Population Dynamics and Molecular Profiling of Microbial Ecosystems during Spontaneous Fermentation of *Garcinia Mangostana* Pericarps

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***Abstract*— The spontaneous fermentation has resultant product has inconsistent microbiological and sensory qualities. This study aims to determine population dynamics properties of fermented *Garcinia Mangostana* Pericarps, characterise the various indigenous species of microorganism present at different stages of fermentation and also to determine the survival of dominant species. The *Garcinia Mangostana* Pericarps fermentation was carried out anaerobically for 100 days at room temperature and sample collection was done on daily basis. Enumeration of microbial population was done on different selective media and pure culture was isolated. The *Genomic DNA* of pure culture was isolated and further PCR amplified using universal primer set. The 16S rRNA sequence analysis was done using Blastn and the phylogenetic tree was constructed. Due to indigenous microflora of spontaneous fermentation, 13 major genera was found which is *Klebslella, Enterobector, Msngovibactor, Escherichia, Cronobactor, Komagataeibacter, Lactobacillus, Bacillus, Meyerozyma, Saccharomycete, Hanseniaaspora, Mycobacterium* and *Pichia*. The predominant microorganisms present in fermented GMP were LAB (Log CFU/mL 2.723579±0.171854 to 3.74846±0.315589). Lactobacillus brevis and Lactobacillus plantarum were the dominant members of LAB. Lactic acid fermentation inhibit many pathogenic bacteria through the production of organic acids, mainly lactic acid, and cause rapid acidification during fermentation cause the dominant species of LAB survive toward end of fermentation. The survival study showed that these pathogens could survive up to 21 days. The diverse indigenous LAB microflora provides a prospective consortium for product development in future improving the quality and safety of fermented *Garcinia Mangostana* Pericarps.**

Keywords*— *Garcinia mangostana, Spontaneous fermentation, PCR, sequencing, Lactic acid fermentation, Lactic acid bacteria, Phylogenetic analysis.

I. INTRODUCTION

Fermentation has claimed to be a popular technique to preserve staple food, fruits, vegetables, herbs and other edible materials as well as improving their nutritional value and sensory quality [1, 2]. Some traditional fermented foods such as kimchi (originally Korea)[3] and saeurkraut [4] become international delicacy. Other traditional fermented foods derived from wheat, starch, milk, maize are known by their local names such as *doklu* (africa) [5], *tape* (Indonesia) [6], *tempoyak* (Malaysia) [7], *kishk* (Egypt). Despite long-time tradition of fermented foods spanning over millenia, their current production method is largely low-tech at household scale with the majority of production processes employ spontaneous fermentation using indigenous microorganisms inhabitating the raw materials. On the other hand, the use of starter culture evolved from repeated 'backslopping' processes to iterate the best-adapted strain [4]. For this reason, fermentation becomes a recommended food preservation method and nourishment programme for the population in impoverished areas [4]. Until recently, the health-promoting aspects of these fermented foods in terms of the presence of probiotics such as lactic acid bacteria (LAB) that is beneficial to cholesterol modulation, immune stimulation and toxin inactivation were revealed. In addition, the bacteriocin secretion of LAB inhibits the foodborne pathogenic growth and preventing diarrhea [6].

Numerous tropical plants have fascinating biological activities with potential therapeutic applications for example Garcinia *mangostana Linn* which belongs to the family of *Guttiferae* and is named ''the queen of fruits". It is found in the tropical rainforest of some Southeast Asian nations like Malaysia, Sri Lanka, Thailand, Indonesia, and Philippines. Citizen in these countries utilise the *Garcinia Mangostana* fruit's component as a traditional medicine for the treatment of suppuration, abdominal pain, diarrhea, infected wound, dysentery, and chronic ulcer. Moreover, mangosteen has been proven to contain various secondary metabolites (e.g. prenylated and oxygenated xanthones) [8, 9]. In 1855, a-mangostin was found among the major xanthones taken from the pericarps of the mangosteen fruit [10]. Subsequently, the structure of this xanthone was construed by Dragendorff [11]. The molecular formula, type and position of the substituent groups of a-mangostin were then determined by Yates and Stout [12]. This compound has been discovered to possess a wide range of biological activities, with anti-inflammatory, anti-tumor, cardioprotective, antidiabetic, antibacterial, antifungal, antiparasitic, antioxidant and antiobesity agents.

