Fermentation of Green Seaweed *Ulva fasciata* Using Six Different Strain of Lactic Acid Bacteria (LAB)

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Abstract. Marine fermented products nowadays are commonly made from fish or shrimp, but marine plant like seaweed which are considered as a valuable source of nutritous food has yet produce popular fermented food. Research correlated with seaweeed fermented-based product intended for consumption is still very few. Therefore, this research aims to investigate the fermentation of green seaweed *Ulva fasciata* using lactic acid bacteria (LAB) to seek potential source of functional food. Six different strains of LAB were used as a starter culture in the fermentation. Previously, spontaneous fermentation was conducted and the colonies appeared were analized using DNA sequencing to see the native LAB presented in the seaweed. However, the result showed that there was no potential LAB that existed in this seaweed therefore, six strain of LAB inoculum were added in the following fermentation: filtered seaweed extract (FSE) and fresh seaweed. FSE was used as an initial trial of fermentation on all strain to see if this strain could survive in the *U. fasciata*. After getting a positive result, fermentation was conducted in the fresh seaweed with the addition of each strain and was treated with and without the addition glucose. All samples were investigated during fermentation by measuring the pH and the number of cell growth and the changes of appearances and odour were also examined. The result showed that all bacteria strain able to grow in the seaweed and was indicated by the increasing number of the cell during the investigated time point. The addition of glucose favours the fermentation by decreasing the pH in all sample.

Keywords: Ulva fasciata · seaweed · lactic acid bacteria · fermentation · sea lettuce

1 Introduction

Seaweeds are considered as a valuable source of nutritious food that contain polysaccharides, protein, mineral and some vitamin. It has long been consumed in various dishes in many countries such as soup or condiment, but so far not popular as fermented product.

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Marine fermented product nowadays are commonly made from fish or shrimp but no food product have yet been produced from marine plant like seaweed. Most of research of seaweed fermentation have focused on bioenergy, such as biodiesel, bioethanol, biomethane and bio-oil. Other research correlated with seaweed fermented-based product intended for consumption is still very few. *U. fasciata* is known as "sea lettuce" because its thallus-leafy part of seaweed resemble lettuce that is thin, broad and rounded or oval at the edge. Although considered as sea vegetables, seaweed is different from the normal vegetables grown in the land. *U. fasciata* mainly consist of complex sugar namely rhamnose that account for almost a half of the total sugar and the rest are xylose, mannose and glucose (Shao *et al.* 2015). This sugar composition is contrast with some land vegetables that are commonly fermented with LAB such as cabbage which contain glucose and fructose as the main sugars (Nei *et al.* 2006). Simple sugar like glucose and fructose are utilized easily by LAB for their metabolism which then converted into organic acid that shape the characteristic of fermented product. The crude polysaccharide of *U. fasciata* consist of total sugar, crude protein, crude fibre and ash. *Ulva* sp. Has a distinct polysaccharide called ulvan which is water soluble polysaccharides and composed of sulphate rhamnose, xylose and glucuronic acid or glucose that present in the same chain (Shefer *et al.* 2017). Ulvan has a gelling property by the presence of boric acid, ion calcium and a Ph between 7.5–8.0 (Lahaya *et al.* 2007). The presence of cellulose and hemicellulose in ulvan cell wall make it cannot be degraded by human endogenous enzyme in the intestine because the presence of cellulose and hemicellulose constituted in the cell wall and thus, belongs to dietary fibers..

Fermentation is a common method to naturally extend the shelf-life and improves the sensory properties and nutritional value of vegetables, fruits, or meat. There are many kinds of fermented food that are widely consumed across the world such as kimchi, pickles, and sauerkraut. LAB is considered to be most involved bacteria in the food fermentation which promote a beneficial effect on health through the inhabitation of pathogenic bacteria and toxin production of other bacteria. *Lactobacilli* and *Lactococcus* are amongst the most important because these bacteria produce bacteriocins, antimicrobial peptides that enable to inhibit the growth of mold which then ensure the stability of the fermentation process (Juodeikene *et al.* 2009). The extensive historical use of natural or selected starter LAB in the food fermentation has contributed to the GRAS (generally recognize as safe) status for human consumption. Metabolic process of LAB also triggers the production of specific flavor and aroma in the product that give a consistent sensory and nutritional quality.

