau through China's Yunnan Province, Myanmar, Laos, Cambodia, Thailand and Vietnam, called the Mekong countries) is the most probable place of origin of fermented fish products in Asia (Ishige 1993; Ruddle 1993).

Global Fermented Foods and Beverages

- About 5000 varieties of major and undocumented minor fermented foods and beverages are prepared and consumed by billions of people in Asia (Tamang et al. 2016a) as:
  - staple, curry, stew, side dish, fried, cooked, paste, seasoning, condiment, pickle, confectionery, salad, soup, dessert, savory, drink, candied, masticator, colorant, taste-maker, alcoholic and non-alcoholic beverages.
- Daily per capita consumption of fermented foods & beverages is about 50-400 g worldwide, representing about 5-40 % of daily meals intake (Tamang 2010).

Properties of Global Fermented Foods and Beverages

Major sensory and physico-chemical properties of fermented foods are 3A : **Acidic, Alkaline** and **Alcoholic** (Tamang, 2010).

- In **Acidic fermentation**, the substrates are kept in air-tight container (less or no oxygen) to allow LAB to grow on starchy materials to get the acidic product. Eg. ***kimchi, gundruk***
- In **Alkaline fermentation**, semi-anaerobic or aerobic condition should be maintained to facilitate the growth of aerobic bacilli (mostly *Bacillus subtilis*) as in ***kinema, natto***, ***pidan***
- In **Alcohol fermentation**, sachharification (starch to glucose) and glycolysis (glucose to alcohol and CO_2) production is obtained as in **beer, alcoholic drinks and beverages.** Eg. ***saké, pulque***

Microbiology of Fermented Foods

- Culturalable and non-culturable microbiome naturally ferment majority of global fermented foods and beverages. (Tamang et al. 2016a).
- Microorganisms transform the chemical constituents of raw materials of plant/animal sources during *in situ*/*ex situ* fermentation, thereby enhancing (Tamang et al. 2016b):
  - nutritional value in some fermented products, enriching with improved flavour and texture, prolonging the shelf live,
  - fortifying with health-promoting bio-active compounds, vitamins and minerals, degrading undesirable compounds,
  - producing antioxidant
  - antimicrobial compounds
  - harbouring probiotic functions (Farhad et al. 2010, Franz et al. 2014, Tamang 2015a).

Lactic Acid Bacteria (LAB)

- LAB are non-sporeforming, Gram-positive, catalase-negative without cytochromes, non-aerobic or aerotolerant, acid-tolerant, and strictly fermentative bacteria with lactic acid as the major end-product during sugar fermentation.
- LAB genera isolated from fermented foods are:

  Alkalibacterium, *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Oenococcus*, *Pediococcus*, *Streptococcus*, *Tetragenococcus*, *Vagococcus* and *Weissella* (MetaMicrobe.com/Lactic Acid Bacteria 2013; Holzapfel & Wood 2014).

- *Lactobacillus plantarum* has one of the largest genomes (> 3,000,000 bp) among the lactobacilli (Siezen et al. 2012).

Bacillus

- ***Bacillus*** **is a Gram-positive, endospore forming, rod-shaped, catalase positive, motile and aerobic to semi-anaerobic growing bacterium (Gordon et al. 1973).**
- **Species of *Bacillus* present in fermented soybean foods are *B. subtilis, B. amyloliquefaciens, B. circulans, B. coagulans, B. firmus*, *B. licheniformis, B. megaterium, B. pumilus, B. natto* and *B. thuringiensis* (Kiers et al., 2000; Kubo et al., 2011).**
- **Some strains of *Bacillus subtilis* produce λ-polyglutamic acid (PGA) which is an amino acid polymer commonly present in Asian fermented soybean foods giving the characteristic sticky texture to the product (Urushibata et al. 2002).**

Micrococcaceae

- **Micrococcaceae are Gram-positive coccii, aerobic, non-sporeforing, non-motile and catalase-positive bacteria with irregular clusters or packets (Schleifer 1986).**
- **Several species of *Staphylococcus, Micrococcus, Kocuria* have been reported from fermented milk products, fermented sausages and meat and fish products such as (Wu et al. 2000, Martin et al. 2006, Cotton et al. 2010)**

Other Bacteria

- Species of *Bifidobacterium, Brachybacterium, Brevibacterium,* and *Propionibacterium* have been isolated from cheese and other fermented milks (Bourdichon et al. 2012).

- *Enterobacter cloacae*, *Klebsiella pneumoniae, K. pneumoniae* subsp. *ozaenae, Haloanaerobium, Halobacterium, Halococcus*, *Propionibacterium, Pseudomonas,* etc., are also present in many fermented foods (Tamang 2010b).

- Species of *Arthrobacter* and *Hafnia* are involved in meat fermentation (Bourdichon et al. 2012)

Yeasts

About 27 yeast genera with several species have been reported from fermented foods and beverages: ***Brettanomyces***, ***Candida, Cryptococcus, Debaryomyces, Dekkera***, ***Galactomyces, Geotrichum, Hansenula, Hanseniaspora, Hyphopichia, Issatchenkia***, ***Kazachstania***, ***Kluyveromyces, Metschnikowia, Pichia, Rhodotorula, Rhodosporidium, Saccharomyces, Saccharomycodes, Saccharomycopsis, Schizosaccharomyces, Sporobolomyces, Torulaspora***, ***Torulopsis, Trichosporon, Yarrowia*** and ***Zygosaccharomyces*** (Tamang and Fleet 2009, Watanabe et al. 2008, Kurtzman et al. 2011, Lv et al. 2013; Shah et al. 2017).

Major roles of Yeasts in Food Fermentations are (Tamang and Fleet 2009):

- Amylolytic and alcohol production in ethnic fermentation.
- Sugar fermentation
- Enzymatic activities: Lipolytic, proteolytic, pectinolytic, glycosidasic, urease.
- Production secondary metabolites and growth factors.
- Inhibitory effect against mycotoxin-producing moulds.

Mycelial Fungi

- Fungi in fermented foods are relatively limited.
- Some common genera of mycelial or filamentous fungi associated with fermented foods and beverages of the world are ***Actinomucor, Amylomyces, Aspergillus, Monascus, Mucor, Neurospora, Penicillium, Rhizopus, Ustilago, Fusarium, Lecanicillium, Scopulariopsis, Sperendonema*** (Hesseltine 1991, Samson 1993, Nout and Aidoo 2002).
- Functional properties of the fungi in fermented foods are (Aidoo and Nout, 2010).
  - Production of enzymes such as maltase, invertase, pectinase, α-amylase, ß-galactosidase, amyloglucosidase, cellulase, hemi-cellulase, acid and alkaline proteases, lipases.
  - Degradation of anti-nutritive factors thus improving bioavailability of minerals.

Gut Microflora

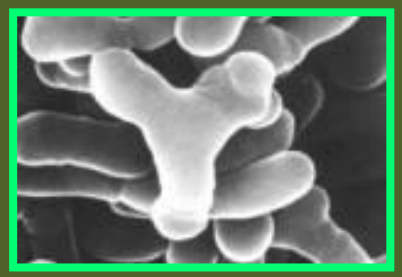
- Human gastrointestinal tract (GIT) houses over 10^{14} microbial cells with over 1,000- 1,500 diverse bacterial types, mostly in the colon (Lepage et al. 2012).
- Colonization of the gut is initiated before birth following ingestion of microbe containing amniotic fluid by the fetus (Aagaard et al. 2014).
- The composition and distribution of Gut microbiota (Purchiaroni et al. 2013) are →
  - in stomach (*Lb. reuteri, Lb. delbrueckii, Lb. gastricus, Lb. antri*),
  - In small intestine (*Lb. reuteri, Lb. bulgaricus, Lb. acidophilus, Enterococcus avium, Ent. dispar, Ent. durans, Ent. faecalis, Ent. faecium, Ent. flavescens, Ent. gallinarum, Ent. hirae, Ent. mundtii, Ent. raffinosus*),
  - in large intestine (*Ent. faecalis, Bacteroides*, *Bifidobacterium*, *Eubacterium*, *Peptococcurs*, *Clostridium*, *Lactobacillus*).

Gut Microbiota and their functions

Qin. et al. (2010)

Beneficial effects

- produce useful substances
- inhibit colonization of pathogens
- maintain integrity of epithelial layer
- enhance immune responses

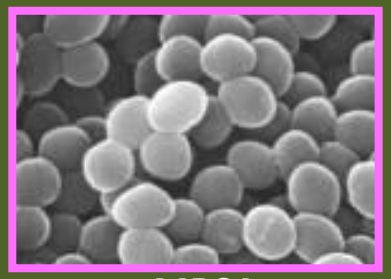


Bifidobacterium

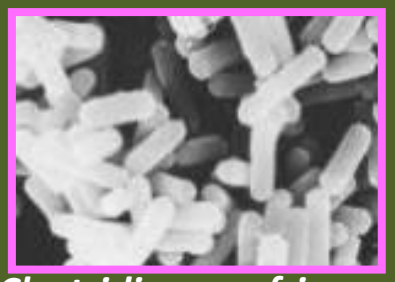
Lactobacillus

Enterobacteriaceae

Bacteroides



MRSA



Clostridium perfringens

Harmful effects

- produce noxious substances
- Invade the host
- disturb integrity of epithelial layer
- induce inflammatory responses

Intestinal microbiota

Types of Global Fermented Foods

Based on substrates, fermented foods (Tamang, 2010):

(1) Fermented Vegetable (LAB)

(2) Fermented Legume (Bacilli, Mould, LAB)

(3) Fermented Cereal (LAB + Yeasts)

(4) Fermented Milk (LAB, Yeasts)

(5) Fermented Fish (Micrococci, LAB, others)

(6) Fermented Meat (Micrococci, LAB, others)

(7) Fermented Root (LAB, Yeasts)

(8) Asian amylolytic starters (Mould + Yeasts + LAB)

(9) Fermented Beverage (Yeasts, LAB)

(10)Miscellaneous Fermented Products (vinegar, *miang, cacao, pidan*) (Acetic acid bacteria, LAB, non-LAB)

Besides edible fermented foods, Silage fermentation, unique fermented fodder fed to animals. (LAB).

Fermented Vegetables

- Most widely studied global fermented vegetable products are *kimchi* of Korea (Chang et al. 2008; Nam et al. 2009), *gundruk,* and *sinki* of India and Nepal, (Tamang 2010), *pao cai* of China (Lu 2010).
- Fermentation of vegetable is mostly dominated by species of *Lactobacillus* and *Pediococcus*, followed by *Leuconostoc, Weisella, Tetragenococcus*, *Lactococcus*.
- Species of LAB have the antimicrobial activities including bacteriocins and nisin production (Tamang et al. 2009).

Fermented Vegetables

Gundruk **गुन्द्रुक of India, Nepal and Bhutan**

Kimchi **김치 of Korea**

Suau cai **酸菜 of China**

Sauerkraut **of Germany**

Fermented bamboo shoot products of India

Microorganisms:

Lb. plantarum, Lb. brevis, Lb. coryniformis, Lb. delbrueckii, Lb. curvatus, Leuc. fallax, Leuc. lactis, Leuc. mesenteroides, Leuc. Citreum, P. pentisaceus, Tetragenococcus halophilus, Enetrococcus durans, Bacillus subtilis, B. licheniformis, B. coagulans.

Ref: Tamang *et al.* (2008). *International J Food Microbiology* 121: 35-40.

Ref: Tamang and Tamang (2009). *Food Biotechnology* 23: 133-147.

Tamang *et al*. (2009). **Functional properties of lactic acid bacteria isolated from ethnic fermented vegetables of the Himalayas.** ***International J Food Microbiology*** **135: 28-33.**

Abstract

A total of 94 strains of Lactic acid bacteria (LAB), previously isolated from ethnic fermented vegetables and tender bamboo shoots of the Himalayas, were screened for functional properties such as acidification capacity, enzymatic activities, degradation of antinutritive factors and oligosaccharides, production of biogenic amines, hydrophobicity and adherence to mucus secreting HT29 MTX cells. **Strong acidification and coagulation activities** of LAB strains were recorded. Most of the LAB strains showed antimicrobial activities against the used indicator strains; however, only *Lb. plantarum* IB2 (BFE 948) isolated from inziangsang, a fermented leafy vegetable product, **produced a bacteriocin** against *Staphylococcus aureus* S1. LAB strains **showed enzymatic activities** and also **degraded oligosaccharides**. Almost all the strains of LAB were **non-producers of biogenic amines** except few strains. Some strains of *Lb. plantarum* showed more than **70 % hydrophobicity**. Adherence to the **mucus secreting HT29 MTX cells** was also shown by strains indicating their probiotic nature.

Fermented Soybeans

Fermented soybeans are of two types:

- **Bacteria-fermented Sticky and non-salted product by *Bacillus* spp. (mostly *B. subtilis*):**

  ***natto* of Japan, *kinema* of India, Nepal and Bhutan, *thua nao* of Thailand, *chungkokjang* of Korea, *sieng* of Laos, *pe poke* of Myanmar, etc. (Tamang et al. 2016).**

- **Mold-fermented soybeans which are mostly fermented by moulds (spp. of *Rhizopus, or Aspergillus*):**

  ***doenjang* of Korea, *tempe* of Indonesia, *sufu* of China, *miso* and *shoyu* of Japan, *daouchi* of China, etc.**

Cultivation of soybeans in the Himalayas

Chinese soybeans 大豆 (***Dàdòu***)

Indian soybeans भटमास

Bacillus-fermented Soybean Foods of India (Tamang 2015)