These bioavalaibility of the compound can be enchanced by 11-16 times by fermenting the whole mangosteen fruit using Saccharomyces boulardii starter culture compared to standard dry mangosteen extract as reported by [13]. The fermentation of *garcinia mangostana linn* pericarp is expected to have more bioavalaibility of this compound thus rendering quicker therapeutic effect. A variety of microorganisms such as Colletotrichum gloeosporioides and Neosartorya spathulata [14] and Saccharomyces boulardii [13] as starter culture are capable of improving the α and β-mangostin content during fermentation. However using Spontaneous fermentation condition will utilises indigenous microflora. Comprehensive review by Anandharaj et al. [15] highlighted the prevalence of LAB species such as *Lactobacillus plantarum, L. pentosus, L. brevis, L. acidophilus, L. fermentum, Leuconostoc fallax,* and *L. mesenteroides* in Asian

fermented fruits and vegetables with respect to their wide ranging health benefits. the fermentation is besieged by lots of unknown microbe which either pathogenic or therapeutic. The product safety is important by determining types of organism exist in end product and which organism are dominance towards end of fermentation.

Mangosteen or *Garcinia mangostana* Linn pericarp is known to be potentially to be developed for therapeutic use and a useful with nutritional, medicinal and health benefits. The Spontaneous fermentation, as adopted in the fermentation of *G. Mangostana* pericarp are neither predictable nor controllable. The temporal and spatial heterogeneity of the microflora during fermentation are likely to affect the metabolic processes that influence the physio-chemical properties and chemical composition of the product. It is expect the fermentation will favour more bioavailable thus rendering quicker therapeutic effect. In spite of all these benefits,

Thus it is essential determine rates of microbial growth and death and it is necessary to enumerate microorganisms to determine the fermentation product safety. The Bacteria also can perform a variety of beneficial functions, With respect to the factors affecting productivity and beneficial function, it is important to isolate new sources of microbe, purify microbe and investigating the factor affecting their activity. Diverse microorganisms have been investigated in an effort to obtain new isolates that are good microbe producers, and in order to increase productivity as well as therapeutic effect.

II. METHODOLOGY

A. G. Mangostana fermentation and sampling

G. Mangostana fruits were purchased from a local market in Shah Alam, Malaysia. The pulps were removed while the pericarps were washed to remove physical dirt and shredded into small pieces. 700 g of shredded paricaps were collected. The pericarps are then blended into smaller size with addition of distil water to ease the process. The lab scale 5L benchtop bioreactor was setup. The prepared pericarps is loaded into 5L benchtop bioreactor about Ten percent (w/v) of each raw material about 700g of pericarp. The distilled water was added to make up 5L and sugar was added at 10 % w/v as initial substrate. The fermentation was carried out anaerobically for 90 days at room temperature. Neither starter culture nor preservative were added. Sample collection was done using basis of day 0, 2, 6, 8, 21, 30, 48, 60, 75 and 90 of the fermentation. The pH reading of fermentation product was collected before the sample taken. 45ml of sample was taken using sterile syringe and fill into sterile Plastic Centrifuge Tubes/