Seaweed *U. fasciata* is popular among people living near coastline to forage and harvest this seaweed to make local dishes. In some countries in Europe such as France, green seaweed is consumed as sea vegetable under trade names of "sea lettuce", same as its common name (Fleurence *et al.* 2016). Although it has been reported that fermented seaweed has been available in the market but, the study of fermented seaweed is very few. Previous study of seaweed fermentation using LAB strain have been reported by Uchida *et al.* (2004), Gupta *et al.* (2010–2012) and Rocchi (2017). The potential of seaweed as functional food based through fermentation process have been developed from three different kind of Irish brown seaweed: *Himathalia elongate, Laminaria digitata,* and *Laminaria saccharina,* tested raw and heated (Gupta *et al.* 2011). The results showed

that *H.* elongata did not support the growth of *L. plantarum* in both treatment while, heated *L. digitata* and *L saccharina* were found to be suitable to support the growth of *L. plantarum* with the maximum specific growth rate of the cell population (μmax) around 0.24 and 0.34 per hour, respectively. This research was continued to explore the potential of *Saccharina latissimi and Laminaria digitata* seaweed as a sole nutrition source of a probiotic bacterium, *Lactobacillus rhamnosus* (Gupta *et al.* 2012). The results demonstrated that *L. rhamnosus* grew well on both species reached nearly 10 log CFU/ml after 16 and 24 h of fermentation. Another study on seaweed fermentation was conducted by Marrion *et al.* (2003) on red alga (*Palmaria palmata*) which contain high amount of xylan and insoluble fiber that make it has a weak digestibility. By giving physical treatment and fermentation with three different microorganisms the digestibility could be improved. Among the treatments given in the study, *Trichoderma pseudokoningii* was reported to hydrolyze polysaccharide most efficiently compared to other mold strains. To see the potential of functional food through fermentation, this research aims to investigate fermentation of green seaweed *Ulva fasciata* using six different strain of lactic acid bacteria. Due to the lack references of spontaneous fermentation of this seaweed and the autochthonous LAB from this seaweed is still unknown, using starter culture of LAB would be favorable both from a hygiene and safety perspective therefore, 6 different strains of LAB were used in the experiment.

2 Material and Method

Green seaweed, *Ulva fasciata* was obtained from Bribie Island Research Center (BIRC) located in Queensland, Australia. This seaweed was cultured in the aerated tank with controlled water supply. Seaweed was collected in the afternoon on 18th September 2018. Prior to collection, the water was removed by squeezing the seaweed and transferred into zip lock plastic bag and put into the cold box with ten ice gel to keep the temperature low during transportation to Microbiology laboratory in the University of Queensland. The sample then stored in the freezer at 20 ° C. The second batch of seaweed was sent from BIRC using Styrofoam box, and the seaweed was put into plastic bag. Once it arrived, it was packed into a smaller vacuum bag and sealed and stored at -80 ° C. Before analysis, it was thawed in the 4 ° C fridge and washed with distilled water and then was squeezed to remove the remaining water. It is then chopped using knife with the size of 0.5–1 cm wide and put in the clean glass jar for fermentation.

Lactobacillus plantarum 299V was utilized for the preliminary experiment of fermentation in fresh seaweed and at the same time, spontaneous fermentation was conducted as a control and at the same time to see whether or not native LAB present in the seaweed *U. fasciata.* Strain *L.plantarum 299V* was obtained from a capsule of Ethical Nutrient IBS (Irritable Bowel Syndrome) Support and the company claim that each capsule contains 20 billion live bacteria. Different treatments were conducted for both samples that includandifferent percentage of NaCl (3 and 6%), incubation temperature (12 ° C and room temperature) and the size of seaweed (cut and uncut). Overall, there are 16 different sample from this treatment. pH was measured each day for 8 days of fermentation and cell counting was conducted at the beginning, middle and end of fermentation. The appeared colonies with different shape, color and size were analyzed to

find out the species by using RAPD-PCR for DNA amplification and DNA sequencing. RAPD-PCR was conducted using the method performed by Dong *et al.* (2017).

