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Indonesian Dadih

INGRID SURYANTI SURONO

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14.1 Introduction

Indonesia is the largest archipelago blessed with one of the richest mega-biodiversities on the globe, rich in ethnic and cultural diversities, and also home to one of the most diverse cuisines and traditional fermented foods. Dadih is an Indonesian traditional fermented milk produced and consumed by the West Sumatran Minangkabau ethnic group of Indonesia as one of the characteristic traditional foods of Minangkabau culture. Native people named it as dadiah, and it is a very popular dairy product in Bukittinggi, Padang Panjang, Solok, Lima Puluh Kota, and Tanah Datar (Surono and Hosono 1996). It serves as a significant dairy product in the diet of the Minangkabau resembling yogurt and is similar to dahi of India.

Dadih is one of the most distinct products in microbial quality and an important fermented milk. It is a yogurt-like product with a distinctive thicker consistency, smooth texture, and pleasant flavor and has provided safety, portability, and novelty to milk nutrients for the indigenous people in West Sumatra. Currently, dadih is consumed as a traditional food, served at weddings and while giving an honorable title "Datuk" in West Sumatra during the ethnic tradition or "adat" ceremony. Generally, dadih is consumed during breakfast with rice after adding sliced shallot and chili ("sambal"), or it is mixed with palm sugar and coconut milk, being served as a topping of steamed traditional glutinous rice flakes, a corn flake-like product, called "ampiang dadih."

The manufacturing method of dadih is quite similar to the dahi of India, except for the heat treatment of raw milk and the starter cultures being incorporated. In dahi making, the raw cow or buffalo milk, or a combination of both, is pasteurized and then fermented using leftover dahi from the previous lot as starter cultures (Indian Standard Institution 1980). In Indonesia, dadih is still a homemade product by the traditional way, involving the milk of water buffaloes. No heat is applied to buffalo milk while manufacturing dadih. The milk is neither boiled nor inoculated with any starter culture. The fresh unheated buffalo milk is placed in bamboo tubes covered with banana leaves, incubated at the ambient temperature (28°C–30°C) overnight, and allowed to ferment naturally until it acquires a thick consistency (Akuzawa and Surono 2002). Various indigenous lactic acid bacteria (LAB) involved in the dadih fermentation may vary from time to time as well as from one place to another due to the natural fermentation without any starter culture involved (Surono and Hosono 2000; Akuzawa and Surono 2002).

Interestingly, even though the process did not implement the hygiene practice, there was no product failure and no food poisoning was reported among people who consumed dadih. Instead, the older generation believes that consuming dadih may provide a beneficial effect to their health. Due to this fact, it is very interesting to explore the indigenous LAB involved during dadih fermentation. Some dadih LAB have antimutagenicity, hypocholesterolemic properties, antipathogenic properties, and immunomodulatory properties (Surono and Hosono 1996; Pato et al. 2004; Surono et al. 2011).

Microbial ecology of dadih is little known. However, recent development in molecular biology techniques and sequencing may allow us to gain more insight on the complexity, beauty, and potential advanced applications of these understudied, invaluable resources for microbial bioprospecting as well as functional foods. Scientific literature and data on production is also limited, and marketing and standard specifications for production and quality control of dadih are not well established. Still, it remains challenging to study dadih preparation and supposed to be well documented on a scientific basis. The technological parameters, the biochemical changes, and the keeping quality of dadih should be further studied, in order to scale up production. Nevertheless, this chapter covers the available aspects of production of dadih.

14.2 Product Description of Dadih

Yogurt-like products are made widely in various regions in the world, including the Mediterranean area, Asia, Africa, and central Europe. Dadih and dahi are Indonesian and Indian yogurts, respectively, made out of buffalo or cow milk that seems to share the same root word. The body and texture of yogurt depends largely on the composition of milk employed in its manufacture, whereas the manufacture of dadih and dahi is simpler than western-type yogurt (Surono and Hosono 2011).

As dadih is made from buffalo milk, it produces thick bodied product due to its higher total solids content as compared to cow milk, as a consequence of higher fat and casein content in buffalo milk (Table 14.1). The higher protein content in buffalo milk results in custard-like consistency at the end of fermentation. In addition, higher fat content enrich the flavor developed in the dadih products. Dadih is

Table 14.1 Composition of Mammals Milks

	PERCENT COMPOSITION OF MAMMALS MILKS					
MAMMALS	FAT	CASEINS	WHEY PROTEINS	LACTOSE	ASH	TOTAL SOLIDS
Cow	3.7	2.8	0.6	4.8	0.7	12.7
Water buffalo	7.4	3.2	0.6	4.8	0.8	17.2
Goat	4.5	2.5	0.4	4.1	0.8	13.2
Sheep	7.4	4.6	0.9	4.8	1.0	19.3
Mare	1.9	1.3	1.2	6.2	0.5	11.2
Sow	6.8	2.8	2.0	5.5	–	18.8