Kinema of Nepal, Sikkim and Darjeeling

Hawaijar of Manipur

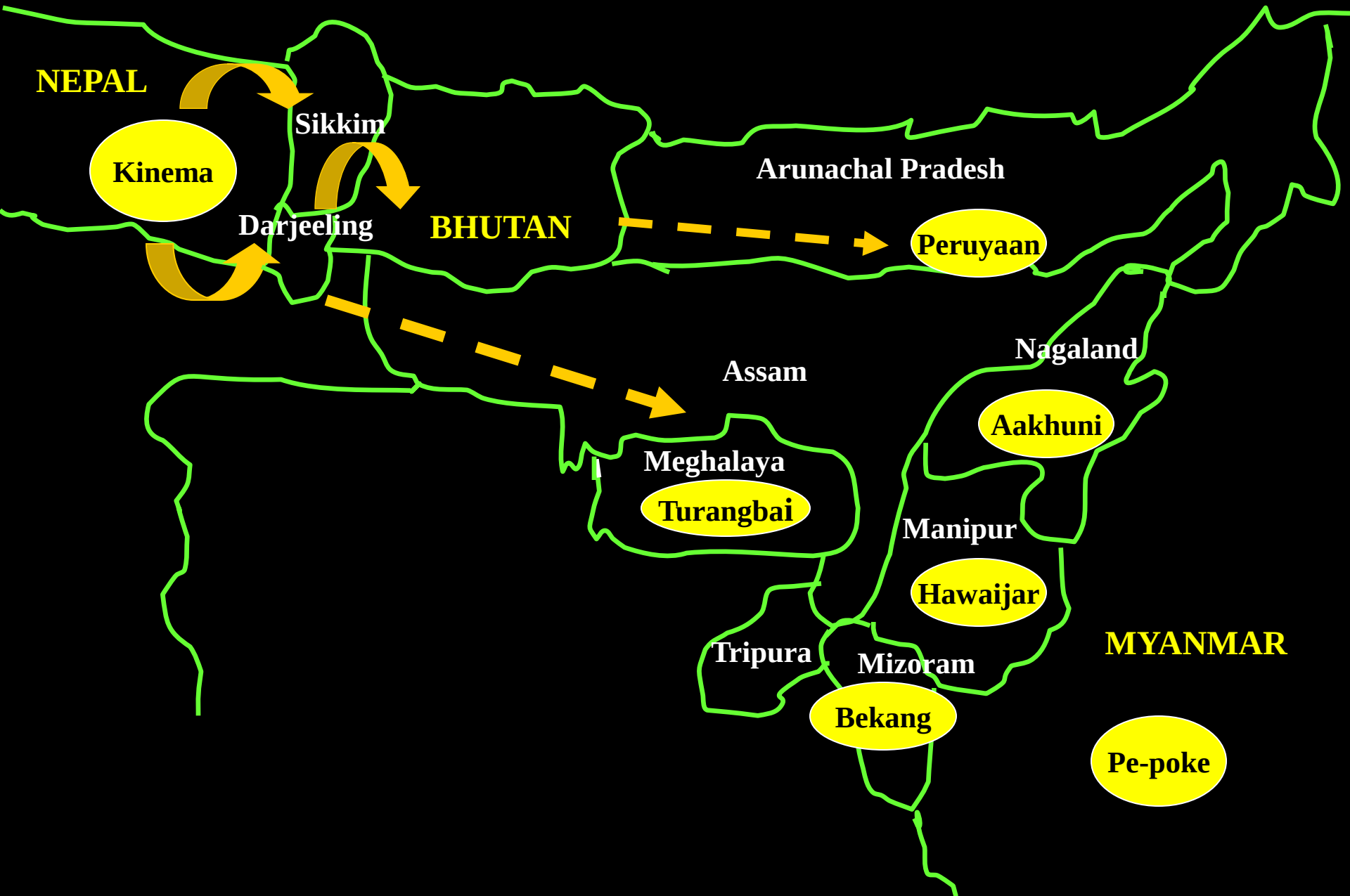
Tungrymbai of Meghalaya

Bekang of Mizoram

Peruyaan of Arunachal Pradesh

Aakhone of Nagaland

Diversity of Fermented Soybeans in the EH



Soybeans

Soaked

Cooked

Lightly cracked

Ash added

Wrapped

Fermented

Kinema

Microorganisms:

Bacilli- *Bacillus subtilis* (functional bacterium); LAB- *Enterococcus faecium;* Yeasts- *Candida parapsilosis* and *Geotrichum candidum* (Tamang 1992, Sarkar et al. 1994).

frontiers in Microbiology

ORIGINAL RESEARCH
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Poly-γ-Glutamic Acid (PGA)-Producing *Bacillus* Species Isolated from *Kinema*, Indian Fermented Soybean Food

*Rajen Chettri, Meera O. Bhutia and Jyoti P. Tamang**

Department of Microbiology, School of Life Sciences, Sikkim University, Gangtok, India

Kinema, an ethnic fermented, non-salted and sticky soybean food is consumed in the eastern part of India. The stickiness is one of the best qualities of good *kinema* preferred by consumers, which is due to the production of poly-γ-glutamic acid (PGA). Average load of *Bacillus* in *kinema* was 10^7 cfu/g and of lactic acid bacteria was 10^3 cfu/g. *Bacillus* spp. were screened for PGA-production and isolates of lactic acid bacteria were also tested for degradation of PGA. Only *Bacillus* produced PGA, none of lactic acid bacteria produced PGA. PGA-producing *Bacillus* spp. were identified by phenotypic characterization and also by 16S rRNA gene sequencing as *Bacillus subtilis*, *B. licheniformis* and *B. sonorensis*.

Keywords: *Kinema*, *Bacillus*, fermented soybean, poly-glutamic acid

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Edited by:
Andrea Gomez-Zavaglia, Center for Research and Development in Food Cryotechnology, Argentina

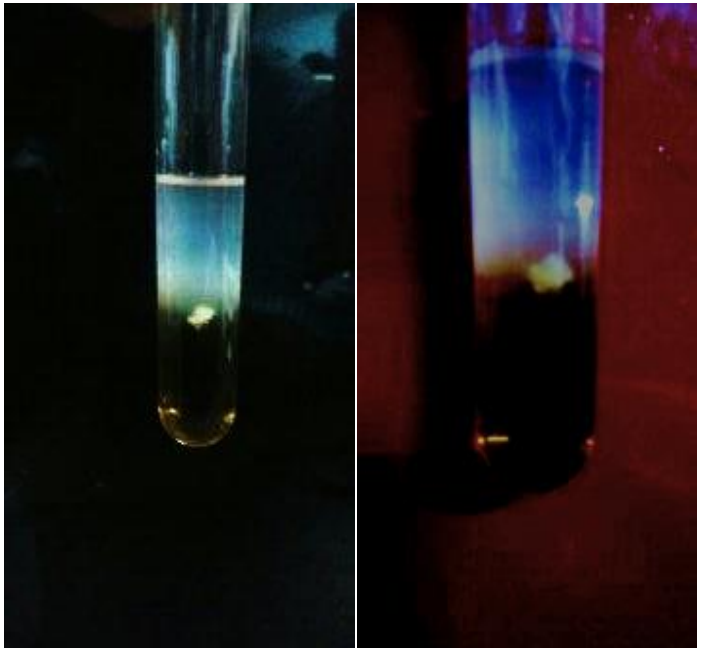

Fig 1.

Clumping of insoluble material presumbly PGA biopolymer produced by *B. subtilis* KAS:B5 after addition of ethanol into PGA medium.

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journal homepage: www.elsevier.com/locate/ijfoodmicro

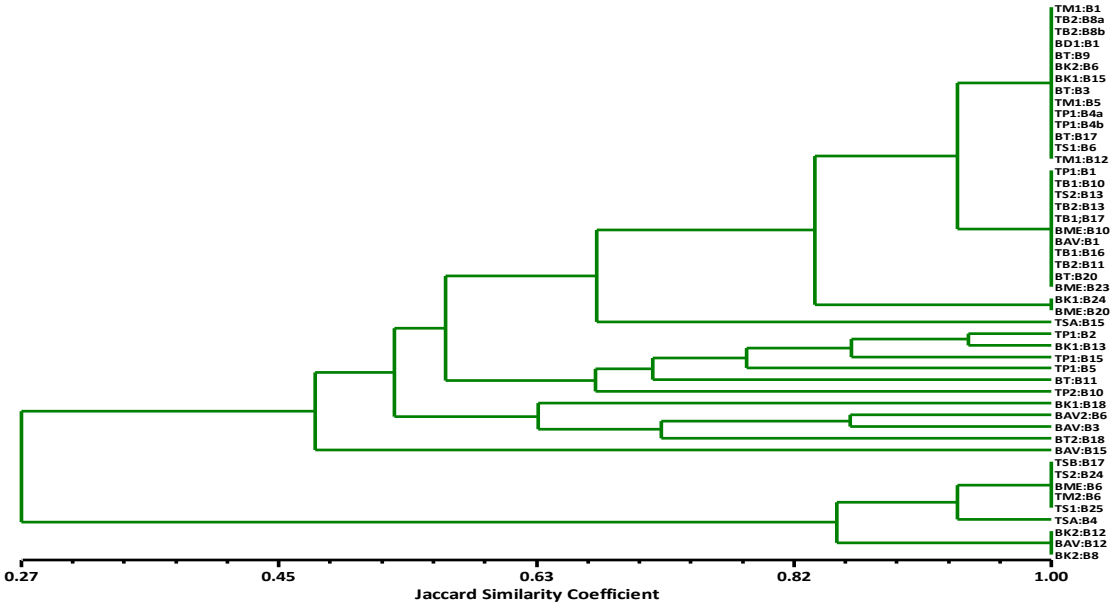
Short communication

Bacillus species isolated from *tungrymbai* and *bekang*, naturally fermented soybean foods of India

Rajen Chettri, Jyoti Prakash Tamang *

***Tungrymbai* of Meghalaya**

***Bekang* of Mizoram**



- Dendogram of combined ARDRA and ITS profiles of *Bacillus* from ***Tungrymbai***: *Bacillus licheniformis* (25.5%), *B. pumilus* (19.5%) and *B. subtilis* (55%).
- ***Bekang***: *B. brevis* (2%), *B. circulans* (7.5%), *B. coagulans* (6.5%), *B. licheniformis* (16.5%), *B. pumilus* (9.1%), *B. sphaericus* (4.6%), *B. subtilis* (51.8%), *Lysinibacillus fusiformis* (2%).

Bacillus-fermented sticky soybean foods of Asia

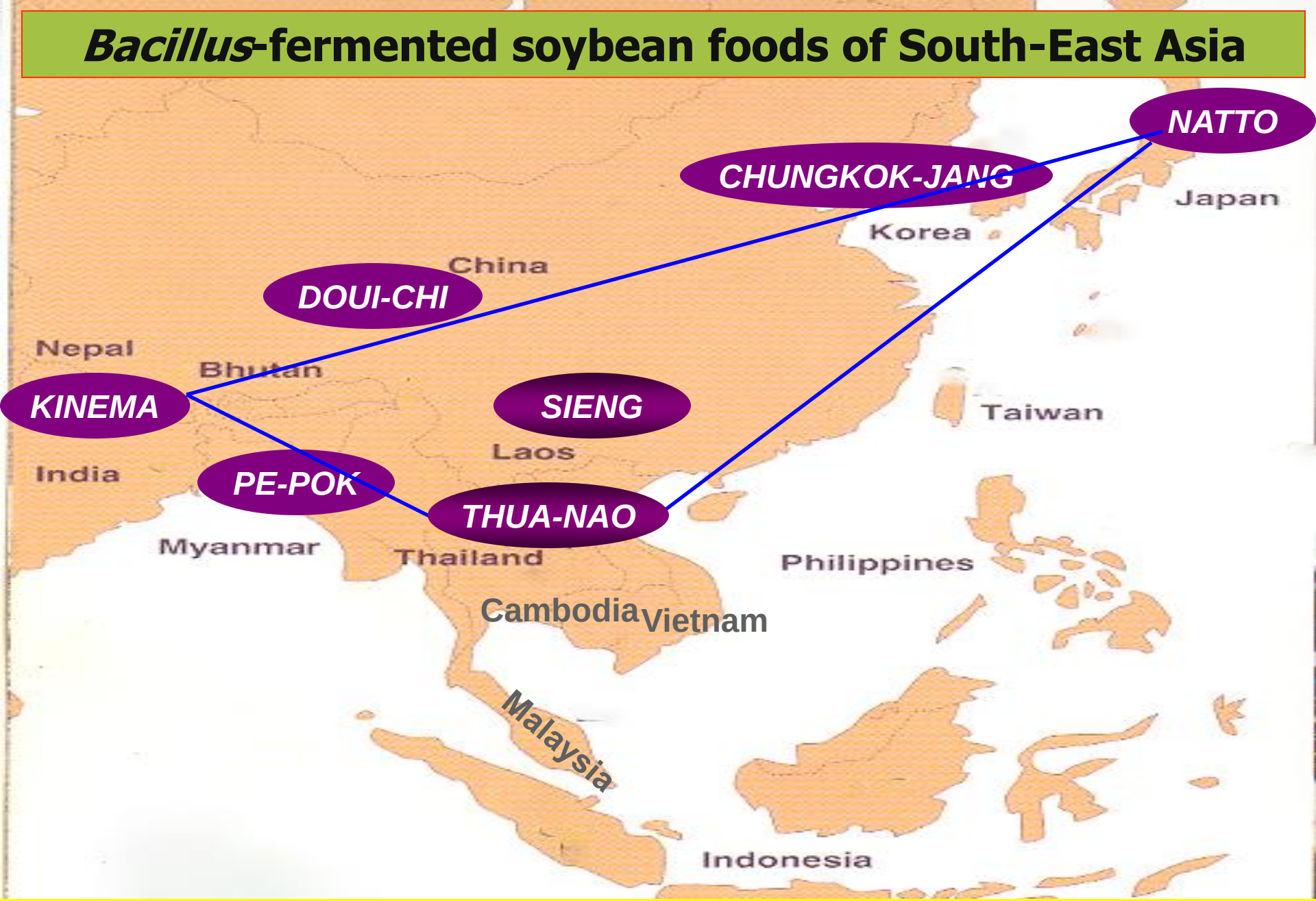
Douchi 豆豉 of China

Thua nao of Thailand

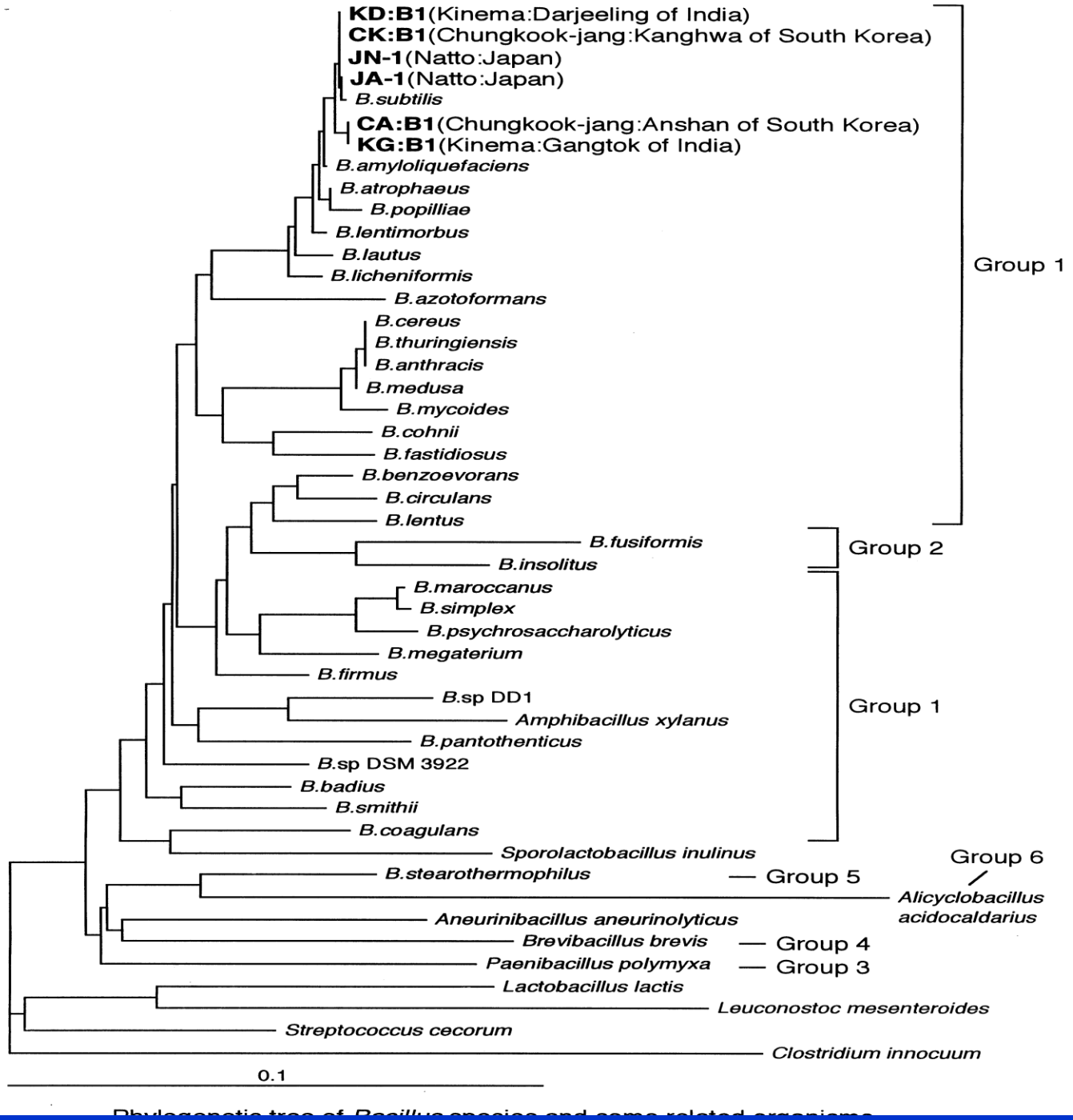
Natto of Japan

Chungkok-jang of Korea

Bacillus-fermented soybean foods of South-East Asia



Kinema-Natto-Thua nao (KNT)-Triangle proposed by Tamang (2010)