After the preliminary study, fermentation in the filtered seaweed extract (FSE) and fresh seaweed were conducted using inoculum from six different strain of LAB isolated from various vegetables and fruits. They are: *Lactococcus lactis 537*, *Weisella confusa 44*, *Weisella cibaria 752, Leuconostoc mesenteroides 109*, *Leuconostoc holzapfelii 733*, and *Leuconostoc lactis 824.*

2.1 Preparing Filtered Seaweed Extract

FSE is obtained through filtration using microfilter as a sterilization method. Fresh seaweed was grounded using mortar with the addition of distilled water, and the ratio between fresh seaweed and distilled water was two third. It was then transferred into 50 ml falcon tube and centrifuged at 5000 rpm for 30 min at 4 ° C and then supernatant was removed into 15 ml Eppendorf and centrifuged again at 17000 g for 10 min using McHugh Electronic Hereus Pico 17. The supernatant was then collected into 30 ml yellow cap tube and filtered using microfilter size 22 μ l and wrapped with aluminum foil and stored in the 20 ° C for the analysis.

2.2 Subculturing the Strain

Glycerol stock of each strain that stored at -80 ° C was thawed at the room temperature and 10 ml of MRS broth in the yellow cap tube was prepared as resuscitation media for bacteria. Sterilized metal loop was put into the cryogenic bottle to take the pure culture. It was then transferred into the yellow cap tube by dipping it in the MRS broth for several times. The bottle was sealed tightly and.

2.3 Preparing Cell Pellets

1 ml from 10 ml overnight culture was taken and transferred into 1.5 ml Eppendorf, the cell suspension was then centrifuged at 12000g for 2 min. The supernatant was removed and the remaining MRS broth was pipetted to ensure no MRS broth left in the cell. The cell pellet of bacteria sticks at the bottom of the Eppendorf. One ml of 0.85% NaCl solution was then added and mixed to wash the cell and this was done twice. Again, the washed cell suspension was centrifugated at 12000g for 1 min and resuspended with 1 ml of 0.85 NaCl solution and mixed thoroughly using vortex. After that, the cell suspension was diluted using 0.85% NaCl 1000 times and this suspension was kept for inoculation to the fresh seaweed.

2.4 Fermentation on FSE

Six different strain of LAB that have been prepared was diluted 10000 times and 50 μl from this suspension was taken to put in the 4950 μl FSE in the yellow cap-tube. The total of 5 ml solution was then centrifuged thoroughly and put in the rack in the 30 ° C incubator. pH measurement was conducted every t = 0, 2, 4, 6, 8, 28 and 36 using

Table 1. Formulation of each fresh seaweed fermentation using 6 different strains

Fermentation sample	Formulation
Control (without glucose)	40 gr seaweed + 60 ml autoclaved water
Control (with glucose)	40 gr seaweed + 1.5 ml of 20% glucose stock + 58.5 ml autoclaved water
Strain x^{1-6} (without glucose)	40 gr seaweed + 60 μl cell suspension + 59 ml and 940 μl autoclaved water
Strain x^{1-6} (with glucose)	40 gr seaweed + 60 μl cell suspension + 1.5 ml of 20% glucose stock + 58 ml and 440 μl autoclaved

pH meter and at the same time point, cell counting was performed using spread plate method. 100 μ l of each sample was taken and spread over the surface using plastic spreader. After that, agar plates were put into anaerobic container and BD Gaspak™ EZ was added and incubated in the 30 ° C for two days to see the result.

2.5 Fermentation on Fresh Seaweed

Fermentation was carried out by inoculating six different strains in the fresh seaweed with and without the addition of 0.5% glucose. Firstly, 20% glucose stock was put into 58.5 ml of solution to be added to the chopped seaweed fermentation. The following table show a more details formulation.