Sources: Fernandes, C.F. et al., Fermented dairy products and health. In: *The Lactic Acid Bacteria: The Lactic Acid Bacteria in Health and Disease*, ed. B.J.B. Wood, 297–339. Elsevier Applied Science, London, 1992; Chandan, R.C. and Shahani, K.M., Other fermented dairy products. In: *Biotechnology, Biotechnology: Enzymes, Biomass, Food and Feed*, Vol. 9., eds. H.-J. Rehm and G. Reed. Wiley-VCH Verlag GmbH, Weinheim, Germany, 1995.

manufactured in bamboo tubes, which is hygroscopic aided in keeping the product from wheying off (Figure 14.1).

The milk composition of various mammals used in yogurt and yogurt-like manufacture is shown in Table 14.1. The nutritive value of water buffalo milk products is higher than cow milk products because of the higher concentrations of protein, fat, lactose, minerals, and vitamins in buffalo milk (Walstra et al. 1999).

Dadih making is carried out by natural fermentation. The buffalo milk was poured in bamboo tubes and kept overnight at room temperature, which stimulate the mesophilic indigenous LAB derived from the fresh raw milk to dominate and grow. Consequently, the fermentation of dadih is much longer than yogurt, 24 and 4 h, respectively, due to different types of LAB involved in the fermentation process at the incubation temperature, 28°C–30°C and 45°C, respectively, besides thicker consistency of dadih.

Hosono et al. (1989) reported that *Leuconostoc paramesenteroides* was the dominant strain of LAB encountered in dadih. Surono and Nurani (2001) found that *Lactobacillus* sp., *Lactococcus* sp., and *Leuconostoc* sp. were dominant in dadih. Surono (2003) reported that among 20 colonies of dadih LAB isolated from Bukittinggi, West Sumatra: five strains were of as *Lactococcus lactis* subsp. *lactis*; three strains of *Lb. brevis*; and three each of *Lb. plantarum*, *Lb. casei*, *Lb. paracasei*, and *Leu. mesenteroides*.

Figure 14.1 Traditional way of dadih making in Padang Panjang West Sumatra using fresh raw buffalo milk in bamboo tube.

The milk is fermented by indigenous LAB of the buffalo milk. Its natural fermentation provides different strains of indigenous lactic bacteria involved in each fermentation (Akuzawa and Surono 2002). The natural indigenous LAB observed in dadih could be derived from the bamboo tubes, buffalo milk, or banana leaves involved in milk fermentation, and buffalo milk has been observed to contribute the most, while bamboo tubes and banana leaves as well as personal hygiene practice may also contribute. Hence, diverse indigenous LAB were reported involved in dadih fermentation due to traditional way of dadih manufacture.

Dadih does not meet any standards such as the national standard for yogurt, Standard National Indonesia 2981:2009, and international standard of yogurt and fermented milk available such as the U.S. Federal Standards of Identity, International Dairy Federation, or Codex Standard for Fermented Milks, which requires pasteurizing the milk, since there is no heat application to the buffalo milk as a raw material of dadih in home industry.

14.3 Nutrition Profile of Dadih

Chemical composition/nutrition profile of dadih may vary from time to time and from place to place of production due to its traditional way of manufacture. However, as dadih is made out of buffalo milk, both protein and fat content are much higher than yogurt made from cow milk, as shown in Table 14.2.

Table 14.2 Nutritional Profile and Microbial Counts of Buffalo Milk Dadih, Dahi, and Cow Milk Yogurt

SAMPLE	PH	TA	PROTEIN (%)	FAT (%)	CARBOHYDRATE (%)	MOISTURE (%)	YEAST COUNTS (CFU/G)
Dadih A[a]	4.1	1.278	5.93	5.42	3.34	84.35	2.0×10^5
Dadih B[a]	4.0	1.32	7.57	6.48	3.79	81.03	2.7×10^9
Dahi[b]	Not detected	0.5–1.1	3.2–3.4	5.0–8.0	4.6–5.2	85–88	1.32×10^8 to 2.46×10^8
Yogurt[c]	3.85	1.49	3.47	3.25	4.66	87.90	1×10^6 to 2×10^6

Sources: [a] Yudoamijoyo, M. et al., *Japan J. Dairy Food Sci*., 32, A7, 1983.

[b] Gandhi, D.N. and Natrajan, A.M., *Preparation of a Good Quality Dahi (Curd) and Probiotic Milk Products*. http://s3-ap-southeast-.amazonaws.com/jigsydney/general/PDF/49212~Dahi-Making.pdf, 2006.

[c] USDA Agricultural Research Service National Nutrient Data Base for Standard Reference Release 27. http://ndb.nal.usda.gov/ndb/foods/show/105?fgcd=&manu=&lfacet=&format=&count=&max=35& offset=&sort=&qlookup=Yogurt%2C+plain%2C+whole+milk%2C+8+grams+protein+per+8+ounce.