Phylogenetic tree of *Bacillus* species and some related organisms

Ref: Tamang *et al.* 2002

Mould-fermented soybean products of Asia

***Shoyu* 醤油 of Japan**

***Tempe* of Indonesia**

Soy-sauce 醤油 (Jiàngyóu) **of China**

***Deonjang* of Korea**

Miso of Japan

Miso soup

Douchi of China

Sufu of China

Asian Fermented Soybeans

- Fermented soybeans are traditionally prepared and consumed exclusively by the Mongolian races of Asian courtiers of China, Taiwan, Japan, Korea, Myanmar, Laos, Thailand, Cambodia, and Vietnam, Bhutan, Nepal and Northeast regions of India, Indonesia, Malaysia, Singapore and Philippines (Tamang and Samuel 2010), may be due to development of typical flavor called *umami* 鮮味 (Kawamura and Kara 1987)
- No such ethnic fermented soybean foods have been recorded in other continents.
- In Africa, *dawadawa/iru/sambala*, a traditional locust bean fermented legume products are prepared and conusmed.

Dawadawa

Primary Benefits of Soybean Fermentation

1. Improvement of flavour and aroma
2. Bio-enrichment of nutritional value (vitamin)
3. Improved digestibility
4. Degradation of anti-nutritive factors
5. Improvement in bio-availability of minerals

6. Low-fat and low-cholesterol food
7. Anti-allergy
8. Antioxidant activities
9. Therapeutic values: prevention of osteoporosis, heart disease
10. Low-cost high plant protein food

Tamang (2015). Health Benefits of Fermented Foods, CRC Press, New York

Fermented Cereals

- Common fermented cereal foods of Europe and Americ are *sourdough*, loaves: *nan*, *idli* and *dosa* of Asia, *ogi*, *pito* of Africa; *pozol* of Mexico (Tamang et al. 2016).

- Yeasts are responsible for the leavening process in sourdough while the LAB determine the souring of the dough (de Yuyst et al. 2009).

Sourdough

Nan of India

Dosa of India and Sri Lanka

Idli of India

Selroti of India, Nepal and Bhutan

Jalebi of India, Nepal, Pakistan

Ogi of Nigeria

Mawé of Benin

Indian *Nan* in Japan - very popular

Indian *Nan* in China - popular now

Fermented Milk Products

Yoghurt

Dahi of India, Bangladesh, Nepal

Cheese

Camembert of France

Fermented Milk Products

Chhurpi (hard and soft) of India, China (Tibet), Nepal, Bhutan

Chhurpi curry

Kefir and Koumiss of Russia

Nunu of Ghana

www.nature.com/scientificreports

OPEN

5 June 2017
4 January 2018
online: 24 January 2018

Bacterial community in naturally fermented milk products of Arunachal Pradesh and Sikkim of India analysed by high-throughput amplicon sequencing

H. Nakibapher Jones Shangpliang[1], Ranjita Rai[1], Santosh Keisam[2], Kumaraswamy Jeyaram[2] & Jyoti Prakash Tamang[1]

Shangpliang et al....Tamang 2018
***Scientific Reports* 8: 1532**

Figure: (a) *Chhurpi* of Arunachal Pradesh (AP); (b) *Chhurpi* of Sikkim; (c) *Churkam* of AP; (d) *Dahi* of Sikkim; (e) *Gheu* of Sikkim; (f) *Mar* of AP.

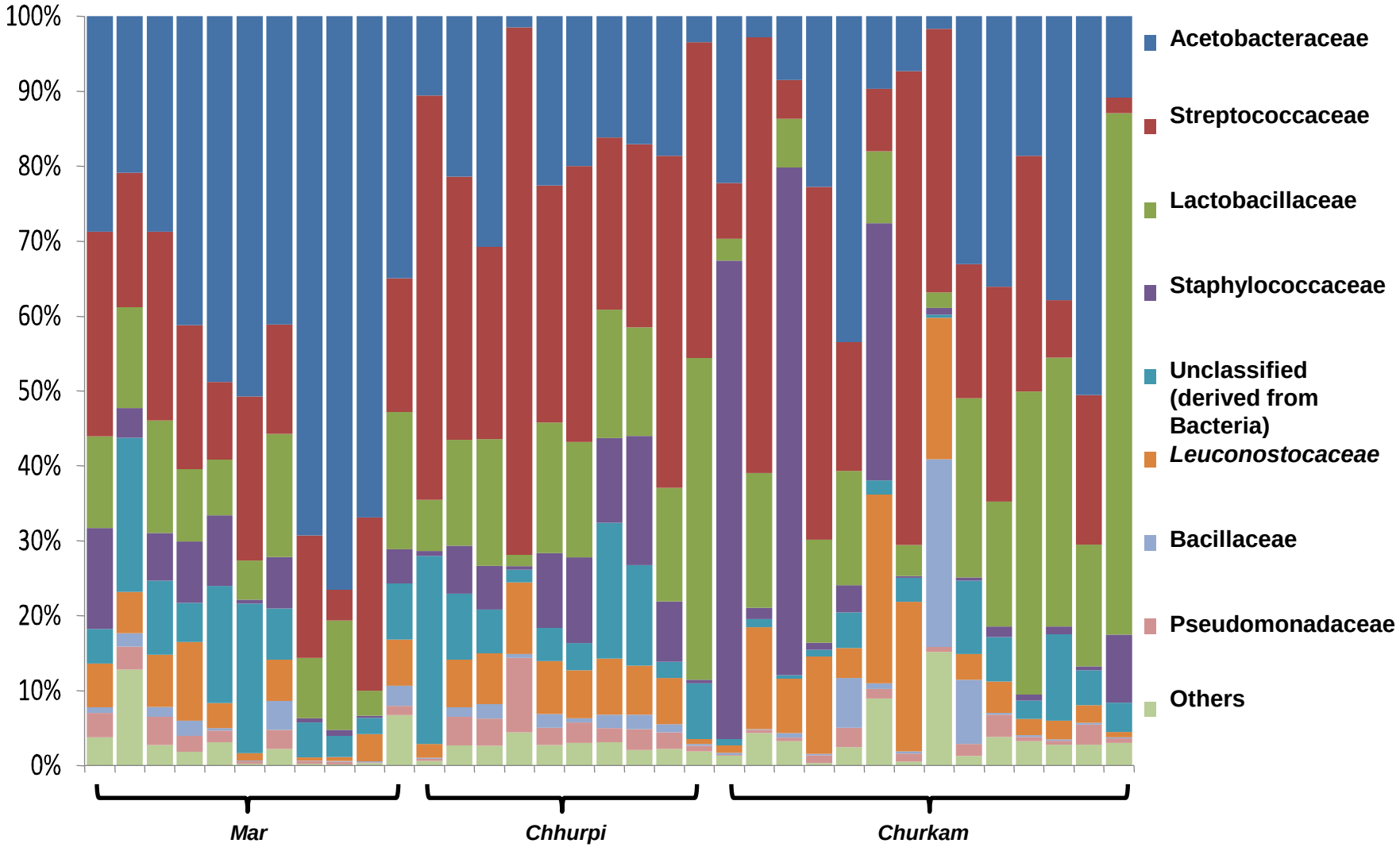


Figure 2: Relative abundance of the eubacterial families present in the NFM of Arunachal Pradesh

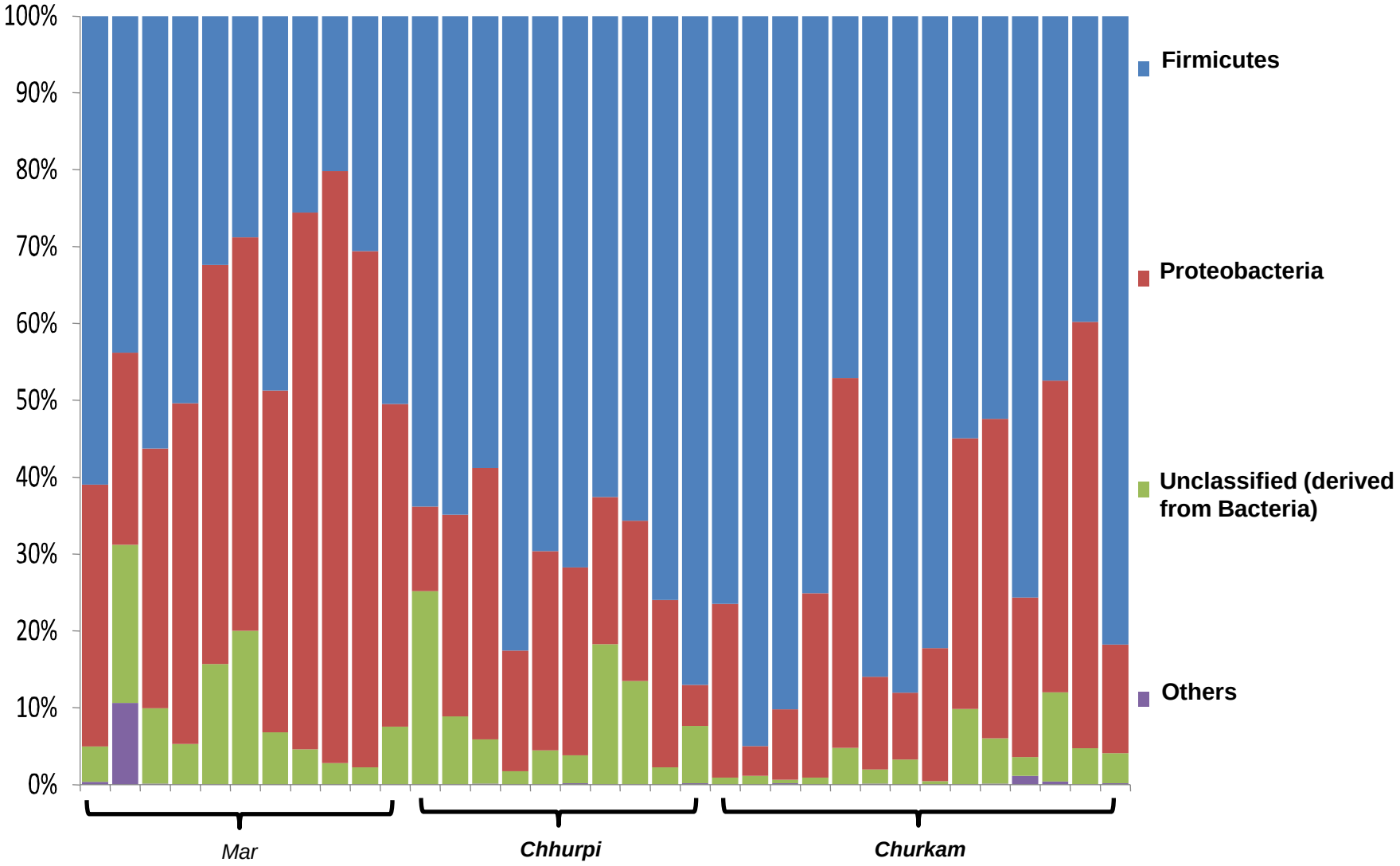
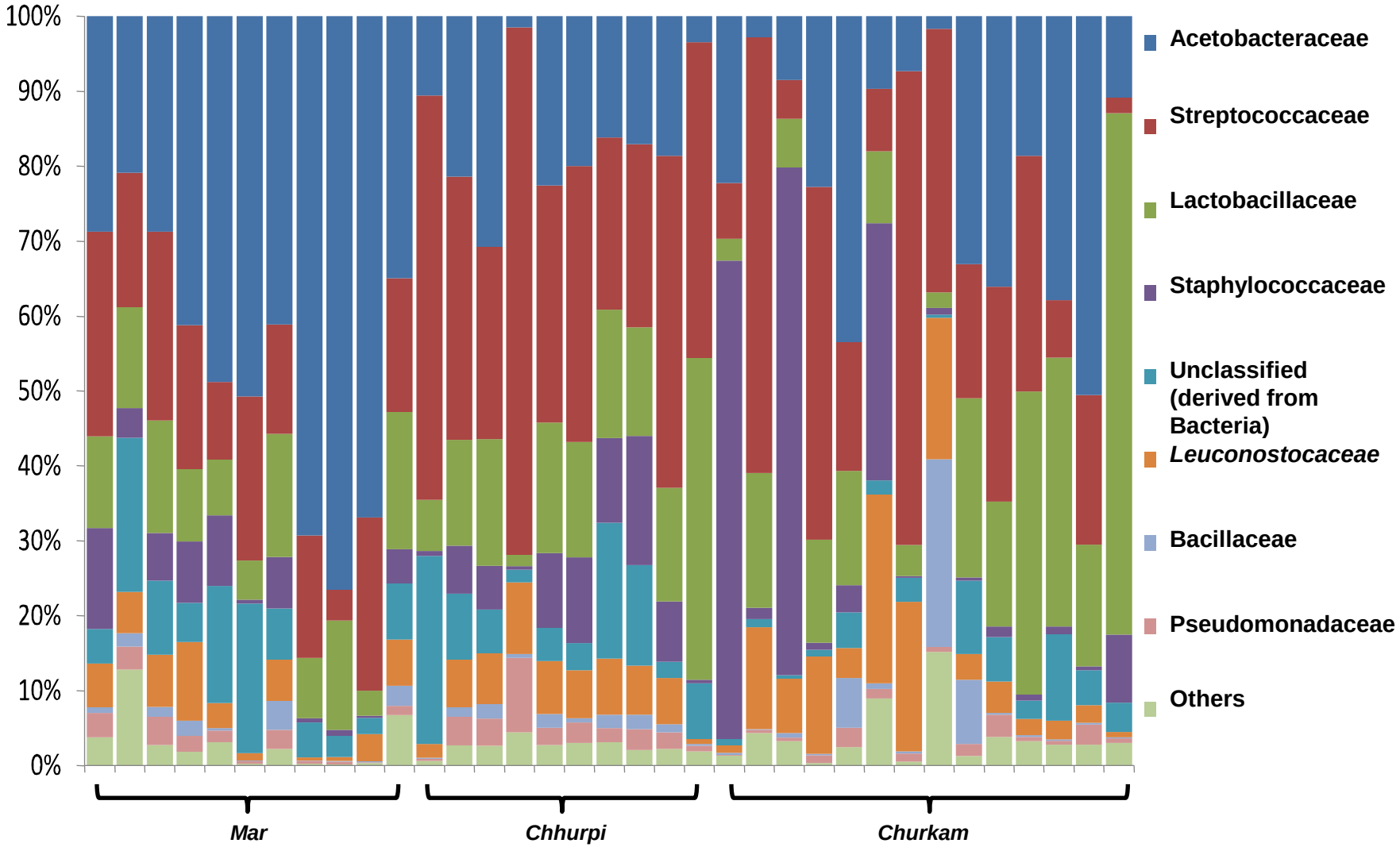


