The Role of LAB during Fermentation on Safety and Quality of Fermented Foods

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The Role of Lactic Acid Bacteria during Fermentation on Safety and Quality of Fermented Foods

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Summary

Indonesia, one of Asian countries, has many kinds of fermented food which coverage area are from Sumatera to Papua. Fermented food product is processed food through fermentation process, which certain microorganisms play an important role during the process. Growth of undesirable wild microorganisms may cause fermentation failure and results undesirable products. Many researches have been done to explore fermented foods from several areas such as *dadih* and *tempoyak* (Sumatera); *ikan peda*, *petis*, *oncom*, *tape*, *tempe* and *kecap* (Jawa); *brem*, *urutan*, *bebontot*, *brengkes* (Bali); *perahancak* and sour horse milk (NTB); *sei* (NTT); and *bekasang* (Sulawesi). From these products are also explored many kinds of useful microorganisms. Most of them are a group of lactic acid bacteria (LAB), which has been used as starter culture and probiotic as well. By using LAB as starter culture showed some benefit such as accelerate the process and ensure quality and safety of the products. During fermentation, LAB produce lactic acid and other metabolites that respectively lower the pH and limit the growth of pathogenic microorganisms. The bacteria also release hydrolytic enzymes (lipases and proteases), which are able to break down macromolecules, such as lipids and proteins, resulting in the production of precursors for specific aroma. The discussion in this paper is focused on the role of LAB on safety and quality of fermented food, especially *urutan* (Balinese fermented or dried sausage), sour horse milk, and pickle of bamboo shoot. The LAB potential isolated from those natural products are potential to use as starter culture. The bacteria as starter culture can control the fermentation and prevent the growth of pathogenic bacteria. *Pediococcus acidilactici* U318 isolated from *urutan* is the bacteriocin-producing bacteria, which could inhibit the growth of undesirable bacteria in the product. The bacteria produce bacteriocin and organic acids, which may suppress the growth of pathogenic bacteria and ensure the product safety. The growth of LAB in horse milk during storage could control the milk safety and the bacteria themselves are promoted as probiotic, which give a positive impact to the human health.

Key words: Lactic acid bacteria, fermented food, *urutan*, sour horse milk

Introduction

Fermented food product is processed food through fermentation process, which certain microorganisms play an important role during the process. Growth of undesirable wild microorganisms may cause fermentation failure and results undesirable product. Microorganisms involved during fermentation produce the expectation product which relate to the characteristics of the end product such as characteristics of physico-chemical, sensory, and safety. During fermentation they lower the pH and limit the growth of pathogenic microorganisms, as well as releasing hydrolytic enzymes (lipases and proteases), able to break down macromolecules, such as lipids and proteins, resulting in the production of precursors for specific flavor of each fermented food. In general, the objective of fermentation is to preserve, reduce toxin, and increase nutrition value and flavor of food products. Fermentation also increases the safety, stability, and acceptability of food fermented products.

Natural fermentation that depended on contribution of indigenous microorganism takes longer time with high risk of failure. At the initial stage of fermentation, microorganisms from raw material, equipment, and environment start to grow, and gradually increase their quantity and compete in using nutrition to produce metabolites. This initial phase of fermentation can be accelerated by back-sloping or inoculation of selected cultures. It has been approved that starter culture isolated from one product is fail doing their activity if applied to different product (Ordonez *et al.*, 1999; Gariga *et al.*, 1996). Using of starter culture that has been developed for more than 50 years is done in order to accelerate process of fermentation, decrease using of food additive (preservative and coloring material), and standardize sensory characteristics of the product (Hugas dan Monfort, 1997).

Indonesia as diversity country has many kinds of foods including fermented foods. Non-fermented foods develop well with variety, and with standardized quality and safety. On the other hand, only a few fermented food products develop with good standard and have been produced commercially, such as *kecap*, *tempe*, *oncom*, and *terasi*. Most of Indonesian fermented foods do not develop well doe to inconsistence of process, so that the quality and safety of the products are not conformed to consumer. Many researches have been done to improve the quality and safety of the products. Fermented food products with standardized and consistent quality and safety may be led to produce them commercially in industrial scale.