Note: Dadih A: collected from a village near Bukittinggi, West Sumatra. Dadih B: purchased from local market in Padang Panjang, West Sumatra.

TA, titratable acidity (as lactic acid).

Buffalo milk yogurt is reported to have higher contents of protein, total solid, carbohydrate, and ash than those reported for cow milk yogurt (Fundora et al. 2001; Lindmark-Månsson et al. 2003), indicating higher nutrient density in buffalo milk dadih as well as dahi. During the dadih fermentation, bacteria convert milk into curd and predigest milk protein. As a result, the hydrolysis of protein in buffalo milk supports the growth of LAB and provides more nutritious dadih to consumers, which is easily digested as compared to the buffalo milk.

14.4 Important Factors and Physicochemical Changes during Dadih Fermentation

Fresh raw buffalo milk, diverse natural LAB cultures, fermentation temperature, and time are the important factors during spontaneous dadih fermentation. Traditional way of dadih fermentation gives many variations in the characteristics, quality, and acceptability of traditional dadih, inevitable due to the unregulated condition of the spontaneous natural fermentation, while in fermentation technology, the milk, microbes, and the environment are supposed to be controlled properly. Moreover, information about detailed characteristics of bacteria or other microorganisms contributing to these fermentation processes is scanty. A good quality dadih is firm with uniform consistency, creamy-white color, pleasant aroma, and acidic taste. The surface is smooth and glossy, and a cut surface is trim and free from cracks and air bubbles.

14.4.1 Fresh Raw Buffalo Milk

Most of the buffalo milk in West Sumatra and nearby areas is produced in the villages by farmers with small land holdings; by landless agricultural laborers, mostly produced in small quantities of 2–4 l; and by small and marginal farmers in numerous and widely scattered villages. Conditions under which milk is produced in the villages are far from satisfactory, mainly due to the economic backwardness of the producers. Hence, significant portion of the milk is being fermented immediately into traditional dadih due to lack of refrigeration and transportation facilities.

The water buffalo, as a domesticated cattle animal of the bovine subfamily, is fed with natural feed grass, which is free from antibiotics. Hence, no antibiotic residue is in the buffalo milk, which may give inhibitory impact on the growth of the natural starter and cause product failures of dadih, as well as no possible antibiotic resistance of indigenous LAB is in the buffalo milk.

The buffalo milk is not pasteurized, and hygiene practice is not implemented properly during dadih making. Buffalo milk contains higher total solids and has 100% more fat content than cow milk, which makes it creamier and thicker, makes it suitable for the manufacture of traditional dadih, and contributes to the aroma development. The nutritional value of yogurt is similar to the milk from which it is made except for the partial loss of lactose due to fermentation.

14.4.2 Natural LAB

The raw milk of different domestic animals is a natural niche and habitat of lactic bacteria (Surono 1996). The diverse indigenous LAB cultures involved in the spontaneous fermentation play important roles in curd formation. A mixture of natural mesophilic starter cultures derived from fresh unheated buffalo milk, containing specific genera, species, and strains of LAB is involved in the manufacture of dadih developing flavor, body, and texture characteristics of the dadih product. As a result of LAB culture growth, transformation of chemical, physical, microbiological, sensory, nutritional, and physiological attributes in basic milk medium is observed. Traditional dadih might be used for strain hunt so that bacterial repository of wider biological diversity to be used as starter cultures as well as beneficial effect to human health can be established.

Diverse microflora has been seen in dadih. Surono et al. (1983) reported the finding of yeast-like fungi at 1.1×10^7 CFU/g, identified as *Endomyces lactis*, which is commonly found in dairy products. Imai et al. (1987) reported that the major bacterial species responsible for dadih fermentation were *Lb. casei* subsp. *casei* and *Lb. plantarum*. LAB in spontaneous fermented dadih from various parts of West Sumatra have been reported. *Leu. paramesenteroides* was the predominant strain of LAB encountered, responsible for producing aromatic compounds such as diacetyl, acetic acid, and other volatile compounds (Hosono et al. 1989). The lactic acid bacterial count of 4.3×10^8 CFU/g was detected in fresh dadih, dominated by LAB, which was 4.0×10^8 CFU/g. *Leu. paramesenteroides* was the dominant strain of the LAB encountered. *Lc. cremoris*, *Lc. lactis*, *Lb. casei* subsp. *casei*, and *Lb. casei* subsp. *rhamnosus* were also found. Several strains belonging to *E. faecalis* subsp. *liquefaciens* were also found in dadih. This fact indicates that the way of manufacturing dadih did not implement hygiene practices, because those microbes belong to the *Enterococci* group.