Figure 1: Relative abundance of the eubacterial phyla present in the NFM of Arunachal Pradesh based on high-throughput illumina amplicon sequencing (Shangpliang et al….Tamang 2018 *Scientific Reports*)



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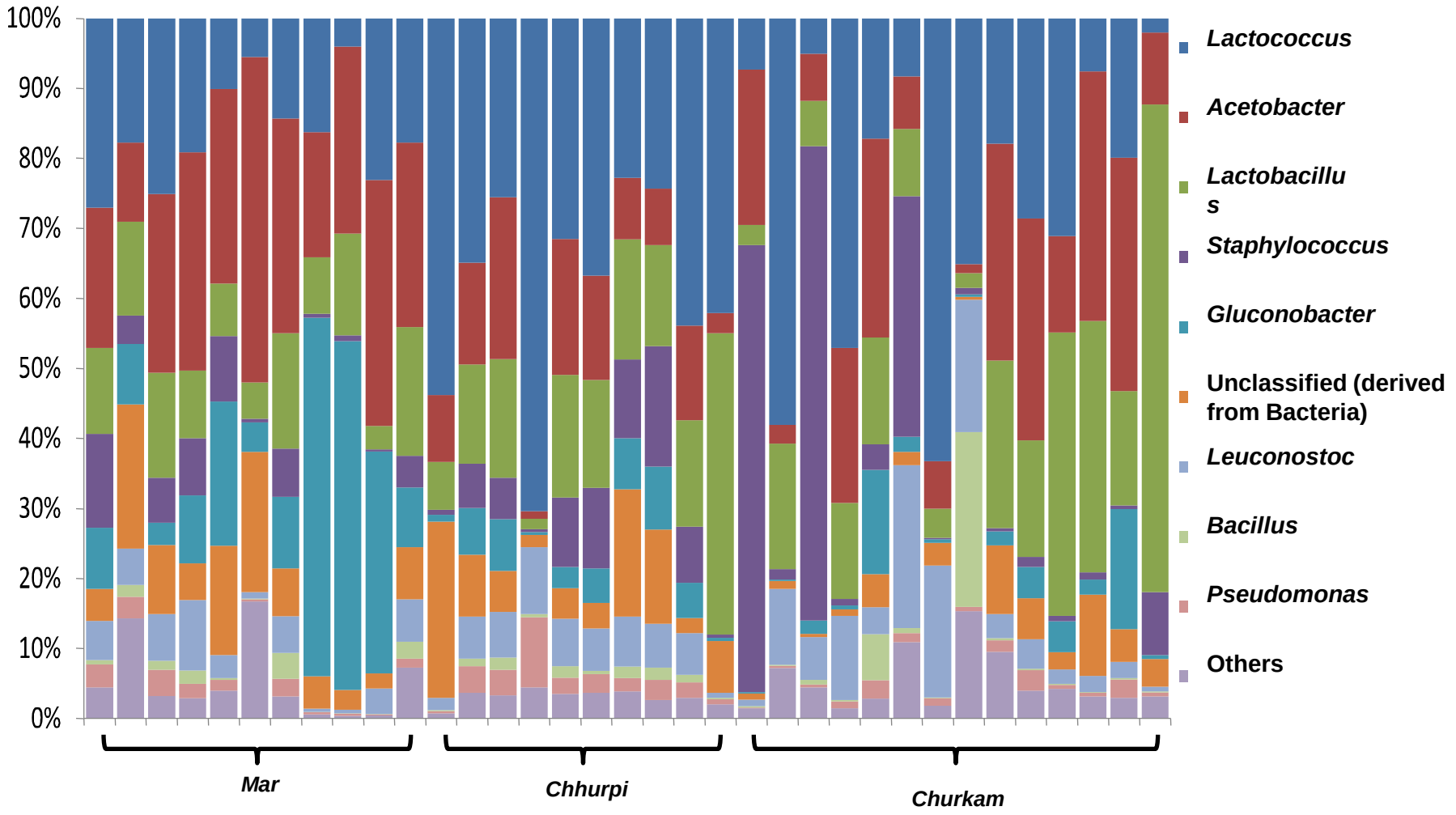


Figure 3: Relative abundance of the eubacterial genera present in the NFM of Arunachal Pradesh

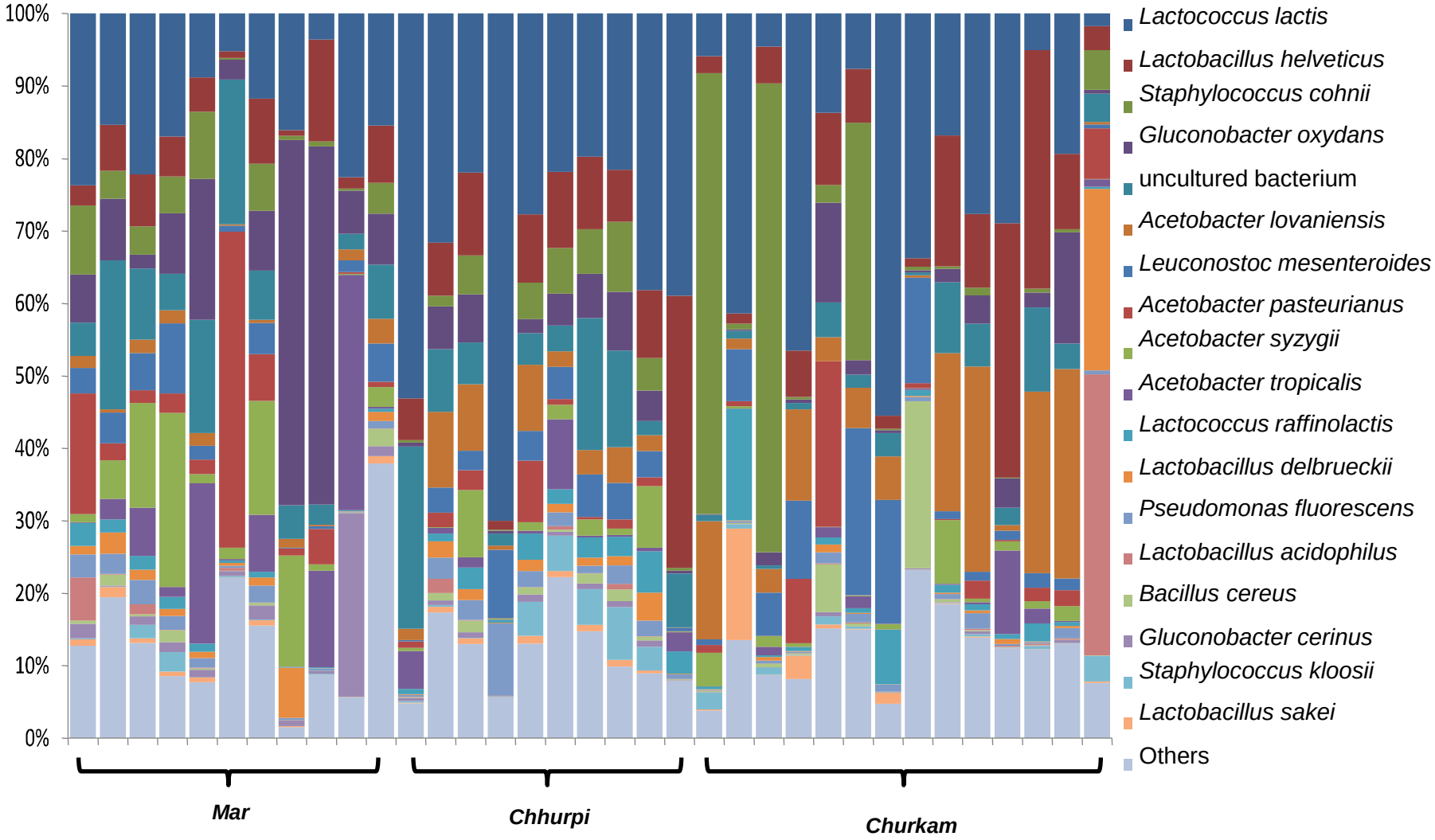
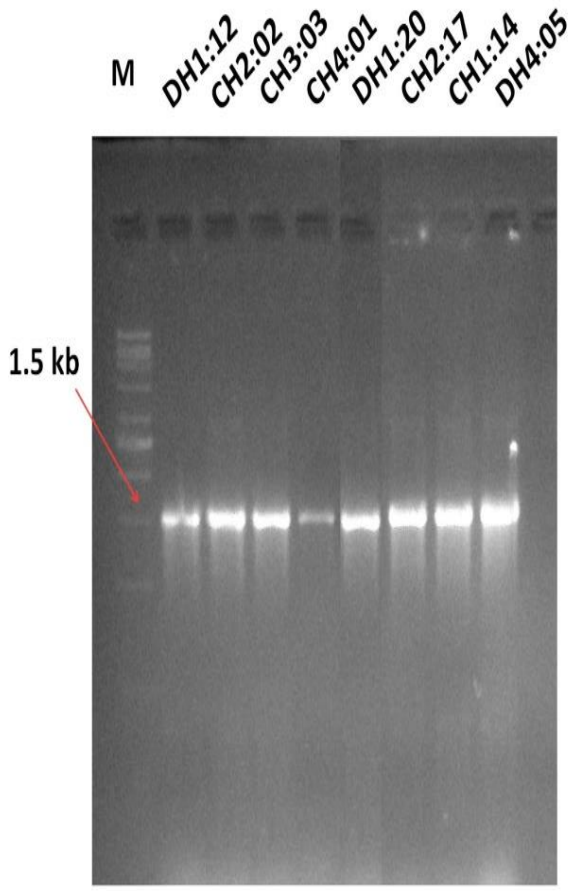


Figure 4: Relative abundance of the eubacterial species present in the NFM of Arunachal Pradesh

Nturally Fermented Milk Products of Bhutan

Figure : (a) - A person is churning milk; (b) - *thaki*; (c) – *datshi*, (d) – *gheu/mar* (e) - *dahi*; (f) – *churkam*.



frontiers in Microbiology

ORIGINAL RESEARCH
published: 01 February 2017
doi: 10.3389/fmicb.2017.00116

Check for updates

Some Technological Properties of Lactic Acid Bacteria Isolated from *Dahi* and *Datshi*, Naturally Fermented Milk Products of Bhutan

*H. N. J. Shangpliang, Sharmila Sharma, Ranjita Rai and Jyoti P. Tamang**

PCR amplification of 16S rDNA of LAB isolates from NFM of Bhutan

- **Based on 16S rRNA gene sequencing isolates of LAB from *dahi* and *datshi* were identified as *Enterococcus faecalis, E. faecium, Lactococcus lactis* subsp*. lactis.***

- **Being the first study of microbiological analysis of the NFM of Bhutan.**

Fermented Fish Products

Two categories of ethnic fermented fish products:

(1) Fish-salt formulations: *ngari, tingtap* and *sidal* of India, fish suce of Thailand

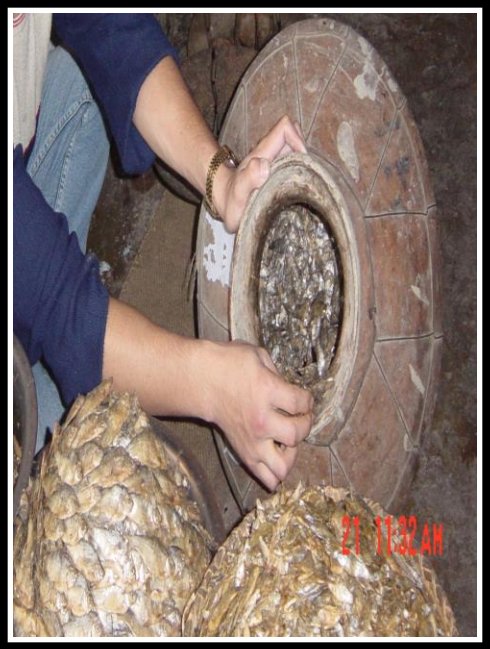
(2)Fish-salt-carbohydrate mixtures: *pla-ra* of Thailand, *burong isda* of Philippines.

- **Thapa, N. (2016). Book: *Microbiology and Nutrition of Ethnic Fermented and Preserved Fish Products of the Eastern Himalayas*. Published by Today and Tomorrow's Printers & Publishers, New Delhi, 116 pages. ISBN: 81-7019-538-8.**

- **Thapa, N. (2016). Ethnic fermented and preserved fish products of India and Nepal. *Journal of Ethnic Foods* (Elsevier) 3: 69-77.**

- **Salampessy, J., Kailasapathy, K. and Thapa, N. (2010). Chapter 10: Fermented fish products. In: *Fermented Foods and Beverages of the World*. (Eds: Tamang, J.P. and Kailasapathy, K). CRC Press, Taylor & Francis Group, New York, pp. 289-307**

Fermented Fish Products



Ngari of India

Sidali of India, Bangladesh

Tungtap of India

Plara of Thailand

Burang isda of Phillippines

Thai Fish sauce

Fish

↓

Sun dried for 5-7 days

↓

Washed

↓

Spread on a bamboo tray to dry

↓

Kept in a closed earthen pot

↓

Pressed tightly by feet, sealed the pot

↓

Fermented at room temperature for 4-6 months

↓

NGARI

Microorganisms:

Lc. lactis subsp. cremoris, Lc. plantarum, E. faecium, Lb. fructosus, Lb. amylophilus, Lb. corynifomis subsp. torquens and Lb. plantarum; B. subtilis, B. pumilus, Micrococcus; **Yeasts-** ***Candida and Saccharomycopsis*****.**

Ref: Thapa *et al.* 2004. *World J Microbiology and Biotechnology* 20: 599-607.