Indonesia, one of Asian countries, has many kinds of fermented food which are coverage from Sumatera to Papua. Many researches have been done to explore fermented food from the area such as *dadih* and *tempoyak* (Sumatera); *ikan peda, petis, oncom, tape, tempe* and *kecap* (Jawa); *brem, urutan, bebontot, brengkes* (Bali); *perahancak* and sour horse milk (NTB); *sei* (NTT); and *bekasang* (Sulawesi). Most of fermented foods from the area of East Indonesia have not been explored yet. Indigenous fermented foods can be developed and improved their safety and quality by using their indigenous cultures.

In general, process of food fermentation involve many kinds of microorganisms, such as bacteria, mold, and yeast, which grow and do the activity together in food material. Most of them are a group of lactic acid bacteria (LAB), which has been used as starter culture and probiotic as well. Using LAB as starter culture showed some benefit such as accelerate the process and ensure quality and safety of the products. During fermentation LAB produce lactic acid that lower the pH and limit the growth of pathogenic microorganisms, as well as releasing hydrolytic enzymes (lipases and proteases), able to break down macromolecules, such as lipids and proteins, resulting in the production of precursors for specific aroma. This paper is limited to discuss the promising LAB that explore from *urutan* and sour horse milk, and their potencies to be used as starter culture and other health benefit.

Strains LAB Isolated from *Urutan*

Urutan as an indigenous product, traditionally made by Balinese people, is fermented under natural conditions. The temperature fluctuates with the average of about 35°C, which is the highest temperature in the day (up to 50°C under sun drying condition) and falls down to the lowest temperature (25°C) at night. Typically, a tropical country is high in humidity with relative humidity ranging from 80-90%.

The use of local types of spices, especially aromatic ginger and turmeric, and the high concentration of garlic make *urutan* intrinsically different with the European-style fermented sausages. The extrinsic and intrinsic factors can affect the growth and distribution of bacterial flora during *urutan* fermentation. Group bacteria of LAB dominate the growth of microorganisms during fermentation. LAB present in *urutan* during fermentation are identified as homofermentative type, which produced abundant lactic acid and a small amount of acetic acid as well as carbon dioxide (CO_2).

The rapid acidification occurred on the first day of fermentation, due to the lactic acid produced by LAB. The lactic acid denatured the meat protein and enhanced the release of water from the product. Consequently, the water activity of *urutan* decreased and the texture of *urutan* became more compact. This process inhibited the growth of *Enterobacteriaceae*, and had a preservative effect on *urutan*.

Aryanta (1996) reported that the genus of *Lactobacillus* and *Pediococcus* were present in *urutan* during fermentation by using phenotypic characteristics. Using these characteristics to identify LAB has some limitation to accurately identify the LAB up to the species or strain level. In my study, I used phenotypic characteristics and 16S rDNA sequence analysis for grouping and identifying the isolates, respectively. From the identification (Table 1) was found that three strains of *Lb. plantarum* were involved in *urutan* fermentation which was shared 52.1% of the total LAB strains in *urutan*. Four strains of *Lb. farciminis* which shared 21.1% of total LAB strains were explored from *urutan*. The obligate heterofermentative strains that present in small amount were identified as *Lb. fermentum* and *Lb. hilgardii*. The heterofermentative LAB shared 4.2% of total LAB strains isolated from *urutan*. All of the cocci shape LAB were tetrad cocci, which were identified as *P. acidilactici* and *P. pentosaceus* contributed 15.5% and 7.0% of all LAB strains, respectively. These strains of LAB are the promising indigenous strains to use as starter culture for improving its quality and safety.