3 Result and Discussion

3.1 *Preliminary* Experiment

The preliminary fermentation result in the grow of colonies in all plates both in the spontaneous fermentation and with the addition of *L. plantarum 299V*. The result of DNA sequencing reveals some different species of bacteria grown: *Bacillus cereus, Lactococcus lactis* subsp. *Cremoris* and *L. plantarum*, with the domination of *L. plantarum* in most of the samples. Unfortunately, DNA sequencing revealed that *L. plantarum* found in the spontaneous fermentation have the exact band as *L. plantarum 299V* which means that contamination occurred from the IBS capsule as well as for *L. lactis* subsp. *Cremoris.* Based on this result, there is no potential LAB that originally comes from seaweed *U. fasciata,* which align with the study of Singh *et al.* (2011). The study found that *Bacillus sp.* And *Marinomonas sp.* Were found naturally to induce morphogenesis in *U. fasciata* and none of LAB strain was reported. Therefore, the next experiment was conducted by adding inoculum of LAB from different sources. Due to very little or no presence of indigenous LAB in the seaweed, acidification did not occur and thus, pH did not decrease significantly. pH measurement showed that in the spontaneous fermentation pH decreased below 5.5 in the second day but, increased again in the following day and reached around pH 6 at day-8 fermentation.

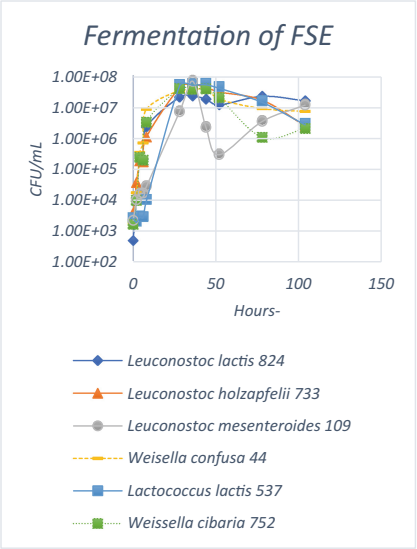

Fig. 1. Fermentation on FSE

The addition of simple sugar such as glucose provide nutrient for the bacteria to grow and release organic acid. *L. plantarum* in the controlled fermentation is generally produced desirable properties in many fermented foods such as pickle and sauerkraut, but using seaweed *U. fasciata* as the substrate in this preliminary study seems results in the other way around (Fig. 1)

3.2 Fermentation of FSE

Fermentation on FSE aimed to test the ability of strain utilizing carbon source in the *U. fasciata.* The preparation of FSE has taken out all fibrous materials in the seaweed and only left the chemical compound that was carried out by the water from the seaweed and the distilled water added in the extraction process. The microfilter with the pore size of 0.22 μl was utilized to remove all bacteria that might present in the FSE. Therefore, the added strain can work solely and their growth can be monitored without interference of other microorganism. The results showed that all strain managed to grow in the FSE. During the first 8 h of fermentation, all strains were in the log phase in which they grew exponentially from the initial count. However, *Le. Mesenteroides 109* and *L. lactis 537* showed a slower growth than other strains in which they reached the highest count after 24 h of fermentation and continue entering their stationary phase with the amount of cell biomass around 10^7CFU/ml. *Le. Mesenteroides 109* has the shortest stationary phase than five other strains, less than 24 h before entering decline phase, but then it increased the number which means that this strain seems to try to adapt with its environment and survive. Whereas, the other strains shared similar trend which increase the number of cells exponentially in the first 8 h of fermentation and remain the same on the first day of fermentation. Longer fermentation time, at the day-12 in the FSE using *Le.*

Mesenteroides 109 showed the formation of clear solid layer on the top of tube that is known as exopolysaccharide (EPS). Studies have been reported that EPS is produced by LAB in the form of layer that protect them against adverse environmental conditions such as dehydration, osmotic stress, antibiotics, extreme temperature and acid (Ruas-Madiedo *et al.* 2002; Looijesteijn *et al.* 2001). Other study such as the one conducted by Matsuzaki *et al.* (2015) and Montersino *et al.* (2008) also confirm the production of EPS from *Le. Mesenteroides* and its potential benefit for immune response.