Fermented and Smoked Meat Products

Fermented meat products are two categories:

- those made from whole meat pieces or slices such as dried meat and jerky;
- those made by chopping or comminuting the meat, usually called sausages (Adams, 2010).

Well documented -fermented sausages (Lücke, 2015), *salami* (Toldra, 2007) of Europe, jerky of America and Africa (Baruzzi et al., 2006), *nham* of Thailand (Chokesajjawatee et al., 2009), and *nem chua* of Vietnam (Nguyen et al., 2013).

American and European sausages

Salami

Traditional Sausages of Asia

Kargyong **of India and China (Tibet)**

Gemma **of India and Nepal**

Chinese sausages

Nham, **fermented pork sausages of Thailand**

Amylolytic Mixed Starters of Asia (coexistence of consortia of filamentous molds, amylolytic and alcohol producing yeast and bacteria for prepration of cereal-based alcholic beverages)

Marcha of India and Nepal

Yao qu of China

Nuruk of Korea

Men of Vietnam

Ragi of Indonesia

Loogpang of Thailand

Ethnic Amylolytic Mixed Starters of South-East Asia

Rice

↓

Soaked in water (6-8 h)

↓

Crushed in foot-driven heavy wooden mortar

↓

← Mixed with herbs, spices and old *marcha*

↓

Made into paste, kneaded into flat cakes

↓

Wrapped in ferns, covered by jute bags

↓

Fermented (25-35° C, 1-3 days)

↓

Sun dried (2-3 days)

↓

MARCHA

Flow sheet of Traditional *Marcha* preparation in Sikkim

Amylolytic starter culture of the Eastern Himalayas and North East India- (A): *Marcha* of Sikkim and the Darjeeling hills; (B): *Marcha* of Bhutan; (C): *Marcha* of Nepal; (D): *Pho* of Bhutan; (E): *Emao/humao* of Assam; (F): *Xaaz pitha* of Assam; (G): *Modor pitha* of Assam; (H): *Hamei* of Manipur; (I): *Thiat* of Meghalaya; (J): *Chowan* of Tripura; (K): *Kherie/Khekhrii* of Nagaland; (L): *Pee* of Arunachal Pradesh; (M): *Phut* of Arunachal Pradesh; (N): *Paa* of Arunachal Pradesh; (O): *Phab* of Arunachal Pradesh; (P): *Dawdim* of Mizoram.

SCIENTIFIC REPORTS

OPEN

Analysis of bacterial and fungal communities in *Marcha* and *Thiat*, traditionally prepared amylolytic starters of India

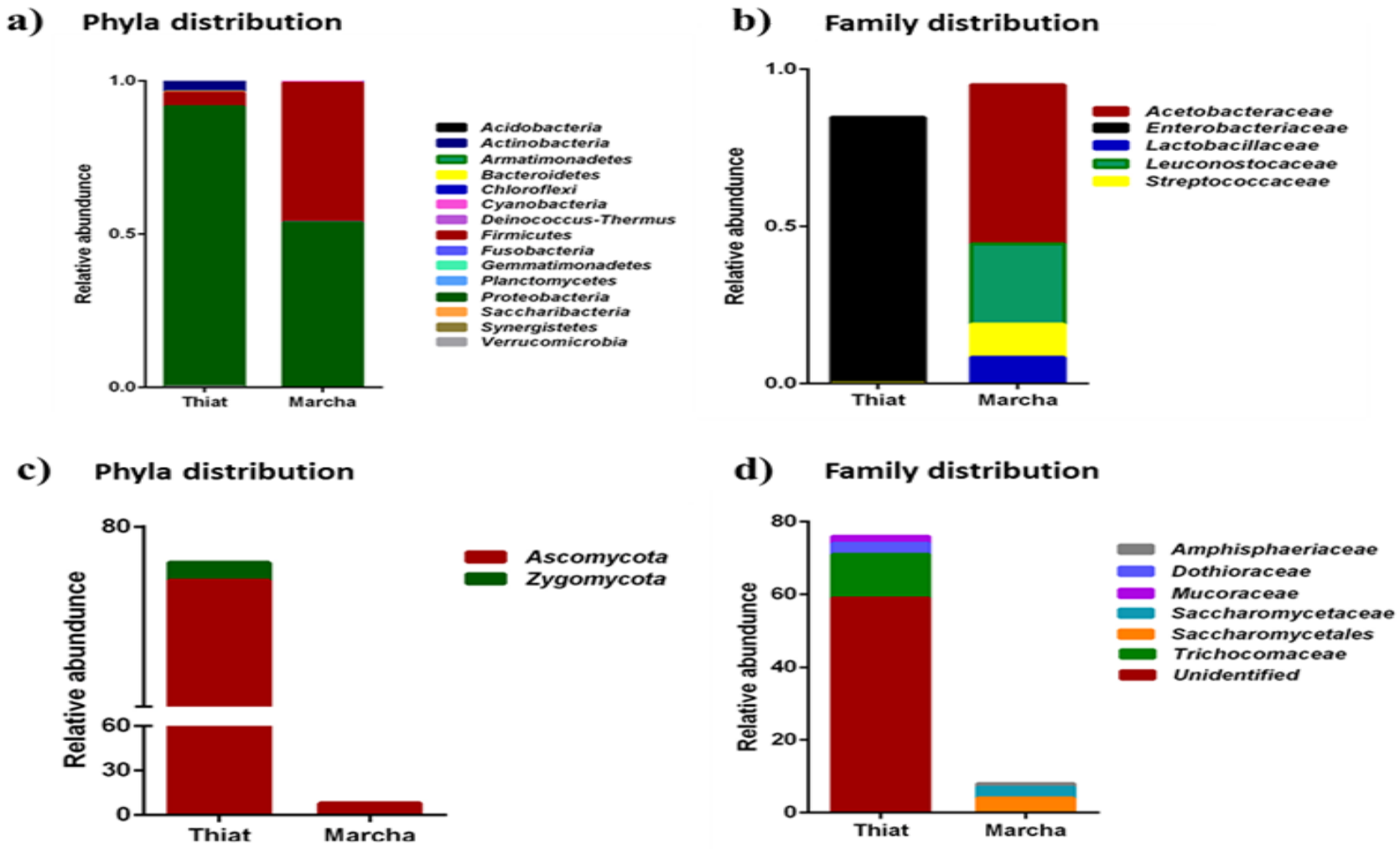
Received: 24 May 2017
Accepted: 29 August 2017
Published online: 08 September 2017

Shankar Prasad Sha[1], Kunal Jani[2], Avinash Sharma[2], Anu Anupma[1], Pooja Pradhan[1], Yogesh Shouche[2] & Jyoti Prakash Tamang[1]