B. Microbiological analysis- Enumeration of microbial population

Each fermented *G. Mangostana* pericarp broth sample was properly mixed to ensure homogenisation of the microbes present in the fermented product. 0.1ml of collected sample was pipetted aseptically into 0.9 ml (1:10 dilution) of sterile peptone water solution in a 1.5ml Sterile Microcentrifuge Tube. A dilution series of 10^{-1} to 10^{-6} was made in peptone water solution and 1 ml of each of these serial dilutions was pipetted in duplicate into appropriately marked Petri dishes. This was done for six different selective media Potato-dextrose agar (PDA), Dichloran Bengal Chloramphenicol (DRBC) agar, Man Rogosa Sharpe (MRS) agar, plate count agar (PCA), Brain Heart Infusion (BHI) agar, MacConkey agar and Urea agar which was pour plated into Petri dishes filled with the serial dilutions and properly mixed for 30 s using L-shaped glass rod. This spread-plate technique is used and the glass rod is sterilized by dipping in alcohol (95% ethanol) and flamed to burn off the alcohol for each spread. All inoculated plates were incubated anaerobically in candle jar at 30 ℃ for 1-2 days (for PDA, DRBC, MRS, PCA) and 37 ℃ for 1-2 days (for MacConkey, BHI and Urea). After incubation, the viable microbial counts of the bacteria, yeasts and mycelia fungi suspended in each serial dilution were determined and expressed in colony forming units per millilitre (cfu.ml-1) of broth sample. The media that were used for the isolation and enumeration of yeast and mould (PDA and DRBC agar), lactic acid bacteria (MRS agar), mesophilic aerobic (PCA), pathogenic bacteria (e.g. Listeria monocytogenes, Staphyloccocus aureus and Escheria coli) (BHI) agar, and Enterobacteriaceae (MacConkey and Urea agar).

C. Chemical analysis - pH measurement

Potentiometric measurements were obtained with the pin electrode of a pH meter that was inserted directly into the bioreaction during fermentation time. Three independent measurements were obtained for each sample. The reported values are the average of three readings.

D. DNA extraction and purification

A tool kits for rapid DNA extraction from pure culture were used according to the protocol described by the manufacturer; Wizard® Genomic DNA Purification Kit (Promega Corporation, USA) for Isolating Genomic DNA from Yeast and Isolating Genomic DNA from Gram Positive and Gram Negative Bacteria.

E. PCR amplification

PCR amplification of V3 region of 16S rDNA gene of each bacterial isolate was carried out using universal primer set of forward primer 27f (5'-AGAGTTTGATCMTGG CTCAG-3') and reverse primer 1492r (5'-TACGGYTACCTTGTTACGACTT-3'). Each PCR mixture of 50 µl total volume consisted of 5 µl of DNA template, 0.25 µM of each primer, 25 µl of REDiant 2× mastermix (1st BASE) which comprised of reaction buffer, 0.06 U/µl of Taq DNA polymerase, 3 mM MgCl2 and 400 µM of each dNTPs and nuclease-free water. Each reaction was carried out using a conventional thermocycler (Eppendorf Mastercycler) at 35 cycles of denaturation at 95 °C for 30 s, followed by annealing temperature at 55.5 °C for 30 s and elongation at 72 °C for 1.5 min. The initial denaturation and final extension were carried out at 95 °C for 5 min and 72 °C for 10 min respectively. The purity of DNA fragments were analysed by running 2%(w/v) agarose gel with 1 × TAE buffer.

For fungal isolates, the 5.8S-Internally Transcribed Spacer (5.8S-ITS) rDNA region was amplified using ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). Other PCR conditions were similar to bacterial isolates analysis.

F. 16S rRNA sequence analysis

The PCR products were submitted to Sanger sequencing by 1st BASE using the same primers used during PCR amplifications. The sequence identity was determined by a BLASTn search at NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) while the phylogenetic tree was constructed using MEGA software 7.0.26 ver.