Total viable LAB were found in the range of 1.42×10^8 to 3.80×10^8 CFU/g in dadih originated from Bukittinggi and Padang Panjang area of West Sumatra (Surono and Nurani 2001). Table 14.3 shows various LAB cultures involved in the manufacture of commercial yogurt and dadih. Dadih involves mesophilic cultures grown at 25°C–28°C and takes 6–18 h to ferment, whereas yogurt production uses thermophilic cultures, which grow faster at 45°C, and requires only 3–4 h fermentation time. Apart from the huge milk microflora, microbial isolates of dadih have also been reported to exhibit probiotic attributes.

Table 14.3 Lactic Acid Bacteria Involved in the Manufacture of Yogurt and Dadih with Major Function as Lowering Acidity, Curd and Texture Formation, Aroma, and Flavor

PRODUCT	PRIMARY MICROBE(S)	SECONDARY MICROBE(S)	INCUBATION TEMPERATURE AND TIME
Dadih	*Lb. plantarum*, *Lc. lactis* subsp. *lactis*, *Lc. lactis* subsp. *cremoris*, *Lc. lactis* subsp. *lactis* var. *diacetylactis*, *E. faecium*, *Lb. brevis*	*Leu. lactis*, *Leu. mesenteroides* subsp. *cremoris*	25°C–28°C/12–18 h
Yogurt	*Lb. delbrueckii* subsp. *bulgaricus*, *Str. thermophilus*	*Lb. acidophilus*, *B. longum*/*bifidum*/*infantis*, *Lb. casei*/*lactis*/*jugurti*/*helveticus*	43°C–45°C/2.5 h

14.4.3 Indigenous Enzymes

Milk contains a large number of indigenous enzymes, with differing functions, stability to processing, impact on dairy products, and significance for consumer safety (e.g., antimicrobial enzymes). Some enzymes are of interest for their beneficial activity (e.g., lactoperoxidase), some for use as indices of processing (e.g., alkaline phosphatase), and some for effects on the quality of dairy products (e.g., plasmin, lipoprotein lipase), which may be either positive or negative for different products. The indigenous proteolytic enzyme in milk is desirable for cheese ripening, but not for the stability of gel formation in yogurt.

Bovine milk contains several proteases including plasmin, plasminogen, plasminogen activators, thrombin, cathepsin D, acid milk proteases, and amino peptidase. Indigenous enzymes in milk can potentially influence the quality of milk and dairy products. The two sets of enzymes, bacterial enzymes mainly derived from LAB and indigenous native protease, may interact during dadih fermentation. The system is presumably optimized to function properly at body temperature.

Neither thermal breakdown of fresh unheated buffalo milk protein nor denaturation and coagulation of milk albumins and globulins occurred as a consequence of no heat application to buffalo milk prior to dadih making, but the custard-like consistency of dadih is formed. In dadih manufacture, fresh unheated buffalo milk is kept overnight at room temperature, which permits protein degradation catalyzed by bacterial or native proteases.

Thickening, gelation, and coagulation of buffalo milk occurs during spontaneous fermentation of fresh unheated buffalo milk attributed to the proteolytic activity from either milk proteases such as plasmin or proteases of bacterial origin. This fact might be due to strong indigenous proteolytic enzymes derived from the buffalo milk itself or from the indigenous natural LAB in the buffalo milk. The presence of natural indigenous proteolytic enzymes from milk showed more rapid fermentation (Gassem and Frank 1991; Kelly and Fox 2006). Lactic bacteria cultures coincidentally may interfere with the milk enzyme system to their advantage.

The presence of alpha-caseins in freshly drawn milk clearly indicates the presence of active plasmin, and hence the activation of plasminogen, in the udder. During incubation of milk, concomitant processes that either increase (i.e., plasminogen activation) or reduce (autolysis) the activity of plasmin occur. Gassem and Frank (1991) reported that yogurt made from milk pretreated with microbial protease had higher firmness, syneresis, and apparent viscosity than the untreated product. Yogurt made from milk treated with plasmin had significantly lower firmness and apparent viscosity and, after 8 days, lower syneresis as compared to the untreated. Proteolysis of milk accelerates fermentation of yogurt.

The proteolytic system is composed of proteinases, which initially cleaves the milk protein to peptides, cleaved by peptidases into small peptides and amino acids. Apart from lactic streptococcal proteinases, there are several other proteinases from non-lacto-streptococcal origin such as serine type of proteinases, for example, proteinases from *Lb. acidophilus*, *Lb. plantarum*, *Lb. delbrueckii* ssp. *bulgaricus*, *Lb. lactis*, and *Lb. helveticus*. Aminopeptidases are important for the development of flavor in fermented milk products (Law and Haandrikman 1997). Production of volatile compounds (e.g., diacetyl and acetaldehyde) contributes to aroma of dairy products.