3.3 Fermentation of Fresh Seaweed

LAB have complex nutritional requirements and are depend on the presence of a fermentable carbohydrate for their growth. LAB able to carry out simple sugar such as glucose into the fermentation pathway and converted into lactate that lower the pH through either homofermentative or heterofermentative pathway. The former produce only lactic acid which can acidify the fermentation substrate and the latter, in addition to lactic acid, ethanol or acetate, CO_2 is produced. The production of this organic acid might enhance the nutritional value and could be as natural preservative because it decreases the pH of fermentation which become one of the most crucial roles because acid environment could inhibit pathogenic bacteria that can cause disease. Although the acidification was considered slow – start decreasing at the third day of fermentation- all samples with the addition of 0.5% glucose managed to lower their pH from around 5.5 to at around 4. Although the significance of decreasing pH of each strain was different, all sample with the addition of glucose demonstrate a lower pH compared to sample without glucose. LAB are also weak proteolytic and lipolytic which is important in the fermentation process especially for producing food. This is because the breakdown of protein and fat are often organoleptically unpleasent in high concentrations. In this study, due to the considerable amount of protein in *U. fasciata* (Labib *et al.* 2020) and the little amount of fermentable carbohydrate that does not dominant in the seaweed component, all sample in the fermented fresh seaweed produced punget odour by the increased time of fermentation. Cell counting for all strain showed that the addition of glucose seems to speed up the growth of all LAB strain. It can be seen from the growth curve, the log period in which the bacteria exponentially grow, sample with 0.5% glucose were almost took a day faster to reach its maximum number compared to samples without the addition of glucose. However, the increased of cell biomass for all samples were quite similar between sample with and without the addition of 0.5% glucose (Fig. 3).

3.4 Control

Sample control with and without the addition of 0.5% glucose demonstrated a quite similar results, except for the pH value. While control without glucose remained at around 5.5, the addition of glucose could drop the pH up to 3.8. The growth pattern of both control demonstrated a long lag phase in which the colony grew after 2 days of fermentation. Compared to samples with LAB inoculum, the bacteria grow in the control sample require a longer time to adapt with the environment or could be influenced by the physiological state of the cell itself. The colonies that growth in both samples was clear and round shape with the smooth edge and small with approximately 1 mm diameter. In