III. RESULTS AND DISCUSSION

A. Bacterial Growth Profile

The results for the spontaneous fermentation of *Garcinia Mangostana* Pericarps showed that over the 100 days period. The changes in bacteria population during spontaneous fermentation of *Garcinia Mangostana* Pericarps is shown in Fig. 1. The population of bacteria in *Garcinia Mangostana* Pericarps significantly increased on the first week with a decreasing during the later stages of fermentation until the day 100. However, only the LAB which from MRS media show a stationary condition more then 1 month of fermentation. At the end of fermentation the lactic acid bacteria and mold & yeast (PDA/DRBC) the only microorganism which dominant bacteria survive in the fermentation. Total plate counts

(TPC) of all viable mesophilic aerobic organisms were shown on PCA agar shows representative of growth trend for all the bacteria.

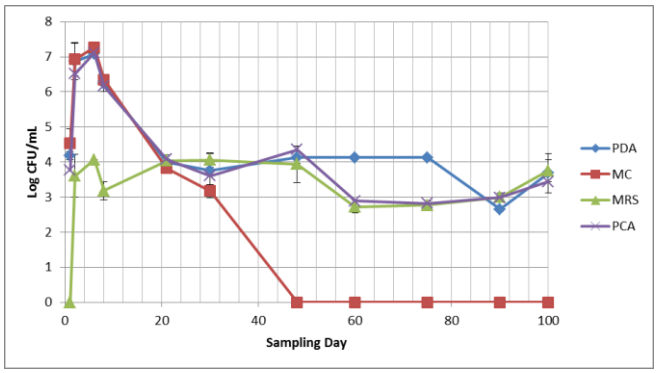

Figure 1: Bactreia growth curve

Total Plate Count-TPC represent the PCA agar as the microbiological growth medium commonly used to assess or to monitor "total" or viable bacterial growth of a sample. The all specific bacteria grow on PCA agar. TPC values steadily increased for the first week and remain stationary until the 6th week of fermentation. Then, the TPC counts start to decrease steadily.

Lactic acid bacteria enumeration-The lactic acid bacteria count is determined using MRS agar. During Early stage of fermentation, the LAB does not shown any growth. However during 2nd day of fermentation, the LAB start to grow with a count 3.6087 log CFU/mL and increasing until day day 48. The growth peak of LAB is at day 6. The LAB shows a steady growth trends with a consistent count compare to other bacteria.

Yeast and Mold Enumeration-The yeast and molds growth was determind using PDA or DRBC agar. The yeast and molds growth profile show that the microorganism increase in count for the first week. The growth peek was seen on the 6th day. On the 8th to 48th days the growth profile shows a decreasing trend then increase growth on the 48th day. However during the 60th and 90th days, the bacteria form a numerous small colony which the count exceed the count limit. Thus, no data available for both day. On day 90 and 100 the growh shows a visible count of increasing trend of growth.

Enumeration of Pathogenic Escherichia coli and Enterobacteriaceae-The growth of pathogenic *Escherichia coli* and *Enterobacteriaceae* was determind using MacConkey agar. The result shows the all growth forms pink colonies which indicated Gram-negative bacteria that can ferment the sugar lactose (Lac+). The intial fermentation show the presence of the bacteria and during the first week the bacteria increase and then decrease after the first toward no growth during day 48 until day 100.

In this study, the bacterial growth curve was demonstrated. From the graph, we may specify the different stages in the growth of the culture as it grows into the stationary phase. However the overall stationary phase does not demonstrated specifically because the sampling time are taken for 100 days. The stationary phases should be seen in the first 15 days. Figure 4.4 shows growth curves of the isolated colonies from spontaneous fermentation of *Garcinia Mangostana* Pericarps. From the graft, the increase of the bacteria population was observed during the first of the spontaneous fermentation of *Garcinia Mangostana* Pericarps. Afterwards, the population decreased.

From the curve, it is clear that the highest cfu was on the 6th day. The yeast & fungi group from PDA was obtain log cfu/ml of 7.0863, while the pathogenic group from MRS was obtain log cfu/ml of 7.2742 and the bacillus group at highest with log cfu/ml of 4.0645. The specific bacteria from selective media PDA, MC, and PCA grow during the zero day of fermentation where by the specific bacteria from selective media MRS shows no growth on the zero day. After the first week, the growth begins to drop assuming the bacteria undergoes nutrient depletion and competitive among other bacteria. However the lactic acid bacteria from MRS media shows a steady state which is stationary phase for more than 40 days of fermentation. Towards the end of fermentation sampling time, the lactic acid bacteria and mold & yeast the only microorganism which dominance in the fermentation.