14.4.4 Bamboo Tubes as Container of Dadih Product

Bamboo tube is used for fermenting dadih, because of its hygroscopic properties, and its bitter taste gives protection against ants. There are two kinds of bamboo tubes, namely, *bamboo gombong* (*Gigantochloa verticillata*) and *bamboo ater* (*Gigantochloa atter*). The native Minangkabau prefers to use *bamboo gombong*. Azria (1986) reported that microbial load of inner part of bamboo tube was 2.5×10^2 to 1.0×10^3 CFU/cm^2, dominated by acid-producing proteolytic bacteria, which may play an important role in dadih fermentation.

14.4.5 Biochemical Changes during Buffalo Milk Fermentation

Understanding of the transformation of buffalo milk into dadih is necessary to appreciate its nutritional and health properties. The major changes brought about in dadih fermentation process due to LAB resulted in specific health benefits documented in literature.

The changes in the milk constituents during dadih manufacture are related to various steps in the fermentation process. Fermentation of lactose may produce different metabolite products depending on the bacteria involved in the fermentation, but they generally include energy for the bacterial cell to grow. Adenosine triphosphate, either lactic acid and/or carbon dioxide, and some volatile compounds will be produced. When LAB get into milk, they convert the lactose into lactic acid, precipitate proteins in the milk, and form curd. The formation of lactic acid changed the taste to sour in dadih products compared with the buffalo milk. Dadih, like yogurt and other fermented milk products, in particular, is low in lactose because of having been fermented, which means many lactose-intolerant individuals can consume it. Fermentation of lactose to form lactic acid is an important means of preventing, or limiting, milk spoilage due to the growth of contaminating bacteria and their enzyme activity.

14.4.5.1 Carbohydrate Lactose is the major carbohydrate in the buffalo milk. A consortium of LAB, which could be homofermentative and heterofermentative natural starter cultures producing lactic acid, with the involvement of beta-galactosidase from lactic starter cultures, results in coagulation of buffalo milk beginning at pH below 5.0 and completing at 4.6. Texture, body, and acid flavor of dadih owe their origin to lactic acid produced during fermentation.

Small quantities of flavor compounds are generated through carbohydrate catabolism, via volatile fatty acids, ethanol, acetoin, acetic acid, butanone, diacetyl, and acetaldehyde. Homolactic starter cultures in dadih such as *Lactobacilli*, *Lactococci*, *Pediococci*, and *Streptococci* yield lactic acid as 95% of the fermentation output, and lactic acid acts as a preservative. Heterolactic starter cultures, such as *Lb. brevis*, *Lb. fermentum*, and *Leuconostoc* sp., contribute to flavor compounds. There are two important roles of lactic acid in dadih manufacture: (1) helps to destabilize the casein micelles and (2) gives the dadih its distinctive and characteristic sharp acidic taste.

14.4.5.2 Protein The proteolytic system in dadih fermentation is composed of proteinases that initially cleave milk protein into peptides and peptidases that cleave peptides to small peptides and amino acids, and the transport system is responsible for cellular uptake of small peptides and amino acids. LAB have a complex proteolytic system capable of converting milk casein into free amino acids and peptides necessary for their growth. LAB are nutritionally fastidious in nature and require several amino acids and vitamins for their growth. The overall proteolytic system of LAB is very weak, but it is sufficient to permit exponential growth in milk. The indigenous enzymes derived from raw buffalo milk may contribute to make amino acids and peptides available supporting the growth of natural LAB starter cultures.

14.4.5.3 Lipids A weak lipase activity originated from microbial contaminants of milk results is the liberation of minor amounts of free fatty acids and volatile fatty acids. In addition, natural lipases from the raw buffalo milk may contribute to the lipid metabolism. Buffalo milk contains about twice as much butterfat as cow milk and higher amounts of total solids and casein (Table 14.1), resulting in creamy textures and rich flavor profiles of dadih. Unfortunately, research on the lipid metabolism during dadih manufacture is scant.

14.4.5.4 Flavor Compounds Lactic acid, acetaldehyde, acetone, diacetyl, and other carbonyl compounds produced by fermentation constitute key flavor compounds of dadih as a result of carbohydrate fermentation, proteolytic enzyme activities, and lipid metabolisms.

14.4.5.5 Cell Mass During fermentation, natural LAB starter multiply to a count of 10^5 to 10^9 CFU/g (Yudoamijoyo et al. 1983; Hosono et al. 1989; Surono and Nurani 2001) and occupy about 1% volume of dadih product. These LAB cells contain cell walls, enzymes, nucleic acids, cellular proteins, lipids, and carbohydrates. Beta-galactosidase activity contributes a major conversion of lactose into LAB in dadih, which is beneficial for lactase deficient people.

14.6 Probiotic Properties of LAB Isolated from Dadih

The latest discoveries on gut microbiota open doors to the new mechanisms of immunity, intestinal permeability, insulin sensitivity, and weight management. Moreover, prevention and treatment of obesity reduce colon cancer, oral health such as bad breath and tooth decay, skin care such as antiaging and biomoisturizer, and manage psychological stress and the gut/brain relation could be managed by gut microbiome. Including dadih, several dairy products have been reported to consist of probiotic bacteria, which when consumed alive and in an adequate amount confer health benefit to the host (FAO/WHO 2002).