Table 1. Similarities of the representative isolates with the reference strains from GenBank based on 16S rDNA sequence[a]

Representa-tive strains	Length of sequences	Closest species match	Type of ref. strains	Accession Number	Simi-larity (%)
U201	1540	*Lb. plantarum*	DSM20205	M58827	99
U102	1573	*Lb. plantarum*	DSM20205	M58827	99
U309	1559	*Lb. plantarum*	DSM20205	M58827	99
U312	1562	*Lb. farciminis*	ATCC29644	M58817	97
U315	1493	*Lb. farciminis*	ATCC29644	M58817	98
U501	1565	*Lb. farciminis*	ATCC29644	M58817	98
U509	1567	*Lb. farciminis*	ATCC29644	M58817	97
U310	1573	*Lb. fermentum*	ATCC14931	M58819	99
U511	1581	*Lb. hilgardii*	DSM20176	M58821	94
U318	1573	*P. acidilactici*	DSM20284	M58833	98
U504	1560	*P. acidilactici*	DSM20284	M58833	98
U208	1542	*P. pentosaceus*	DSM20336	M58834	99

U306	1581	*P. pentosaceus*	DSM20336	M58834	99

[a]Antara *et al*., 2002.

Using of Indigenous Strains as Starter Culture

Two strains of LAB, *Lb. plantarum* U201 and *P. acidilactici* U318, were experimented to produce *urutan* as single culture and co-culture starter. The experiment result showed that using of indigenous starter culture could suppress the growth of *Enterobacteriaceae* and group of micrococci (Fig. 1). *Enterobacteriaceae* were not detected after 24 h and 72 h fermentation in *urutan* inoculated with *L. plantarum* U201 and *P. acidilactici* U318, respectively. The inhibition of *Enterobacteriaceae* was more effective using a mix starter culture containing *L. plantarum* U201 and *P. acidilactici* U318 in which the *Enterobacteriaceae* was below our detection level (less than 10^2 cfu/g) within 24 h fermentation. On the contrary, *Enterobacteriaceae* could survive throughout the fermentation when the LAB species were not inoculated into the meat batter. On the other hand, micrococci bacteria decreased after 24 h fermentation in *urutan* inoculated with *L. plantarum* U201. In *urutan* inoculated with *P. acidilactici* U318, micrococci bacteria increased at the initial stage and sharply decreased after 48 h fermentation. In the control lot, micrococci bacteria increased in the initial stage and survived in high numbers until the end of fermentation. At the end of fermentation, the population of micrococci bacteria could not be detected in *urutan* inoculated with *P. acidilactici* U318 (Fig. 2B). Nevertheless, *L. plantarum* U201 did not totally inhibit the micrococci bacteria and its population was detected at a level as high as 4.0 x 10^2 cfu/g at the end of fermentation (Antara *et al*., 2004).

Enterobacteriaceae and micrococci were found in high numbers in *urutan* produced by spontaneous fermentation. The presence of high *Enterobacteriaceae* count caused hydrogen sulphide odours of sausage, which diminished its overall acceptability (Gariga *et al*., 1996). Our result (Antara *et al*., 2004) showed that used of starter culture could control the growth of these bacteria. The use of *P. acidilactici* U318 and *L. plantarum* U201 either as a single or co-culture starter culture, suppress the growth of *Enterobacteriaceae* and micrococci bacteria in *urutan* up to a safe level. The rapid growth of LAB at the initial stage of fermentation is beneficial in lowering the pH of *urutan*, which is responsible for reducing or eliminating the undesirable bacteria, such as *Enterobacteriaceae* in *urutan*. In addition, the results revealed that *P. acidilactici* U318 showed complete inhibition of the growth of micrococi at the end of fermentation. Meanwhile, about 10^2 micrococcal cells were still encountered when *urutan* was inoculated using *L. plantarum* U201. Since the later stage of *urutan* fermentation was dominated by tetrad forming cocci cells (presumably *P. acidilactici* U318), such inhibition might be due to the bacteriocin activity produced by this strain (Antara, 2004).