The concurrent increase in yeasts and LAB could be due to a symbiotic relationship in which LAB provide an acidic environment which enables the proliferation of yeasts. The yeasts in turn provide growth factors like amino acids and vitamins for the growth of the LAB [17]. Yeasts and LAB are described as the principal microorganisms in the majority of traditional fermented foods, cereals for the production of indigenous Nigerian foods [18], Kimchi [19], Tempoyak [20]. The composition of the microbial populations of both the traditional food and fermented *Garcinia Mangostana* Pericarps has consistently shown the presence of both LAB and yeasts during the different steps of the production process. The association of yeasts and LAB could also result in the production of metabolites which could impact on the flavour and taste of fermented products [21].

There was a significant increase in all bacterial counts during the early stages of fermentation and steady decrease after the first week of fermentation. However, the LAB count shows a steady stationary trend during fermentation time. The LAB is favourable in fermented product which provides the probiotic effects. For other bacteria the decline growth could be attributed to the low pH and competitive factor which the dominance bacteria like LAB and yeast uptake all the nutrient. The dead or injury bacteria are viable but metabolically inactive and cannot perform cellular division. The cells can recover once the stress causing agent is removed and they can then regain their normal capabilities [22]. The key preservative effect of LAB is through the production of lactic acid [23] which decreases the internal pH of bacterial cells, thus denaturing proteins and causing a loss in cell viability. Therefore, the action cause inhibition to the growth of food spoilage organisms as well as pathogens which can cause food poisoning and disease [24].

B. Changes in pH during the Fermentation

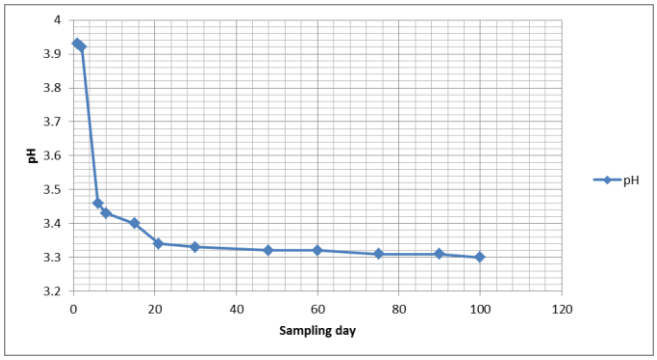

Figure 2 : Change in pH which occurred during the fermentation of Garcinia Mangostana Pericarps.

The change in pH during the fermentation is represented in figure. The pH value are initially high, around 3.9 and decreasing with time with a stationary value around 3.3. This indicated that the fermentation undergo the lactic acid fermentation. Lactic acid fermentation can inhibit many pathogenic bacteria through the production of organic acids, mainly lactic acid, and cause rapid acidification during fermentation [25]. The number of bacteria which are inhibited depends on the type of organisms, the properties of the substrate, the temperature, and the amount of dissociated acid. The result show a pH range 3.3 was obtain uniformly. This pH range is sufficient to inhibit the growth of many pathogenice bacteria such as E. coli and Entrobacteriaceae. It is important to determine the safety of this fermentation product

and it should not contain of a pathogen which could possibly cause illness once comsumed. The finding from Kunene et al., [25] found that the inhibition of Enterobacteriaceae occurred because of the acidic environment created by LAB. It's similar for E.coli which inhibit in acidic environment . The result from microbial analysis show present e.coli because of the product was contained as the final product was exposed to the surrounding environment. This acidic environment means that this fermented *Garcinia Mangostana* Pericarps produce was safe for assumption.