Sha et al…Tamang 2017. *Scientific Reports* 7: 10967

***Marcha* of Sikkim and the Darjeeling hills**

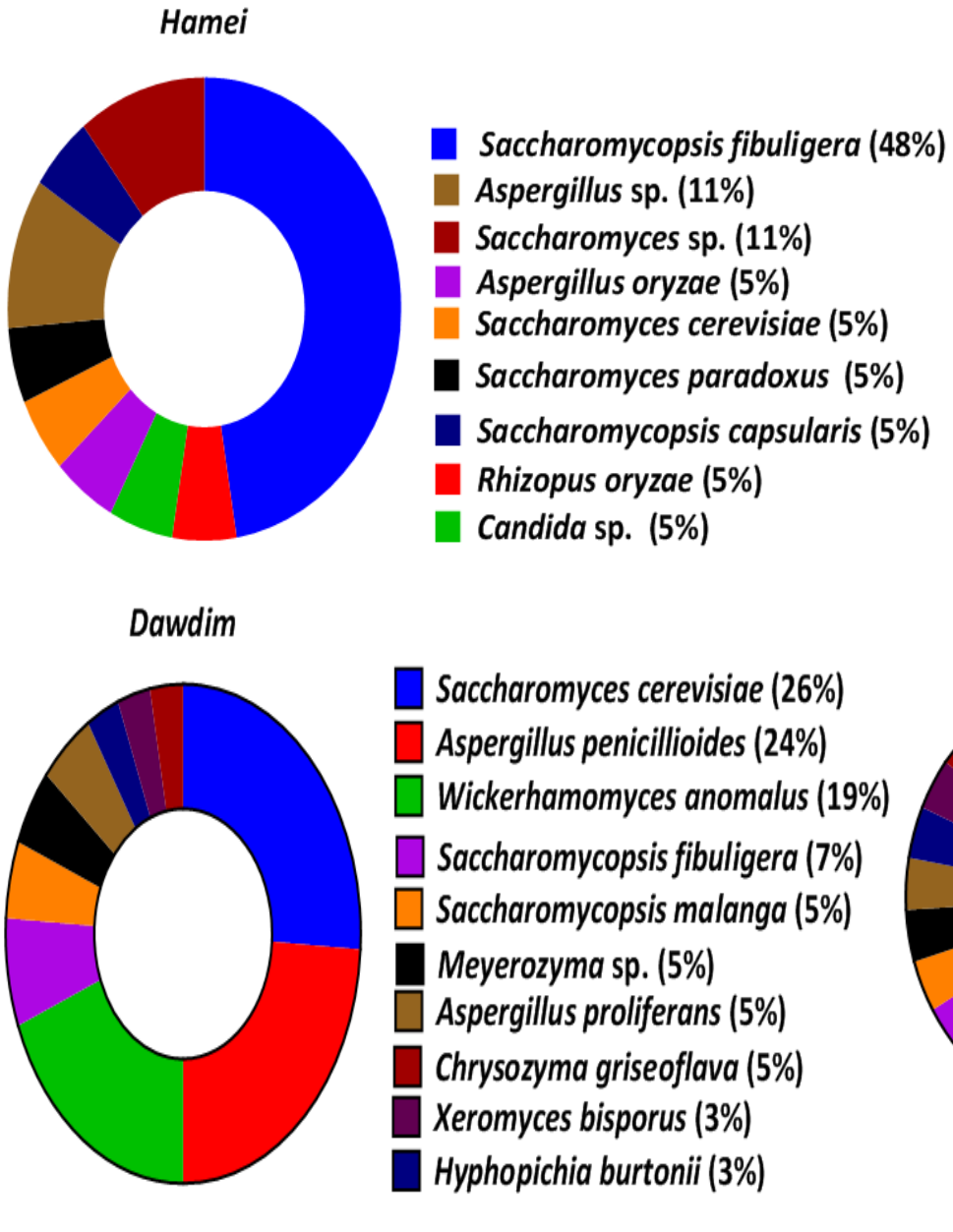
***Thiat* of Meghalaya**



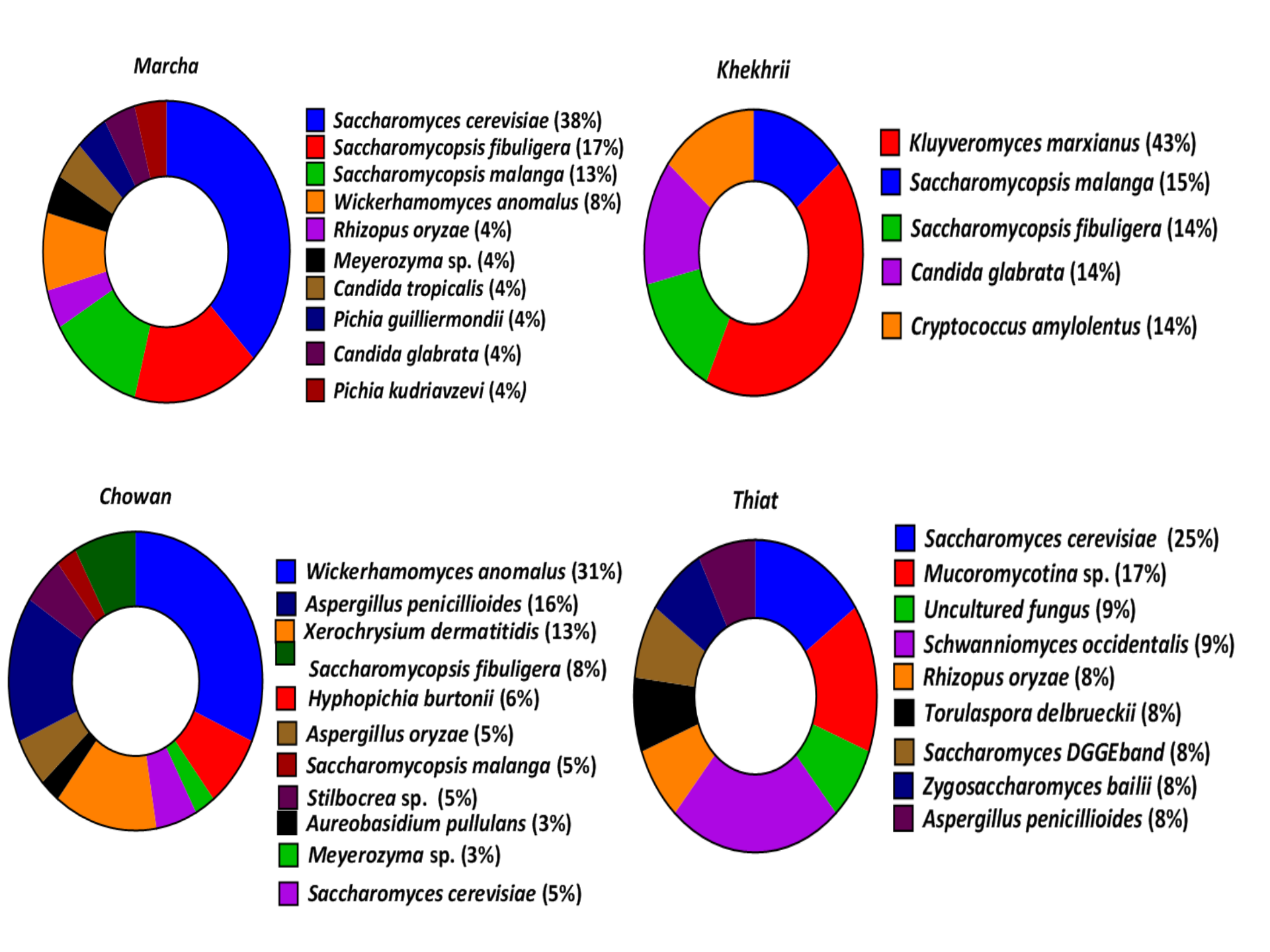
NGS: Amplicon sequencing method

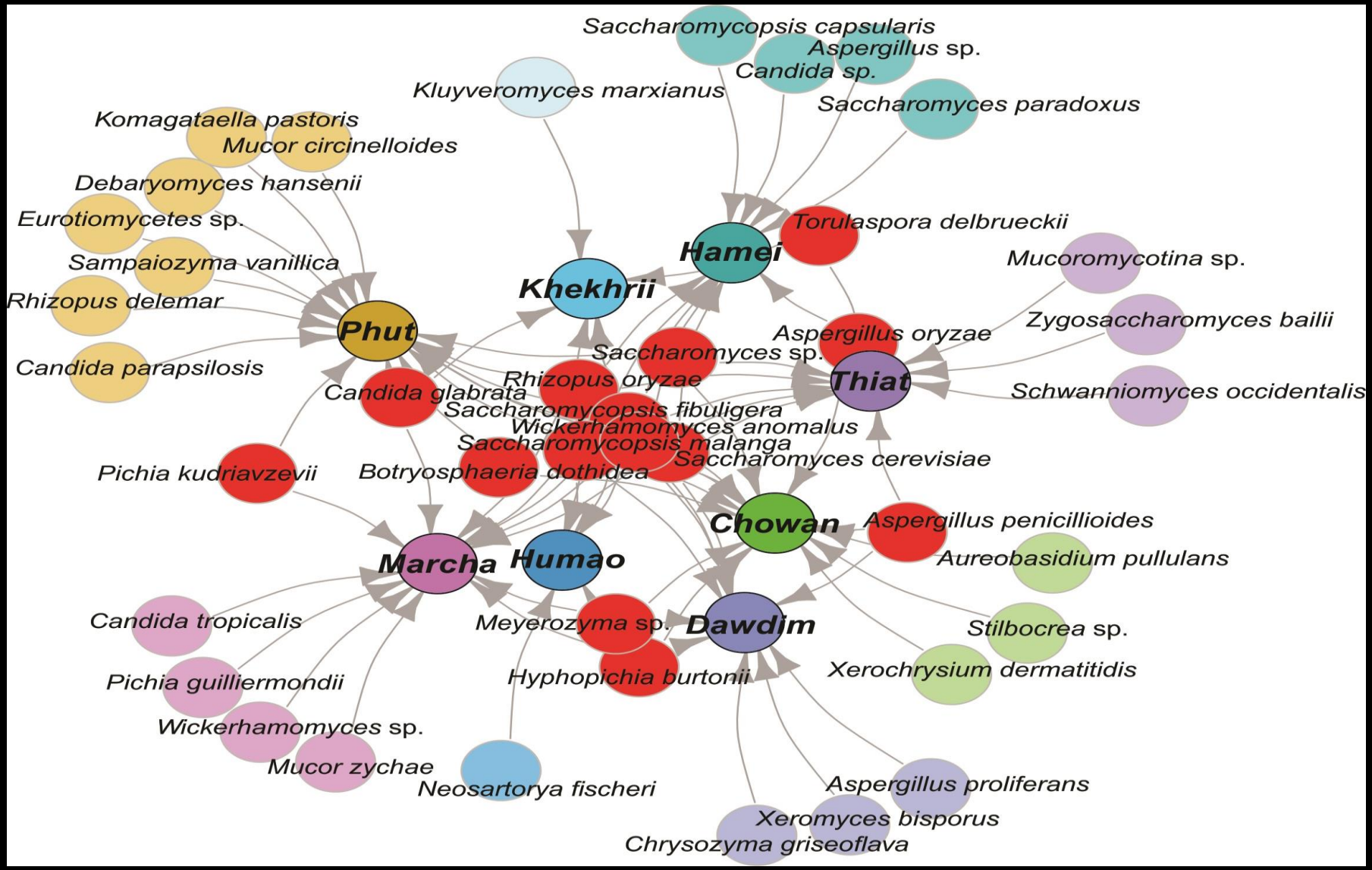
Figure 1. Taxa distributions of phylum and family at different phylogenetic level in *thiat* and *marcha*: (a) bacterial phyla; (b) bacterial family; (c), fungal phyla and (d) fungal family.

(Sha et al…Tamang 2017. *Scientific Reports*)

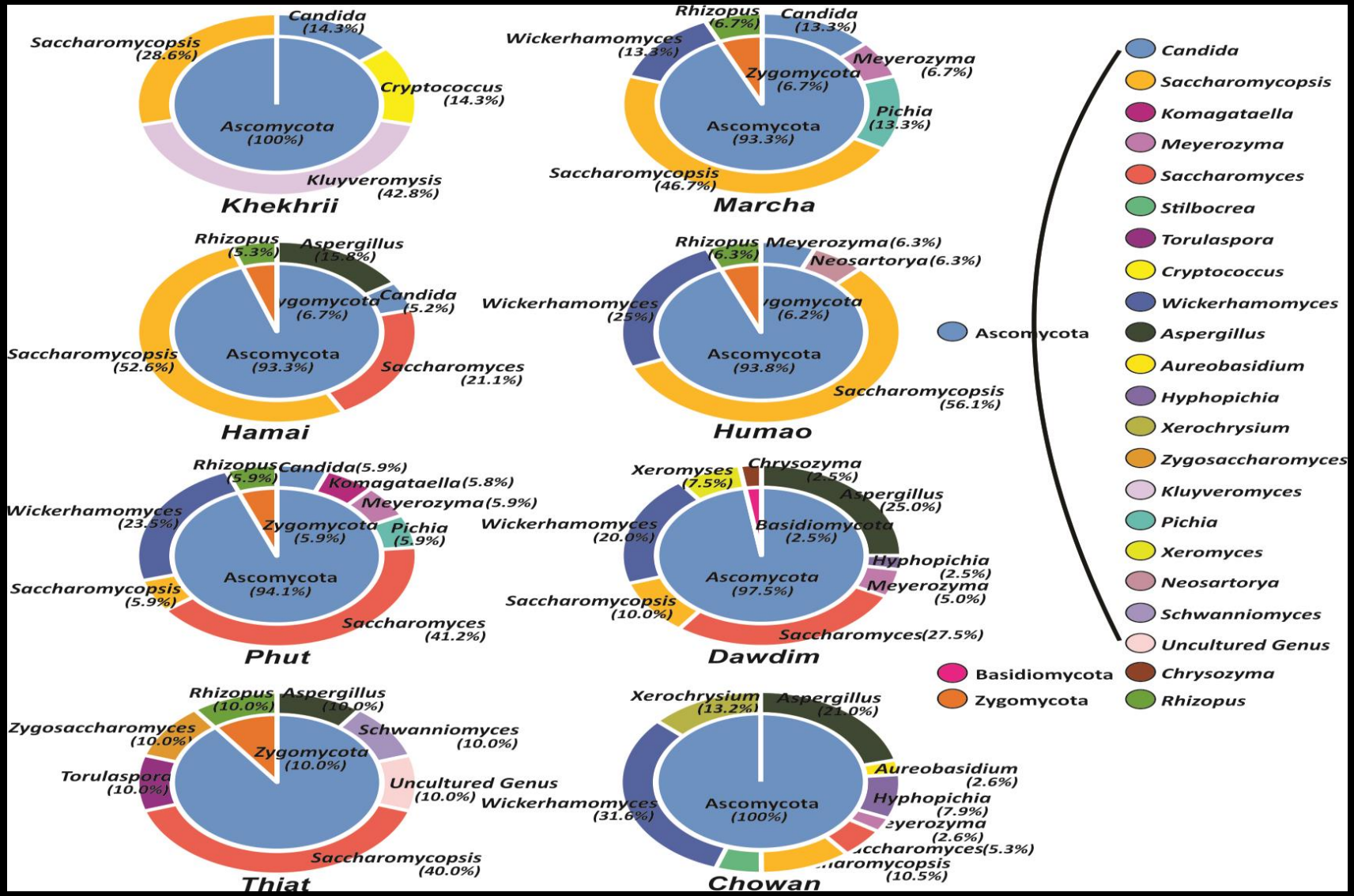


Diversity of yeasts and molds in forty samples of amylolytic starters of North East India by PCR-mediated DGGE analysis (Sha and Tamang, 2018, communicated *Sc. Reports*)

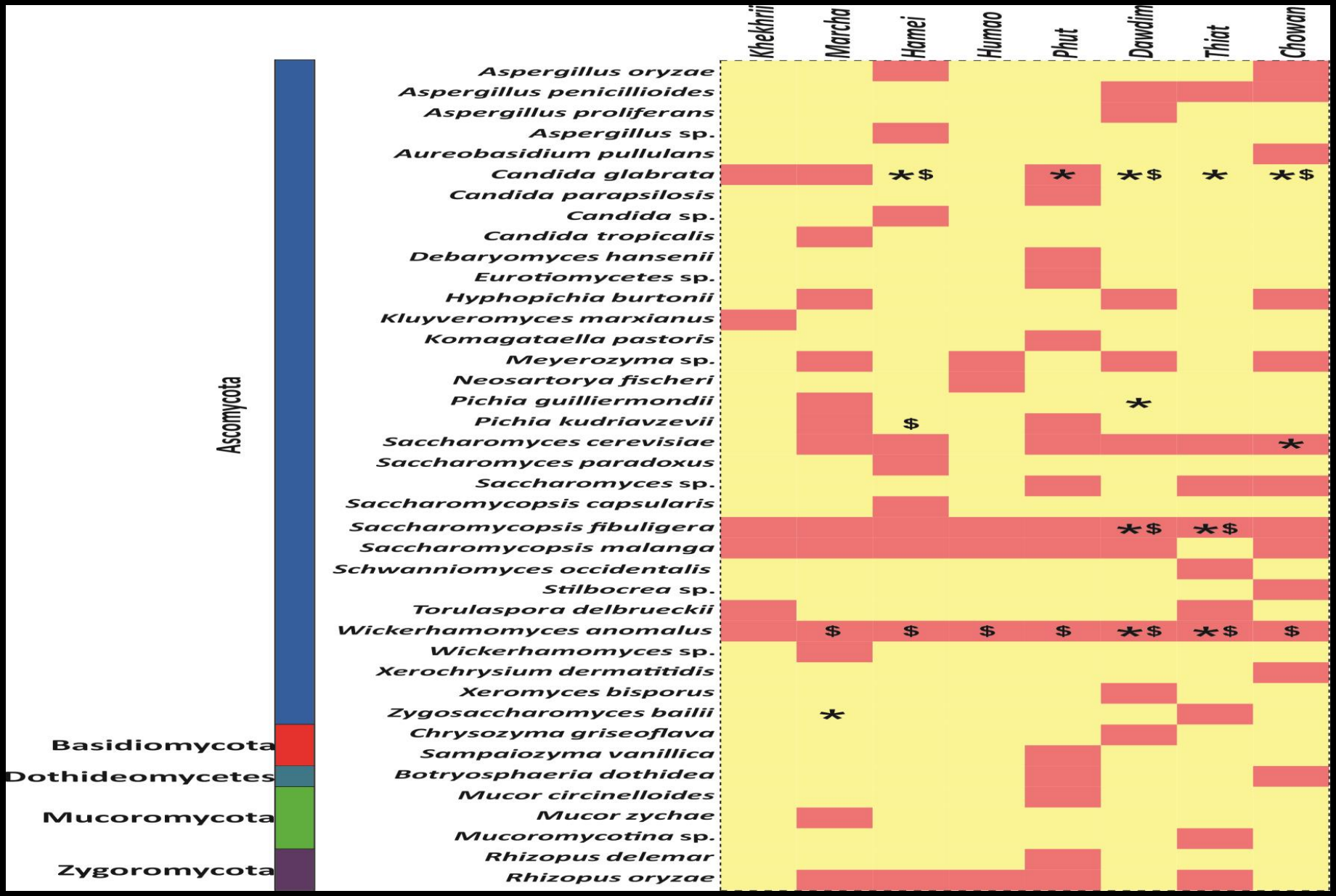

Marcha
Saccharomyces cerevisiae (38%)
Saccharomycopsis fibuligera (17%)
Saccharomycopsis malanga (13%)
Wickerhamomyces anomalus (8%)
Rhizopus oryzae (4%)
Meyerozyma sp. (4%)
Candida tropicalis (4%)
Pichia guilliermondii (4%)
Candida glabrata (4%)
Pichia kudriavzevi (4%)
Khekhrii
Kluyveromyces marxianus (43%)
Saccharomycopsis malanga (15%)
Saccharomycopsis fibuligera (14%)
Candida glabrata (14%)
Cryptococcus amylolentus (14%)
Chowan
Wickerhamomyces anomalus (31%)
Aspergillus penicillioides (16%)
Xerochrysium dermatitidis (13%)
Saccharomycopsis fibuligera (8%)
Hyphopichia burtonii (6%)
Aspergillus oryzae (5%)
Saccharomycopsis malanga (5%)
Stilbocrea sp. (5%)
Aureobasidium pullulans (3%)
Meyerozyma sp. (3%)
Saccharomyces cerevisiae (5%)
Thiat
Saccharomyces cerevisiae (25%)
Mucoromycotina sp. (17%)
Uncultured fungus (9%)
Schwanniomyces occidentalis (9%)
Rhizopus oryzae (8%)
Torulaspora delbrueckii (8%)
Saccharomyces DGGEband (8%)
Zygosaccharomyces bailii (8%)
Aspergillus penicillioides (8%)



Graphical representation of all species identified in PCR-DGGE of 26SrRNA gene after sequencing. (Sha and Tamang, unpublished)

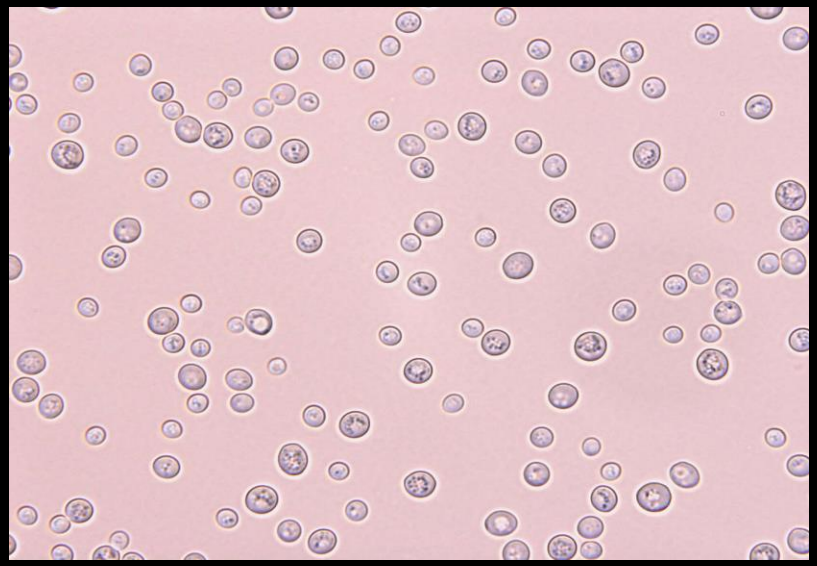


Genus and phylum level distribution of yeast and molds diversity in amylolytic starters. of all species identified in PCR-DGGE of 26SrRNA gene after sequencing. (Sha and Tamang, unpublished)