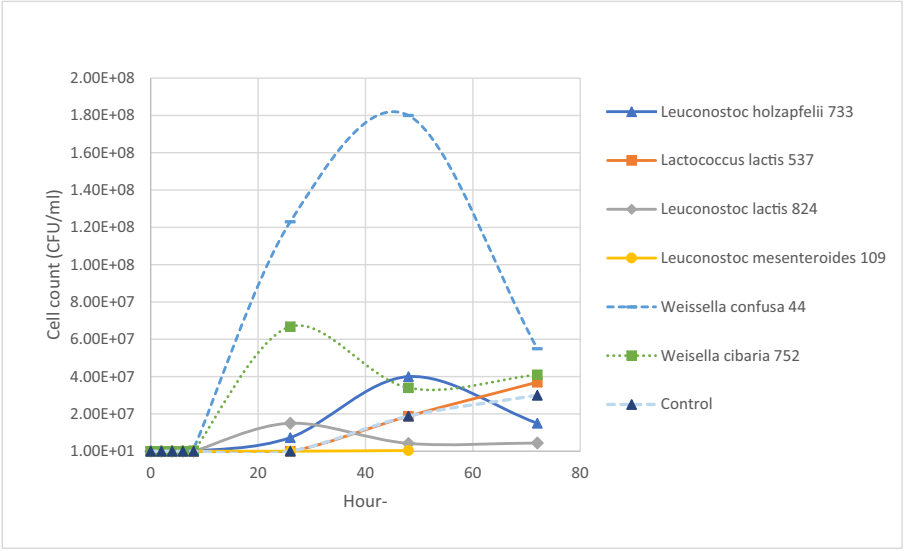

Fig. 2. Fermentation of fresh seaweed without glucose

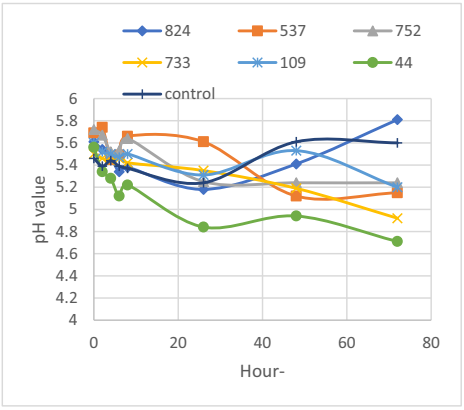

Fig. 3. Fermentation of fresh seaweed without glucose

terms of colour and size, these colonies were different from the six-strain tested which means that this bacteria is the native indigenious from the seaweed *U. fasciata*. Gram staining showed that this bacteria strain is considered as gram-positive due to the pink colour under microscope. The shape is cocci and tent to clumps with high number of cells. Because it able to grow in the MRS agar that is selective for lactic acid bacteria, and the treatment of adding 0.5% glucose showed a significant decrease in pH value, it can be predicted that these bacteria probably belong to group of LAB. However, to convince this outcome, a molecular identification including DNA sequencing should be done. A pure culture of this bacteria has been stored in the 40% glycerol stock and was

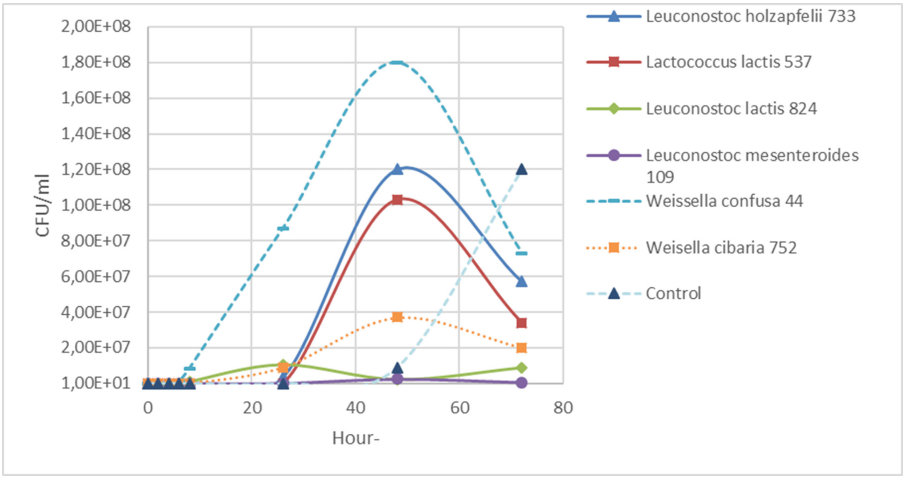

Fig. 4. Fermentation of fresh seaweed with the addition of 0.5% glucose

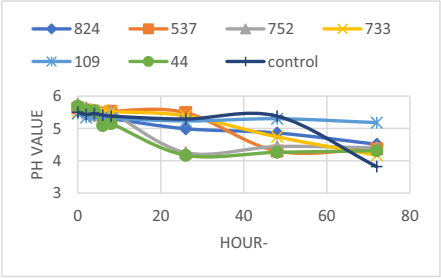

Fig. 5. Fermentation of fresh seaweed with the addition of 0.5% glucose

kept in the -80 ° C for further study. Color changes was occurred in the second day of fermentation with the addition of glucose, while sample without glucose remain green and slightly change in the third day of fermentation with pungent odour (Fig. 5).