The raw material of sausage, especially fresh meat, contains diverse bacteria. The growth of undesired bacteria has to be reduced or eliminated to an acceptable level and it can be achieved throughout fermentation. Since the fermented sausage may still be inhabited by pathogenic bacteria which some times causes food borne diseases, therefore its safety depends on successful fermentation and the use of starter culture is one of the premier options. *P. acidilactici* has also been demonstrated to be able to reduce pathogenic bacteria in salami fermentation (Kang and Fung, 2000) as well as in fish sausage fermentation (Aryanta *et al*., 1991).

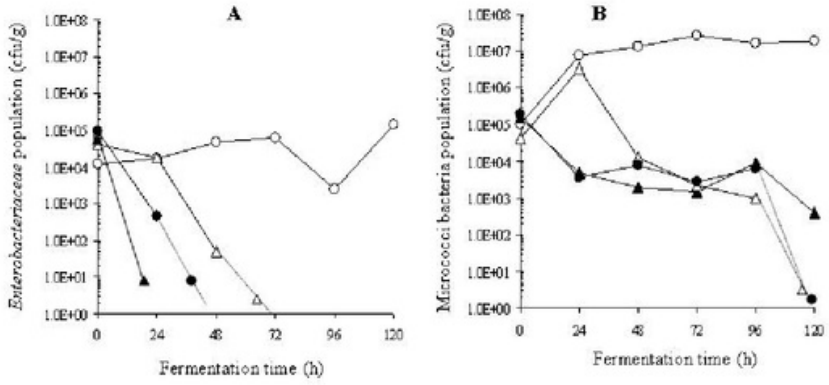
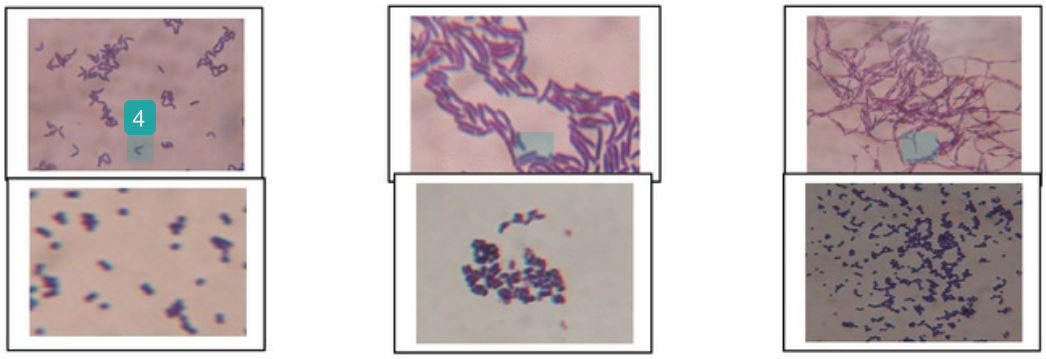


Fig. 1. Growth of *Enterobacteriaceae* (**A**) and micrococci bacteria (**B**) during *urutan* fermentation. Symbols: control (○); inoculated with *Lb. plantarum* U201 (●), inoculated with *P. acidilactici* U318 (△), inoculated with co-culture of *Lb. plantarum* U201 and *P. acidilactici* U318 (▲).

Strains LAB Isolated from Sour Horse Milk

Traditionally, fermented milk products have already been known and consumed by Indonesian for long time, such as *dadih* in West Sumatera (Sugitha, 1996; Sugitha, 2004), *dangke* in South Sulawesi (Suryono, 2003), fermented horse milk in West Nusa Tenggara (Hermawati *et al*., 2004). The indigenous microorganisms that play important role in fermentation process are lactic acid bacteria (Oberman, 1985; Aryanta, 1995). These bacteria are used widely in fermented food as starter cultures, such as in dairy products (Parente *et al*. 1997; Fitzsimons *et al.* 1999), fermented vegetables (Sanchez *et al.* 2000; Kalac *et al.* 2000), alcoholic beverages (Patarata *et al.* 1994; Pattison *et al.* 1998), and fermented meat (Hammes and Hertel 1998; Antara *et al*. 2002). Some species of LAB have been used in industry scale as culture starter and also as probiotic (Goldin, 1998; Salminen *et al*., 1998).