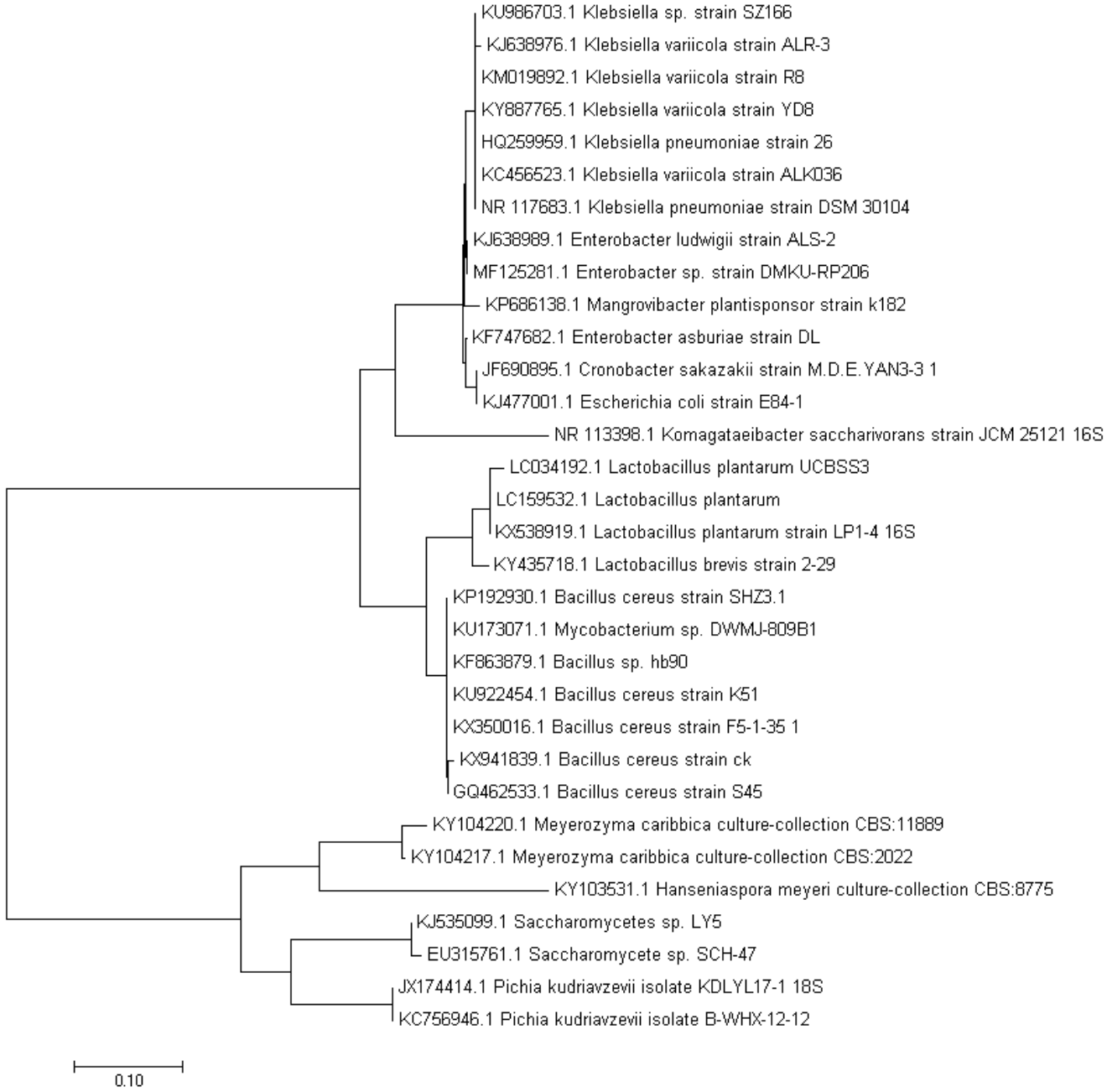

Figure 3: Phylogenetic of fermented *Garcinia Mangostana* pericarp colony relationships among 32 Texa strains based on 16S rRNA gene sequences. The tree was constructed using the NJ method.