3.5 L. Lactis 537

Strain *L. lactis* is commonly used in the dairy products, but it can be found naturally in the vegetable and fruits. According to Vinderola *et al.* (2019), *L. lactis* has a homofermentative pathway that does not produce gases. It able to grow in the temperature ≥ 37 °C in a wide range of fermentable substrate. In this study, the strain was obtained from fresh herb and was a nisin positive. The colonies size of *L. lactis* was the smallest among other strains and this is also confirmed by the finding of Azhar *et al.* (2017) which studied *L. lactis* producing nisin-like bacteriocin. Unlike other strain tested, this strain took longer time to grow optimally on the MRS agar and found to grow solely because of its nisin production which may inhibit other bacterial strain from growing.

pH measurement showed that addition of glucose increased the pH by more than one point. Sample without glucose remained at above 5, while the addition of glucose was close to 4.5. Both samples inoculated with this strain, with and without the addition of glucose showed a decline trend in the first 8 h of fermentation but then continue to grow exponentially. This trend explained that the culture strain might need to adapt to the environment before exponentially grow. The number of cell count in the sample with glucose was higher than without glucose after 8 h of fermentation which indicate that the addition of simple sugar helps them to grow more optimum. The changes of color of fermented seaweed using this strain with 0.5% glucose was noticeable after 24 h of fermentation. By the 7 days of fermentation, it became more brownish and more intensive. Different from this, the sample without glucose showed a lighter color and only slight brownish in the 7 days of fermentation. There was no strong smell detected for both sample with and without the addition of glucose.

3.6 Le. Lactis 824

The addition of sugar using this strain affect much on the fermented seaweed profile but not much on the cell count. The presence of pungent odor in the sample without glucose could be associated with high pH value. Punget odour was quite strong on the sample without glucose, which might be associated with the high concentration of ammonia resulted from protein breakdown. pH measurement also showed the value of almost 6 with the presence of moulds on the surface of the seaweed sample. This might indicate that the grow of *Le. Lactis 824* could be interfered by thIS microorganism so that it could not produce organic acid that acidify the sample. On the other hand, the addition of 0.5% glucose decreases the pH of fermentation into 4.5 and the unappealing smell was not as strong as the sample without glucose. Although both samples showed a noticeable pH value, cell count for both sample with and without the addition of glucose using this train showed no significant difference amount of biomass. Growth curve showed almost similar pattern for both samples, the cell grew exponentially within 24 h and start entering the stagnant phase after that. Moreover, during fermentation period both samples increase the cell count by 4 log from the initial count to the end periode of cell count testing.

3.7 W. Cibaria 752

W. cibaria has been reported to be associated with various type of sourdough due to the formation of EPS called dextran and oligosaccharides during sourdough fermentation (Galle *et al.* 2010). *W. cibaria* is obligate heterofermentative which produce either CO_2 or combination of lactic and acetic acid as the end product of sugar metabolism. Therefore, when it was grown in the MRS broth, gas was heavily fulfilled the tube. The addition of sugar decreased the pH of the fermented seaweed up to 4.3 but did not affect much on the cell biomass. In the first hour of fermentation both sample's count showed a decrease number but then increased exponentially and stagnant after 2 days of fermentation. During the first day of fermentation both samples, with and without glucose showed a decrease number of cell count which indicate the cell adapt with the environment.

At the t = 26 of cell counting, sample with glucose showed a lower number of CFU than sample without glucose and at the same time the three were two different colonies grew on the plate, which may indicate the competition of nutriean between this strain with *W. cibaria 52* which then result in the lower count. The first colonies were round in shape with entire edge and rises convex. While another one was having a bigger size with irreguler size shape and undulate edge. From its appearance, the former was similar to the colonies grow from the previous experiment which is original inoculant of *W. cibaria 752*. While the latter, could be the bacteria that were dormant in the seaweed and manage to grow after well-adapted with the environment. To ensure this, molecular analysis including DNA sequencing should be conducted.

3.8 W. Confusa 44

Growth curve in Fig. 2 and Fig. 4. For this strain showed the highet number of cell count among other strain added both in the sample with and without the addition of 0.5% glucose. Within the same periode of fermentation time, cell count showed the highest number among others, which reached up to 10^8 CFU/ml. However, the initial count (t = 0) was also started in high number (10^3CFU/ml) which means that it increased by 5 log during the fermentation period. This increase was similar for both treatments (with and without glucose) as well as the growth curve, meaning that the strain able to utilize carbon that naturally present in the seaweed and the addition of glucose did not significantly favour the fermentation process.