Exploration of LAB from fermented food Indonesia origin and using them as probiotic may contribute in the development of science and technology. Widiada, *et al.* (2006) have isolated and identified 6 species of LAB from fermented horse milk of Bima namely *Lactobacillus acidophilus*, *Lb. brevis*, *Lb. plantarum*, *Lb. salivarius*, *Lb. delbrueckii* dan *Lactococcus lactis* (Fig. 2).



(d) (e) (f)

Fig. 2. Morphologycal performance of LAB isolated from horse milk of Bima: (a) *Lb. brevis*, (b) *Lb. plantarum*, (c) *Lb. acidophilus*, (d) *Lb. salivarius*, (e) *Lb. delbrueckii* subsp.*delbrueckii*, dan (f) *Lc. lactis* subsp. *lactis*.

Species of LAB isolated and identified were monitored during storage of milk. During storage of horse milk at room temperature was occurred the changes of milk characteristics such as pH of milk decreased during storage caused by increasing of acidity of the milk. The changes of acidity caused the succession of the LAB growth during storage (Fig. 2.). All species population grew on the first day storage, and then change on the next day of storage. On the 10 days storage three species, *Lb. salivarius*, *Lb. acidovilus* and *Lb. brevis*, were still available in the milk and two other species were disappeared. The milk stored for 20 and 30 days one species of *Lb. salivarius* was disappeared, and two species of *Lb. acidovilus* and *Lb. brevis* were still available in milk. This succession was very interesting because only two species of LAB could survive in the milk that stored for more than 20 days.

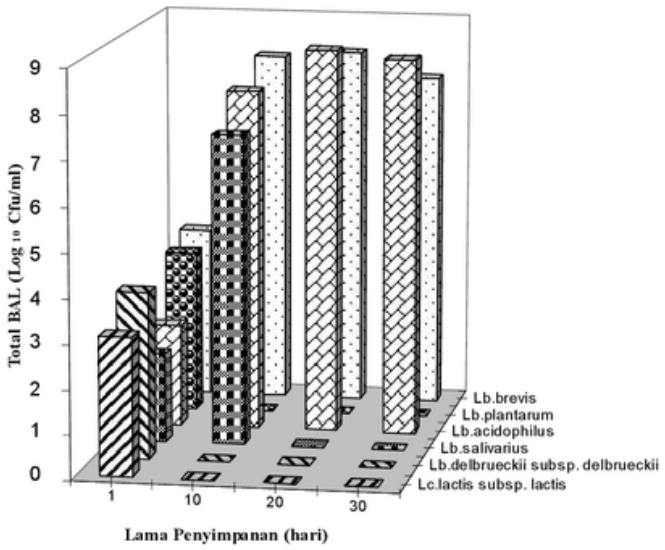


Fig. 3. Succession of LAB species in horse milk during storage.

Indigenous Strains as Probiotic

From identification strains of LAB isolated from sour horse milk, there are six strains identified as promising strains tested as probiotic. The acidity (pH) and survival time, survival in medium contain bile salt, ability of LAB adhere and colonize on mucosa intestinal epithelium, ability to reduce blood cholesterol, and pathogenity are the indicators used to select microorganism (bacteria) as probiotic.

Result of the experiment showed that all of the LAB strain tested could not survive on extremely acid medium (pH 1.5). Only *Lb. brevis* KBa could survive on pH 2.0 medium for 2 and 3 h. And on the pH 2.5 medium two strains of *Lb. brevis* KBa and *Lb. acidophilus* KBc could grow for 2 and 3 h, and the others were death. Determination of growth ability of LAB in medium contain bile salt is based on the condition of human intestinal track. In general, all six LAB strains could grow well in the medium which contains 0.750% and

1.875% bile salt. In medium with 3.75% bile salt only *Lb. acidophilus* KBc and *Lb. brevis* KBa can grow well. One strain LAB tested, *Lb. acidophilus* KBc, could grow in extremely bile salt concentration (5.625%).