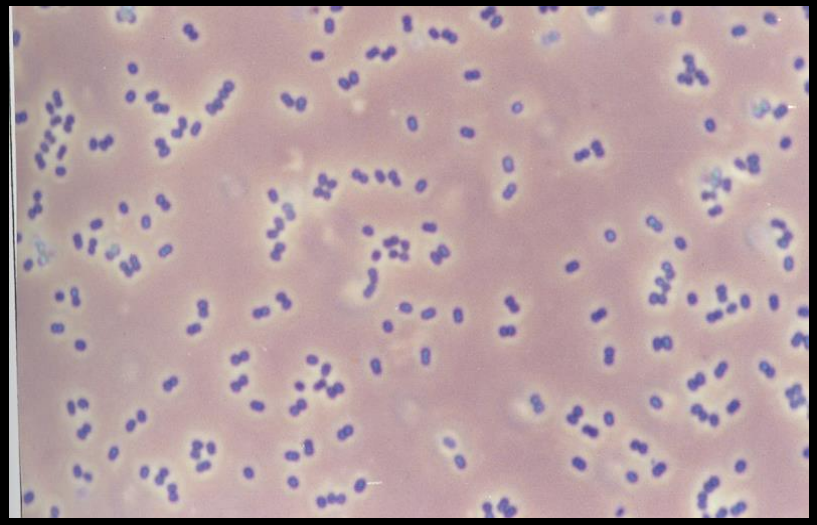
Heatmap showing the consensus species diversity observed during PCR-DGGE, Biolog identification hits and ITS-region gene sequencing of yeast isolates (Sha and Tamang, unpublished)

Yeasts and LAB in *marcha* and *hamei* (Tamang et al. 2007. IJM)

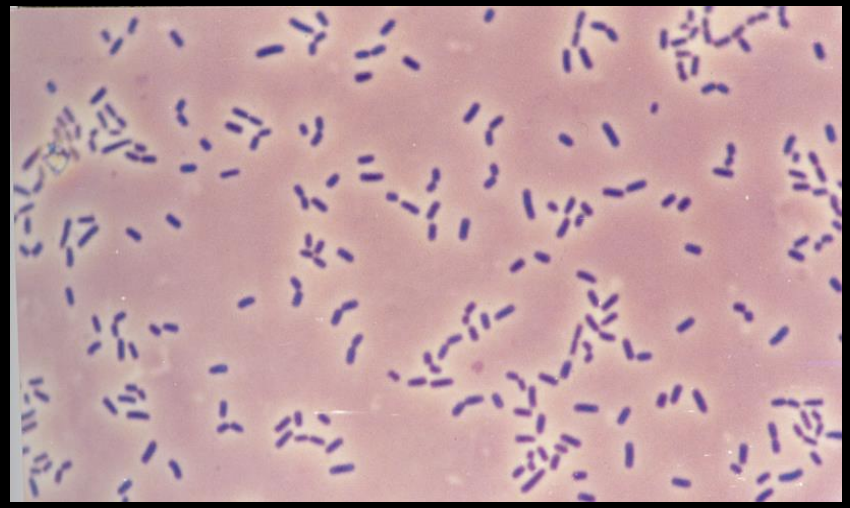


Saccharomyces cerevisiae

Saccharomycopsis fibuligera



Pediococcus pentosaceus

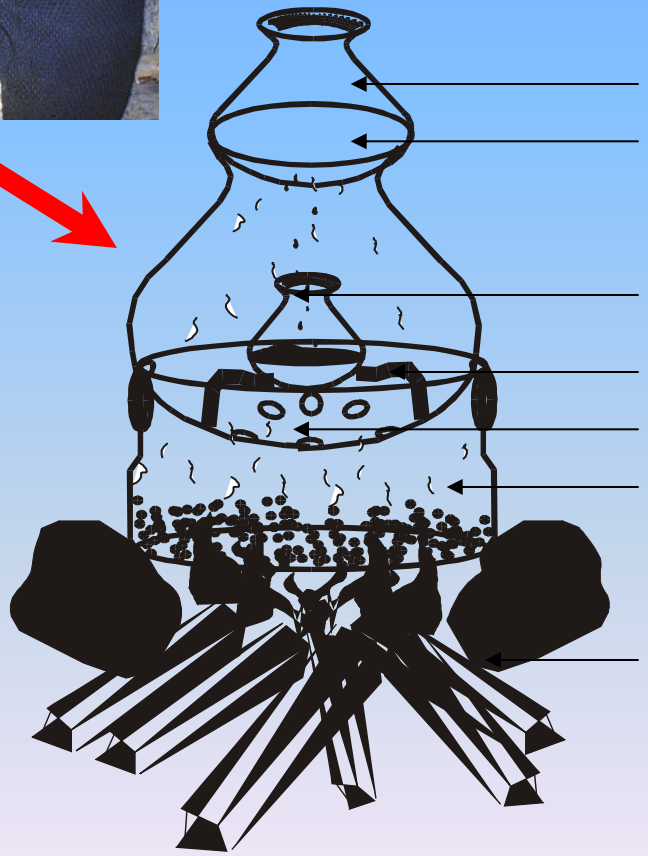


Lactobacillus plantarum

Alcoholic beverages of the Himalayas

Jaanr/Chee

Raksi -distillation apparatus



Metallic condenser

Plastered with mud

Metallic collector called *poini*

Tripod iron stand called *odhan*

Perforated container called *phunga*

Metallic container

Firewood

***Saké* of Japan- National drink of Japan**

***Makgeolli* of Korea**

Export during 2010 = > 10 million US $; most popular alcoholic drink in Korea

***Jaanr and raksi* of Nepal and India**

***Baijiu* 白酒 of China**

Pulque **of Mexico**

Tapé **of Indonesia**

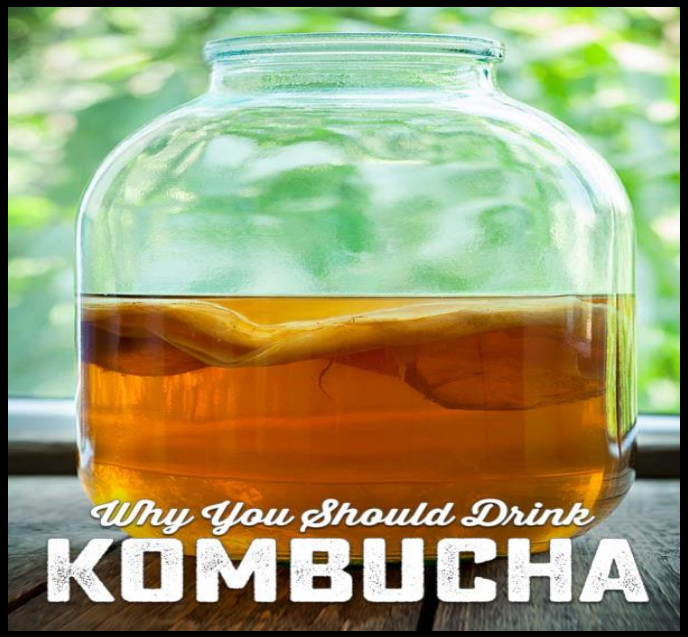
Tchoukoutou **of Africa**

Wine **of Europe**

Sustainable Development of Cassava Product Using *Ragi* (Starter Culture) in Indonesia for *Tapé*

Tapé of Indonesia

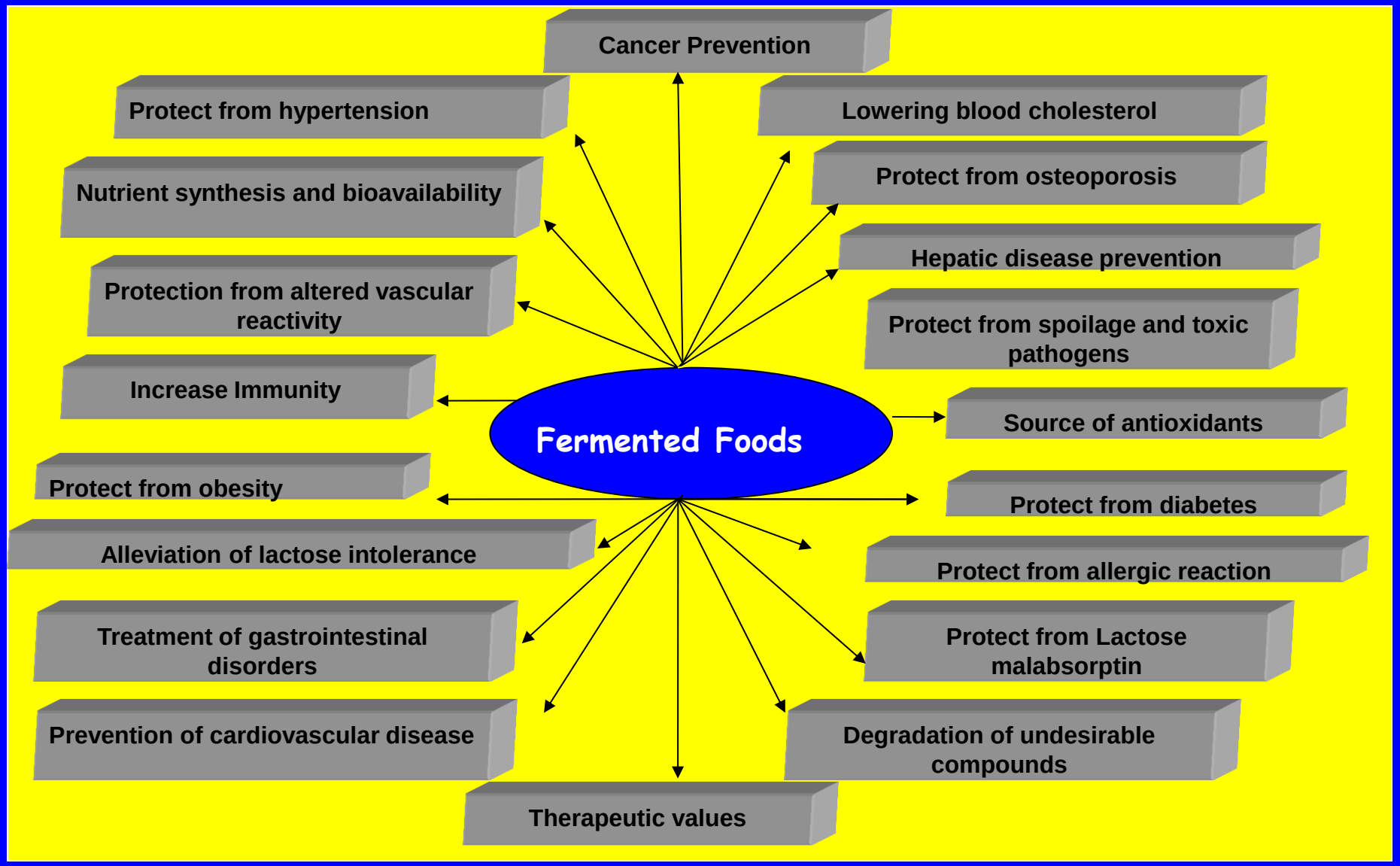
***Miang,* fermented tea of Thailand**



Cacao tree with pods

Heap (cacao) fermentation on banana leaves

Health-promoting Benefits of Fermented Foods



Ref: Farhad, Kailasapathy and Tamang (2010). Health Aspects of Fermented Foods. In: *Fermented Foods and Beverages of the World*. (Eds: Tamang, & Kailasapathy K). CRC Press, New York, 391-414.

Bioactive compounds in some fermented foods and their health benefits (Tamang et al. 2016b)

Bioactive Compounds	Synthesized in Fermented Foods	Health Benefits	Reference
Genistein	*Doenjang*	Reducing body weight	Kwak et a
Vitamin K	Fermented vegetables	Reducing blood pressure Appetite stimulant	Breidt et a
Isocyanate and sulphide Indole-3-carbinol	*Kimchi*	Prevention of cancer, detoxification of heavy metals in liver, kidney and small intestine	Park and
Ornithine		Anti-obesity efficacy	Park et al.
Capsaicin, Allicin		Suppression of *Helicobacter pylori*	An et al. (
HDMPPA (an antioxidant)		Therapeutic application in human atherosclerosis	Kim et al.

Nattokinase, antibiotics, vitamin K	*Natto*	Antitumor, immunomodulating	Nagai (2015)
GABA (non-protein amino acid)	*Nham*	Reducing hypertension, preventing diabetes	Ratanaburee et al. (2013)
Vitamin C	*Saurkraut*	Scurvy	Peñas et al. (2013)
Isothiocynate		Prevention of cancer	Higdon et al. (2007)
Glucosinolates		Activation of natural antioxidant enzymes	Martinez-Villaluenga et al. (2012)
Antioxidant genestein, daidzein, tocopherol, superoxide dismutase	*Tempe*	Prevents hyperlipidemia, diabetes type 2, cancer (breast and colon), cognitive decline and dementia, prevents the damage of pancreatic beta cell.	Astuti (2015)

Phenolics-Resveratrol		**Anti inflammatory**	**Jeong et al. (2010)**
Phenolics - Resveratrol, Flavonoids - Quercitin, Ethanol, Vitamins C, E, Mineral Selenium	***Wine (red)***	**Prevent cardiovascular diseases, heart attacks and mortality rate**	**Walker (2014)**
Melatonin, Resveratrol		**Antioxidant and anti-aging property**	**Corder et al. (2006)**
Resveratol		**Anti-diabetic**	**Ramadori et al. (2009)**
Biomarkers of cancer initiation	***Yogurt***	**Reduction of harmful fecal enzymes. Prevention of bladder and colon and cervical cancer.**	**Chandan and Kilara (2013)**

Health Benefits

- Today, some of these fermented foods are commercialized and marketed globally as health foods or functional foods or therapeutic foods.
- However 90 % of health-benefitted naturally fermented foods and alcoholic beverages in the world are still at home production under traditional conditions.
- Very few clinical trail for health benefit claims of ethnic fermented foods have been conducted.

Post-Graduate Syllabus (Microbiology) of Sikkim University (since 2008)

Third Semester: Paper 9: Food Microbiology 4 Credits Full Marks 100

Unit I: Taxonomy and microorganisms associated with fermented foods

Taxonomic Tools (phenotypic, biochemical and molecular) and Approaches (Culture dependents and culture independent techniques) to study microorganisms associated with fermented foods; Brief account of major groups of microorganisms associated with fermented foods: milk, vegetable, cereal, meat, fish, legumes, amylolytic starters and alcoholic beverages.

Unit II: Foodborne illness and Food safety

Food poisoning and mycotoxins in foods. Characteristics, pathogenesis and clinical features of foodborne diseases caused by *Clostridium botulinum, Escherichia coli, Listeria monocytogenes, Salmonella* and *Shigella.*

Hazard Analysis and Critical Control Point (HACCP) System- definition and application.