Compared to other five strains, the seaweed inoculated with *W. confusa 44* have more intense discoloration, especially with the addition of glucose. pH measurement showed that the addition of 0.5% glucose decrease the pH of the fermented seaweed at around 4.0. Although at the end of fermentation time it was increased slightly but the value still lower than sample without glucose. The odour of fermented seaweed using this strain was still unappealing but the punget odour was less strong than other samples.

3.9 L. Holzapfelii 733

Study of strain *Le. Holzapfelii 733* in the fermentation of seaweed has never been conducted so far. However, this strain was reported to find naturally in the fermented food product such as in the spontaneously fermented buckwheat and teff sourdough and was detected after five days of fermentation (Moroni *et al.* 2011). The addition of sugar influence both pH value and cell biomass of fermented seaweed using this strain. Sugar was converted to be organic acid that decrease the pH gradually to nearly 4.0 at the end of fermentation periode. Cell counting displayed a significant increase of sample with the addition of glucose, went from 10^3 from the initial count to 10^8 in the second day of fermentation. This means that during two days the cell biomass increased by 5 logs. On the other hand, although the sample without glucose also demonstrated high count of 10^7, the initial count was already high at 10^4 which means increased by 3 logs. The appearance and smell of fermented seaweed using this strain was quite similar to other samples, desirable flavour was not formed yet.

3.10 Le. Mesenteroides 109

Le. Meseteroides is often associated with fermented vegetable and is considered to be the most important organism during the early and middle stage of kimchi fermentation. It has been reported that the capacities of adhesion in the human colorectal was stronger than *L. rhamnosus* (Lee *et al.* 2016). Compared to other strains tested, *Le. Mesenteroides* showed a less effective of utilizing sugar in the seaweed *U. fasciata.* This can be seen from the growth curve both with and without the addition of glucose which show that this strain was positioned in the most bottom due to the number of cell count. pH measurement demonstrated almost no difference of sample with and without the addition of glucose 0.5% which was similar at just above 5, which means that the addition of glucose did not acidify the seaweed. This also correlate with the number of cell biomass that was low, meaning that neither carbon from seaweed nor from the additional glucose could support enough for its growth. Study of fermentation using *Le. Mesenteroides* on cabbage and onion showed that pH decreased significantly. The former took 3 days of fermentation to lower the pH from 6 to around 3.7 while, the latter took only 13 h to decrease the pH from 6.0 to 4.1 (Johanningsmeier *et al.* 2007). In this study, fermentation of seaweed *U. fasciata* using this strain seems unfavourable or may took a longer time as the chemical properties are significantly different from cabbage and seaweed.

4 Conclusion

Overall, it can be concluded that all strains tested in this study were able to utilize sugar in the seaweed *U. fasciata* both with and without the addition of 0.5% glucose. The growth curve showed that all strains were exponentially increased its number starting from the first day of fermentation. The addition of 0.5% glucose speed up the growth of all strain in the sample that reach their highest cell count at the second day of fermentation before entering decline phase. However, cell counting showed that all samples with the addition of 0.5% glucose did not seem to impact on the biomass of culture significantly because the CFU/gr seaweed of the strains were relatively similar to the samples without glucose. The addition of glucose showed a decrease of pH value for all sample including control sample. All samples decreased by above one point from the initial measurement to the third day of fermentation, only sample with the addition of strain *Le. Mesenteroides* remained the same as without glucose. The appearance also showed a faster change in term of color and odour compared to the samples without glucose.

5 Recommendation

The finding of this study can be improved by the use of more variety and concentration of sugar as it is essential for lactic acid fermentation. Additional treatment may also be applied on the fresh seaweed such as heating or pressure prior to fermentation to help breaking down the seaweed component. Moreover, analysis of volatile compound could be conducted to find what flavour compound are formed in the seaweed fermentation. Flavour and odour are important introduced by the microorganism involved in the fermentation which then responsible for the flavour formation of fermented product.

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