The ability of LAB adhere and colonize on mucosa intestinal epithelium was tested in vivo using mice as trial animal. Re-isolated administrated LAB was used as indicator on this experiment. The experiment result showed that *Lb. acidophilus* KBc and *Lb.brevis* KBa could be re-isolated from mice feces and wall of intestinal mucosa after 7 days administration. Effect of LAB administration for 4 weeks on blood serum cholesterol is shown in Table 2. *Lb. acidophilus* KBc and *Lb. brevis* KBa may reduce rabbit blood cholesterol significantly ($P < 0,05$) up to 54.15% and 48.95%, respectively.

Table 2. Effects of LAB administration on blood serum cholesterol of rabbit[§]

Treatment[x]	Cholesterol concentration[y,z] (mg/dl)					
	SP	HK	M1	M2	M3	M4
KN	35.7^{a}	35.3^{a}	34.3^{a}	33.3^{a}	34.0^{a}	36.0^{a}
KP	37.3^{b}	303.3^{a}	294.7^{a}	289.0^{a}	284.0^{a}	280.7^{a}
LA	36.0^{e}	278.0^{a}	255.3^{ab}	218.3^{bc}	171.3^{cd}	128.7^{d}
LB	29.7^{d}	296.7^{a}	260.7^{a}	195.7^{b}	158.0^{b}	143.3^{c}

[x] KN: no treatment control; KP: hypercholesterol control; LA: hypercholesterol and *Lb.acidophilus* KBc administration; LB: hypercholesterol and *Lb.brevis* KBa administration.
[y] SP : before treatment; HK: hypercholesterol treatment; M (week).
[z] Value followed by different character on the same row states significantly different ($P<0,05$).
[§] Antara *et al.*, 2009.

Table 3. Safety of LAB isolates on mice (*Mus musculus*)[§]

Group	Mice status	Hystopathological Changes	Reisolated bacteria
Positive control *Past. Multocida*	Death	Lever : congestion, netrophile cell infiltration, detection of colony Kidney : congestion on glomerulus Intestine : villi erotionerosi, netrophilium cell infiltration, and necrosis of epithelium cell	Isolated
Strep. Zooepidemicus	Death	Lever : congestion, netrophile cell infiltration, detection of colony Kidney : congestion on glomerulus Intestine : villi erotionerosi, netrophile cell infiltration, and necrosis of epitel cell	Isolated
Negative control	Life	Normal, there was no demage	Not isolated

Lb. acidophilus KBc	Life	Normal, there was no demage	Not isolated
Lb. plantarum KBb	Life	Normal, there was no demage	Not isolated
Lb. salivarius KBd	Life	Normal, there was no demage	Not isolated
Lb. brevis KBa	Life	Normal, there was no demage	Not isolated
Lb. delbrueckii KBe	Life	Normal, there was no demage	Not isolated
Lc. Lactis KBf	Life	Normal, there was no demage	Not isolated

§ Antara *et al*., 2009.

Clinical observatioan of the mice administrated by LAB showed that the mice were not sick or death after 14 days LAB administration. On the other hand, the mice treated by pathogenic bacteria (positive control) were death after 3th day (Table 3). Result of observation also showed that the LAB administrated did not invade the lever, kidney, lung and hearth of mice, and also there was not pathological change of the organ. On the group of mice that orally administrated by pathogenic bacteria (*Past. multocida* and *Strep. zooepidemicus*) found that the damage and infiltration of netrophilium cell, villi erosion, necrosis of epithelium cell, congestion, and there were colonies of the bacteria found in lever tissue. Clinical observation on trial mice showed that there was not found death or sick mouse and damage of organ, and there was no re-isolated LAB from mice organ. This evident approve that the strain LAB isolated from Bima horse milk were safe and could be used as safe food.