14.6.1 Probiotic Properties of LAB Strains from Dadih

Even though no heat is applied to buffalo milk while manufacturing dadih, and no good hygiene practice is implemented, there is neither product failure nor foodborne disease incidents reported among people who consumed dadih. Instead, the older generation believes that consuming dadih may provide a beneficial effect to their health. This fact has inspired more exploration of the powerful indigenous LAB involved during dadih fermentation, excluding the contaminants and the pathogens from the milk itself as well as environmental surroundings. The selection of new natural probiotics that inhibit or displace a specific pathogen can be used for further assessment, product development, and human clinical interventions on prevention or treatment of infection caused by the pathogen. This would promote human health and have a positive economic impact, especially in developing countries such as Indonesia.

Many criteria have been suggested for the selection of probiotics, including safety, tolerance to gastrointestinal conditions, ability to adhere to the gastrointestinal mucosa, and competitive exclusion of pathogens (Collins et al. 1998; Ouwehand et al. 2002; Collado et al. 2005).

Surono and Nurani (2001) found that *Lactobacillus* sp., *Lactococcus* sp., and *Leuconostoc* sp. were dominant in dadih. Further, to explore the probable probiotic isolates, Surono (2003) screened the 20 colonies of LAB isolated from dadih originated from Bukittinggi, West Sumatra, and 10 strains of dadih lactic bacteria, *Lc. lactis* subsp. *lactis*. *Leu. mesenteroides* and *Lb. casei* each had a moderate survival rate (in the range of 4.83–5.49 log CFU/ml) for 2 h at pH 2.0, while commercial starters such as *Lb. casei* Rolly C, *Str. thermophillus* Rolly T, and *B. breve* Rolly B also had the same range of survival, which was 5.34, 5.41, and 5.33 log CFU/ml, respectively, and reduced two to three log cycles from the initial concentration of viable counts.

Similarly, bile-salt tolerance of testing of dadih lactic bacteria strains *Lc. lactis* subsp. *lactis* exhibited good tolerance. Additionally, one strain of *Lc. lactis* subsp. *lactis* had good survival rate (8.11 log CFU/ml) in the presence of lysozyme after 60-min incubation.

Adhesion to the intestinal mucosa would allow colonization, although transient, of the human intestinal tract (Alander et al. 1999) and has been related to the ability to modulate the immune system, especially during its development (Schiffrin et al. 1997). Hence, adhesion is one of the main selection criteria for new probiotic strains (Havenaar et al. 1992; Salminen et al. 1999).

Collado et al. (2007a) reported that all the five strains of dadih origin showed good adhesion property, and the most adhesive was *Lb. plantarum* strain IS-10506. All LAB strains isolated from dadih fermented milk were able to significantly reduce the adhesion levels of all the pathogens tested. *Lb. plantarum* IS-10506 and *E. faecium* IS-27526 had the highest inhibition abilities. Taken together, *Lb. plantarum* IS-10506 and *E. faecium* IS-27526 have the best adhesion and inhibition properties (Collado et al. 2007a) and show inhibitory, competitive, and displacing properties against pathogens. Hence, they are promising candidates for future probiotics.

Pathogen inhibition by LAB plays an important role to protect against infection through a natural competitive barrier against pathogens in the gastrointestinal tract. Dharmawan et al. (2006) reported that among 10 LAB strains isolated from dadih on human intestinal mucosal surface, there were autoaggregation between *E. faecium* IS-27526 and *E. coli* as well as *S. typhimurium* and *H. pylori*, which might be another mechanism by which probiotics prevent the attachment of pathogens directly on the intestinal surface (Bibiloni et al. 1999; Ouwehand et al. 1999; Tuomola et al. 1999; Canzi et al. 2005). The competence of both strains IS-16183 and IS-7257 (renamed as IS-10506) in competing with *E. coli* was also reported (Collado et al. 2007a).

Aggregation ability is related to cell adherence properties (Vandevoorde et al. 1992; Boris et al. 1997; Del Re et al. 2000). Collado et al. (2007b) reported that all five strains isolated from Indonesian fermented milk product tested show higher percentages of autoaggregation. *Lb. plantarum* IS-10506 and *E. faecium* IS-23427 and IS-27526 presented the highest adhesion to hydrocarbons and also the highest autoaggregation abilities. Further, Surono et al. (2010) reported a significant increase of viable fecal LAB of rats after 3 days of administration with *Lb. plantarum* IS-10506 and *Lb. plantarum* IS-20506 at 1.2×10^{10} to 1.6×10^{10} CFU/day each, by 3.25–3.5 and 0.35–0.65 log cycles, respectively, and they continued the increment after 7 days, by 1.8–2.0 and 2.1–2.3 log cycles, respectively. The abilities of dadih LAB isolates in detoxifying mutagens and cyanobacterial toxins have been reported. The mutagen absorbed and bound to the cell wall, while the cyanobacterial toxin was being metabolized (Hosono et al. 1990; Surono and Hosono 1996; Surono et al. 2008; Surono et al. 2009).