Unit III Fermented foods and beverages

Methods of production, microbiology and nutrition: Fermented vegetables (*any one*): *gundruk, sinki, kimchi, sauerkraut*, *soibum*. Fermented legumes (any one): *kinema, natto, chungkukjang, shoyu, dawadawa, tempe*. Fermented cereals (any one): *dosa, idli, selroti, nan, sourdough, kenkey.* Fermented milks (any one): *dahi, yogurt, chhurpi,cheese, shrikand.* Fermented fish (any one): *ngari, nam pla, tungtap, sidra, jeot kal.* Fermented meat (any one): sausage, *kargyong, nham, salami, nem-chua.* Asian amylolytic starters (any one): *marcha, ragi, bubod, nuruk, hamei, loogpang, koji*. Alcoholic beverages (any one): *kodo ko jaanr, sake, Bantu beer, pulque, chicha*.

Unit IV: Probiotics

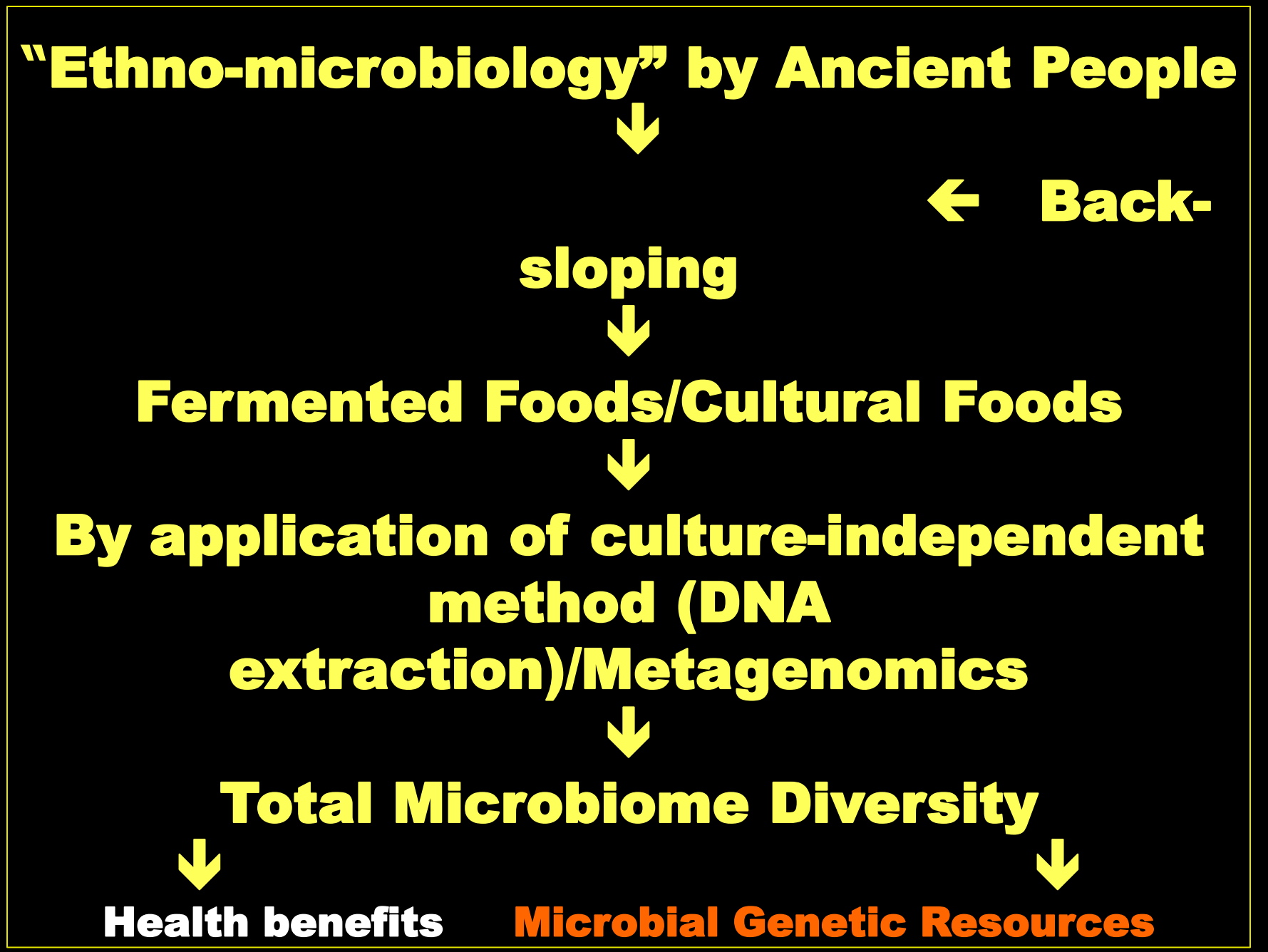
Probiotics: Definition, characteristic, Gut microbiota, beneficial effects of probiotic bacteria; prebiotics and synbiotics.

Paper 12: Laboratory Course-V (40 Lectures) 4 Credits

1. Microbiological evaluation of fermented food (any local product).
2. Microbiology quality assessment of any non-fermented food sample (pathogenic bacteria).
3. Microbiological analysis of milk.

RECOMMENDATIONS

- Development of fermented foods may boast sustainable development of food security and enhances the economy in global food market.
- More clinical trials and validation of health claims of ethnic fermented foods.
- Basic training on molecular microbial taxonomy, using culture independent technique, determination of functional properties and extraction of important bioactive compounds from fermented foods.
- Incorporation of ethnic fermented foods into academic programmes at master and doctoral level in University, such as Sikkim University (India), Wageningen University (Netherlands), Tokyo Agricultural University (Japan), Cornel University (USA).


"Ethno-microbiology" by Ancient People
Back-sloping
Fermented Foods/Cultural Foods
By application of culture-independent method (DNA extraction)/Metagenomics
Total Microbiome Diversity
Health benefits
Microbial Genetic Resources

PhD Students

Tamang

HIMALAYAN FERMENTED FOODS

Microbiology, Nutrition, and Ethnic Values

HIMALAYAN FERMENTED FOODS

Microbiology, Nutrition, and Ethnic Values

Jyoti Prakash Tamang

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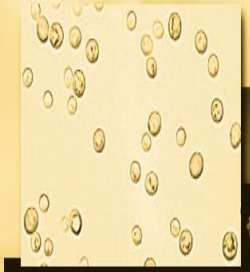

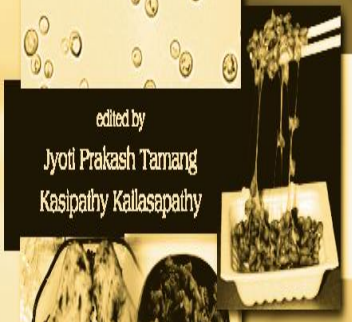

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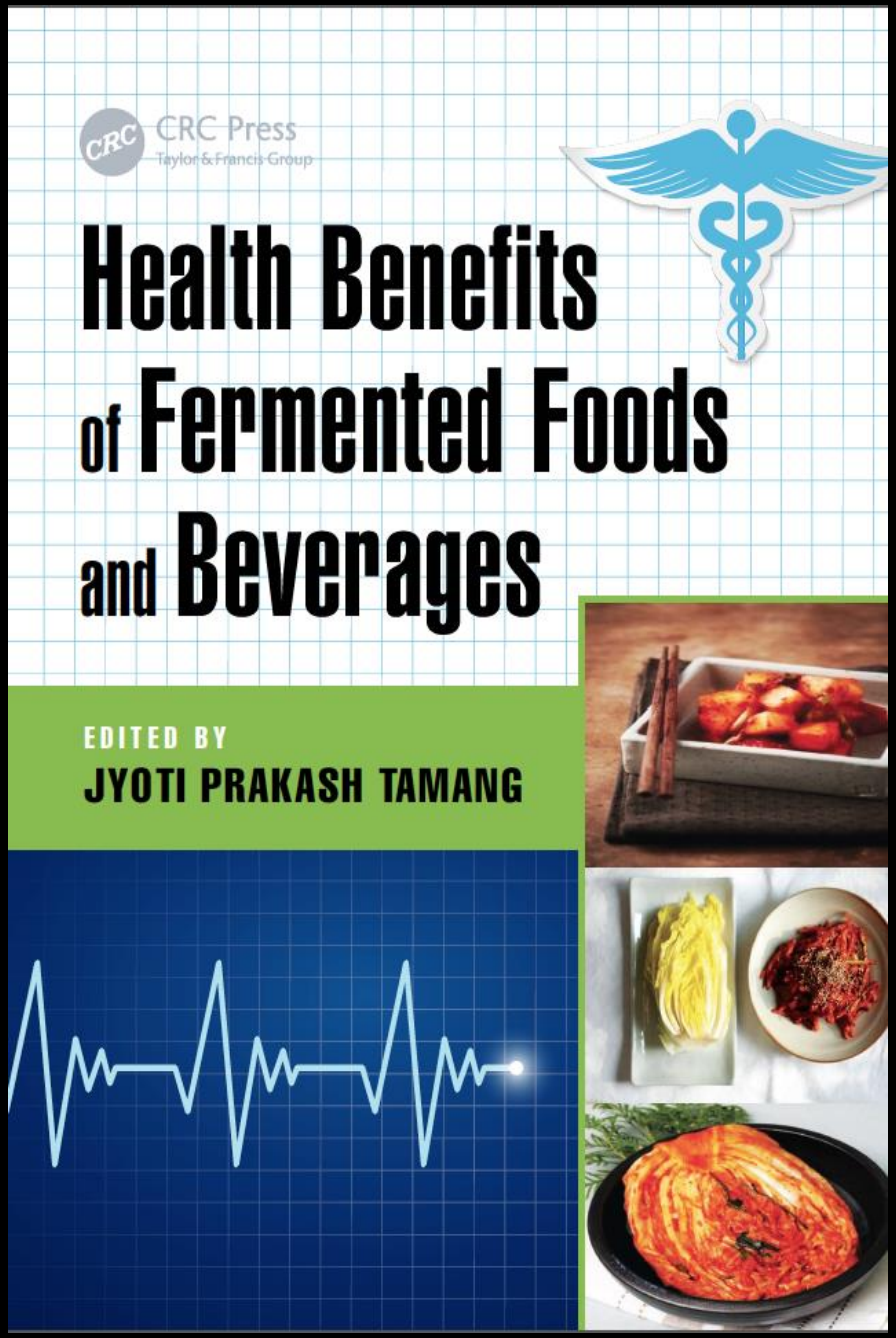
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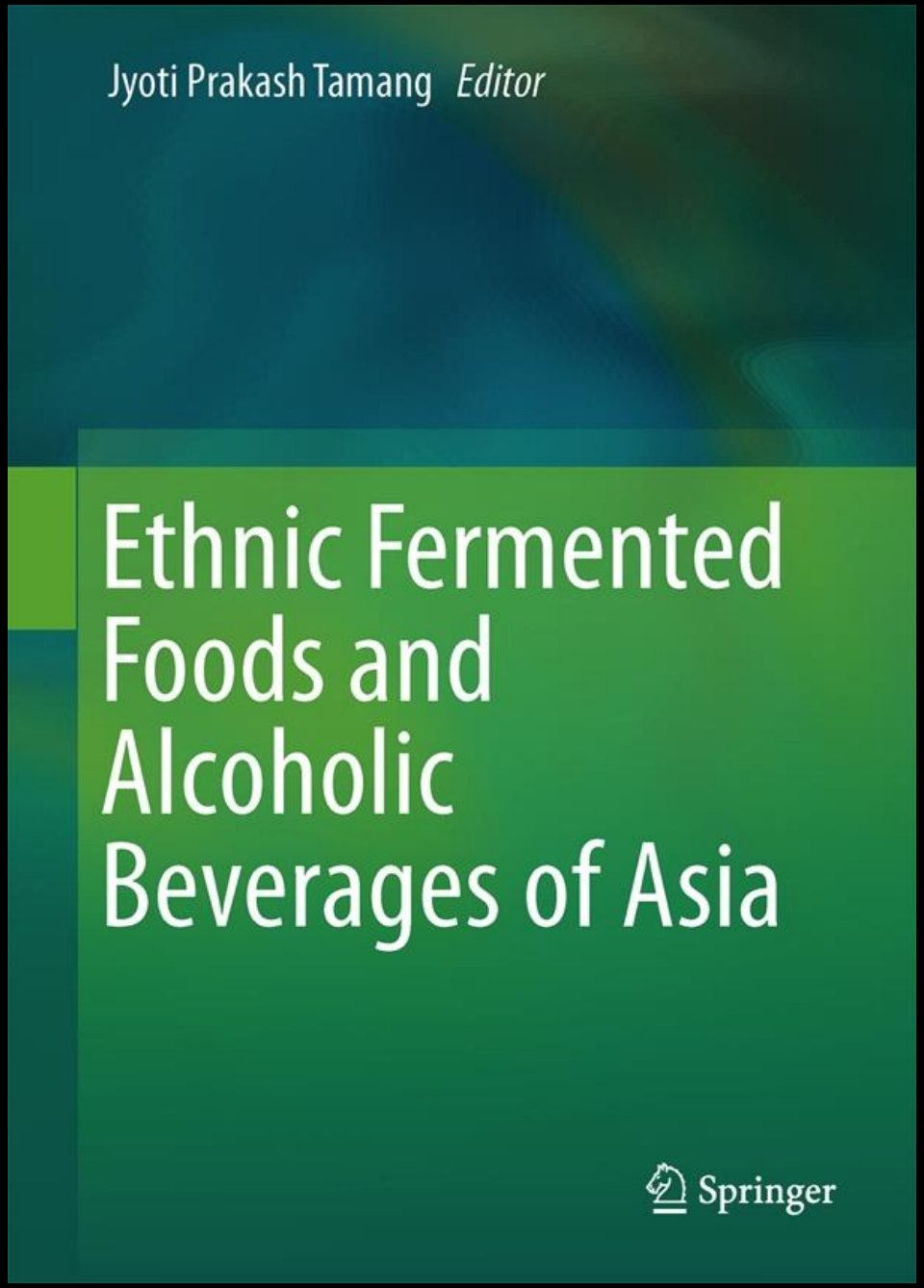
Jyoti Prakash Tamang Editor
Ethnic Fermented Foods and Alcoholic Beverages of Asia
Springer

Welcome to new Research Topic (2018) of *Frontiers in Microbiology* (Impact factor: 4.076):

Insights of Fermented Foods and Beverages: Microbiology and Health-promoting Benefits

(Editors: Jyoti Prakash Tamang, India; Patricia Ester Lappe Oliveras, Mexico and and Baltasar Mayo, Spain).

Contribute your original papers/critical reviews related to microbiology and health benefits of fermented foods and beverages to our Research Topic.

Visit Research Topic public homepage: https://www.frontiersin.org/research-topics/8011/insights-of-fermented-foods-and-beverages-microbiology-and-health-promoting-benefits

Thank you!

धन्यवाद्

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