In general, six of LAB strains isolated from Bima horse milk (*Lactobacillus acidophilus* KBc, *Lb. brevis* KBa, *Lb. plantarum* KBb, *Lb. salivarius* KBd, *Lb. delbrueckii* KBe and *Lactococcus lactis* KBf) are safe microorganisms used in food. They could grow in medium with bile salt in concentration of 0.75%. Two species out of six, namely *Lb.brevis* KBa dan *Lb.acidophilus* KBc, could survive in low pH medium (pH2.5) for 3 h. These two species could adhere and colonize on mice intestinal mucosa epithelium, and could reduce blood serum cholesterol of rabbit in hypercholesterolmia condition up to 54.15% dan 48.95% for *Lb.acidophilus* KBc and *Lb.brevis* KBa, respectively. These strains of indigenous LAB accomplish requirement used as probiotic.

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The Role of LAB during Fermentation on Safety and Quality of Fermented Foods

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SUNDAY, July 24, 2016

No	Time (WIT)	Activity	PIC
1	08.30 - 09.00	Registration	Rahma
2	09.00 - 09.05	Opening	MC
3	09.05 - 09.15	Welcome Speech	G.N. Wididana
4	09.15 - 09.25	Welcome Dancing	Lidya
5	09.25 - 10.00	Speech from Rector of Unas and Keynote Speech: Prof. Dr. Ernawati Sinaga Prof. Dr. Takashi Hayase	Dr. Sri Endarti R.
6	10.00 - 10.10	Coffea Break	Tri Suharti
7	10.10 - 11.10	Presentation dan Discussion Session I : 1. Kanit Muangnil, M. Sc., MBA 2. Prof. Yasuhiro Ishibashi, PhD 3. Dr. Shintani Masaki **4. Prof. Dr. Nyoman Semadi Antara**	Moderator: Dr. Sri Endarti R.
8	11.15 – 12.30	Presentation dan Discussion Session II : 1. Dr. Mahendra 2. Dr. Xu Hui Lian 3. Mr. Paul Daly 4. Dr. T. Nakamichi	Moderator : Dr. Sri Endarti R.
9	12.30 – 13.30	Break (Lunch and Pray)	Kadek Shiro
	13.30 - 15.10	Presentation Call Paper Paralel	Gautama W.
10	13.30 - 13.40	Presentation Call Paper 1	
11	13.40 - 13.50	Presentation Call Paper 2	
12	13.50 - 14.00	Presentation Call Paper 3	

13	14.00 - 14.20	Discussion Presentation	
14	14.20 - 14.30	Presentation Call Paper 4	
15	14.30 - 14.40	Presentation Call Paper 5	
16	14.40 - 14.50	Presentation Call Paper 6	
17	14.50 - 15.10	Discussion Presentation	
18	15.10 - 15.30	Coffea Break	Kadek Shiro
	15.30 - 17.10	Presentation Call Paper Paralel	Gautama W.
19	15.30 - 15.40	Presentation Call Paper 7	
20	15.40 - 15.50	Presentation Call Paper 8	
21	15.50 - 16.00	Presentation Call Paper 9	
22	16.00 - 16.20	Discussion Presentation	
23	16.20 - 16.30	Presentation Call Paper 10	
24	16.30 - 16.40	Presentation Call Paper 11	
25	16.40 - 16.50	Presentation Call Paper 12	
26	16.50 - 17.10	Presentation Call Paper 13	
27	17.10 - 17.20	Discussion Presentation	

MONDAY, July 25, 2016

No	Time (WIT)	Activity	PIC
1	08.00 - 08.30	Registration	Rahma/Tri Suharti
2	08.30 - 08.40	Briefing	Gautama W.
3	08.40 - 08.50	Prepairing	
4	09.00 - 11.00	Bokashi Oil Factory	Kadek Shiro
5	11.00 - 13.00	Paddy Terrace at Tegalalang Ubud, Bali	K.Tisnawati
6	13.00 -14.00	Lunch and Pray	Tri Suharti
7	14.00 -16.00	Tanah Lot	Kadek Shiro
8	16.00 -17.00	Krishna (Souvenir's shop)	K.Tisnawati
9	17.00 - End	Finished	

ORGANIZED BY :

FACULTY OF AGRICULTURE - FACULTY OF BIOLOGY
COMMUNITY EMPOWERMENT CENTRE UNAS