Hosono et al. (1990) reported the ability of 36 LAB strains isolated from dadih to bind mutagenic compounds such as amino acid pyrolysates, namely, 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-b] indole (Trp-P1), 3-amino-1-methyl-5*H*-pyrido[4,3-b] indole (Trp-P2), and 2-amino-6-methyldipyrido[1,2-a:3',2'-d]imidazole (Glu-P1). *Leu. paramesenteroides* R-62 showed the highest binding ability toward Trp-P1, Trp-P2, and Glu-P1 at 99.74%, 99.65%, and 45.2%, respectively. Surono and Hosono (1996) reported the antimutagenic properties of milk cultured with LAB from dadih against mutagenic properties of terasi, an Indonesian condiment, and *Lb. casei* subsp. *casei* R-52 found to have the most. Surono et al. (2009) also reported that *E. faecium* IS-27526 exhibited in vivo antimutagenic property toward Trp-P1.

Microcystins are the main toxins produced by cyanobacteria, cyclic peptides classified as hepatotoxins, and tumor promoters. A provisional guideline level with a limit of 1 μg of microcystin-LR (MC-LR) per liter in drinking water has been established for the protection of human health protection (WHO 1998). Microcystins are chemically stable compounds (Harada 1996; Lahti et al. 1997). Conventional drinking water treatment has only limited efficacy in removing dissolved MC-LR (Svrcek and Smith 2004).

Surono et al. (2008) reported high MC-LR removal by viable *Lb. plantarum* from dadih. *Lb. plantarum* strains IS-10506 and IS-20506 at 8.6×10^{10}–1.2×10^{11} CFU and 7.6×10^{10}–1.6×10^{11} CFU viable cells, respectively, showed MC-LR removal performances at both 22°C and 37°C, but MC-LR removal at 22°C was higher after 30-h incubation, with 75% and 81% of 100 μg/l MC-LR, respectively. Another study (Nybom et al. 2008) reported the highest removal of 95% by *Lb. plantarum* strain IS-20506 (37°C, 10^{11} CFU/ml) with 1%–2% glucose supplementation and 75% in PBS as compared to other probiotic commercial strains tested.

Many other researchers reported hypocholesterolemic activity of dadih. Hosono and Tonooka (1995) reported that *Lc. lactis* subsp. *lactis* biovar. diacetylactis R-43 and R-22 of dadih origin showed high-cholesterol-binding abilities, 33.91% and 29.73%, respectively. Surono (2003) reported that *Lc. lactis* subsp. *lactis* strain IS-10285 and IS-29862 possess taurocholate-deconjugating abilities. Pato et al. (2004) found that rats fed with fermented milk made from *Lc. lactis* subsp. *lactis* strain IS-10285 showed significant ($p < .05$) lower total bile acids in serum.

All these attributes show dadih as potential health-benefitting product.

14.6.2 Novel Probiotics

The aforementioned probiotic properties of several strains isolated from dadih may provide the evidence that how strong the indigenous LAB derived from the fresh raw buffalo milk are in combating the contaminants, both spoil bacteria and pathogens, during the spontaneous fermentation of dadih. The results of research are confirming each other and demonstrating how important molecular identification for probiotic candidate. Moreover, several teams from different labs in Indonesia, Singapore, Japan, and Finland had been involved in dadih research.

Lb. plantarum strain IS-10506 and *E. faecium* had been proved to have in vitro and in vivo probiotic properties.

14.7 Dadih Probiotic for Human Health Promotion

Presently, many dairy products are popular for their therapeutic attributes. Every geographical region have at least one significant dairy product that impart health benefits. Dadih, Indonesian traditional fermented buffalo milk, is believed by the natives to be beneficial for consumers' health. This might be contributed by the probiotic properties exerted by the indigenous LAB present in dadih. Human trials have been conducted to Indonesian subjects based on in vitro and in vivo probiotic properties on two probiotic strains from dadih for their health-promotion properties.

14.7.1 Immune System and Nutritional Status

Several studies on health promotion had been conducted on *Lb. plantarum* IS-10506 originated from dadih as listed in Tables 14.4 and 14.5. Surono et al. (2011) reported a pilot randomized controlled trial study on *E. faecium* IS-27526 isolated from dadih. The supplementation of lyophilized *E. faecium* IS-27526 (2.31×10^8 CFU/day) in 125 ml ultra high temperature low-fat milk for a period of 90 days significantly increased total salivary secretory IgA (sIgA) level and bodyweight of the children ($p < .05$) compared to the placebo. Changes of total salivary sIgA levels were significantly higher in underweight children supplemented with probiotic. Weight gain was observed significantly in children with normal bodyweight supplemented with probiotic. A 90-day randomized doubleblind placebo-controlled pre–post trial has been conducted to four groups of Indonesian children aged 12–24 months, namely, placebo, probiotic, zinc, and combination of probiotic and zinc. Groups of 12 children each were supplemented with microencapsulated *Lb. plantarum* IS-10506 of dadih origin, at 10^{10} CFU/day as probiotic and 20 mg zinc sulfate monohydrate (8 mg zinc elemental) was supplemented as zinc. Blood and stool were collected at baseline and end line. Fecal sIgA was assessed by enzyme-linked immuno assay and serum zinc concentrations by ICP-MS. Fecal sIgA increased significantly in probiotic group, 30.33 ± 3.32 μg/g ($p < .01$), and in probiotic and zinc group, 27.55 ± 2.28 μg/g ($p < .027$), as compared to placebo group, 13.58 ± 2.26 μg/g. Changes of serum zinc concentration in the combination of probiotic and zinc group

Table 14.4 List of In Vitro, In Vivo, and Human Clinical Trials of Scientific-Based Evidences on Probiotic *Lb. plantarum* IS-10506 from Dadih

PROPERTIES	TYPES OF RESEARCH	REFERENCE(S)
Adhesion, colonization of strain IS-10506, increased viable fecal LAB of Balb/c mice, suppression of the allergic reaction by establishing new balance of Th1/Th2	In vivo	Endaryanto (2007)
Adhesion, colonization of strain IS-10506, increased viable fecal LAB of Sprague–Dawley rats	In vivo	Surono et al. (2010)
Adhesion, colonization of strain IS-10506, increased viable fecal LAB of apparently healthy adults	Human clinical trial	Surono et al. (2009)
Adhesion, colonization, co-aggregation, pathogen exclusion of strain IS-10506	In vivo	Dharmawan et al. (2006), Collado et al. (2007a, 2007b)
Combination of strain IS-10506 with zinc, increased fecal sIgA, zinc, and selenium level for 12–24 months of apparently healthy children	Human clinical trial	Surono et al. (2014)
Increased fecal sIgA of children living with HIV received antiretroviral therapy	Human clinical trial	Brahmantya (2013)
Strains IS-10506 and IS-20506 repaired brush border damage induced by LPS *E. coli* by repairing the brush border protein of Wistar rats	In vivo	Ranuh Gunadi Reza (2008)
Strain IS-10506 increased CD4+ cell counts of adult living with HIV strain IS-10506	Human clinical trial	Surono et al. (2010)
Strain IS-10506 modulate immune response of model infection of elderly Wistar rats induced by LPS *E. coli*	In vivo	Kadir (2013)
Strain IS-10506 significantly increased TGF-β and IL-4 of children living with HIV received antiretroviral therapy	Human clinical trial	Radhiah (2013)
Strain IS-10506 significantly reduced blood lipopolysaccharide level in HIV-infected children with antiretroviral therapy		Dwiastuti (2013)

showed the highest elevation at end line. A combination of probiotic *Lb. plantarum* IS-10506 at 10^{10} CFU/day and 8 mg zinc elemental supplementation showed potential ability in improving zinc status of preschool children. Supplementing probiotic *Lb. plantarum* IS-10506 and zinc for 90 days resulted in a significant increase of humoral immune response as well as improved zinc status of the young children (Surono et al. 2014).

Table 14.5 List of In Vitro, In Vivo, and Human Clinical Trials of Scientific-Based Evidences on Probiotic *E. faecium* IS-27526 from Dadih

PROPERTIES	TRIAL	REFERENCE(S)
Adhesion, colonization of strain IS-27526, increased salivary sIgA and bodyweight gain of apparently healthy young children	Human clinical trial	Surono (2011)
Adhesion, colonization of strain IS-27526, increased viable fecal LAB of apparently healthy young children, increased serum IgA	Human clinical trial	Riewpassa (2004)
Adhesion, colonization of strain IS-27526, increased viable fecal LAB of young children, increased fecal sIgA of young children	Human study	Catur Adi (2011)
Adhesion, colonization, co-aggregation, pathogen exclusion of dadih strains	In vitro	Dharmawan et al. (2006), Collado et al. (2007a, 2007b)

14.8 Concluding Remarks

Recent advancement led to investigate the attributes of traditional foods. Dadih is an important traditional food of Indonesia. It consists of many natural microflora that make it nutritious and a healthy product. Dadih contains various LAB microflora with probiotic attributes. However, if the flora of dadih is changed, similarly the composition of dadih also changes. Standard protocol and microflora are yet to be formulated to make this product more significant. Further investigation is needed to explore the complete characteristics of this Indonesian product.

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