C. BLASTn Analysis

Bacteria's DNA was extracted using promega Genomic DNA Purification kit and using agarose gel electrophoresis to check the purity of the isolated DNA. Genomic DNA was visible in the gel as a single band (Figure 4) and this preparation was used for further gene isolation experiments.

The nucleotide sequences of the genome were identified using First BASE Laboratories Sdn. Bhd. service. The sequence then exposed to a homology search against NCBI DNA database using BLAST (Basic Local Alignment Search Tool). The result for all of pure culture sequence is tabulated. The analysis of 16s-rRNA sequencing shows that various of microorganism was found. The pure culture from PCA agar (total plate count) show a result of DNA sequence of *Klebsiella* species for early sampling day from day 0 to day 8 with a similarities of 98 to 96 %. On day 21, *Mangrovibacter plantisponsor* was obtain similarities of 98 to 96%. For day 48 to 90, bacteria from *Bacillus* species was found with a similarities 98 to 97 %. It is show that throughout fermentation day, various of bacteria changing its dominancy for the survival in the medium. The existence of pathogenic bacteria during early stage show the bacteria are most during that stage and the replaced by the bacillus species which dominance in process.

Meanwhile, for the selective media sample (PDA agar), the blast analysis show that fungi *Pichia kudriavzevii, saccharomycete sp.,*

Hanseniaspora lachancei, and *Meyerozyma caribbica* show an average of high similarities over 99 to 100 %. The selective media which grow a spefic fungi group show a result of 4 types of fungi exist in the fermentation.

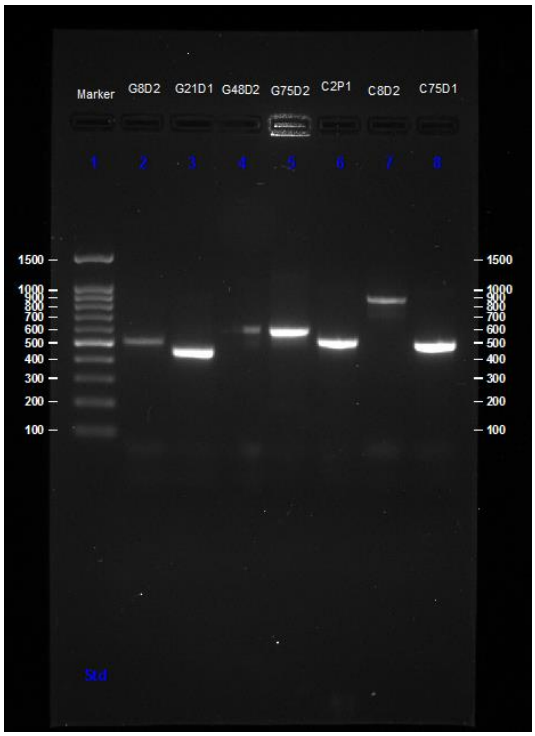

Figure 4: Agarose gel pattern of genomic DNA

For the selective media MRS, few of the DNA sequence show a poor DNA sequence. The similarities obtain are between 88 to 97 %. The overall blast result show specis form *Lactobacillus plantarum, Mycobacterium sp., Komagataeibacter saccharivorans, Lactobacillus brevis,* and *Bacillus cereus* which come form bacillus genus. The result obtain is cohenrent with the selective media MRS. The bacillus genus is desireable in this fermentation because of the LAB could enchance the bioavalaibility of the compound.

As for the Macconkey agar, the species from *Klebsiella variicola, Enterobacter sp., Enterobacter asburiae, Escherichia coli,* and *Cronobacter sakazakii* was obtain form blast analysis. This bacteria are undesirable because contain pathogenic effect to health. Throught out the fermentation these bacteria should not active in low ph. However, during isolating bacteria via selective media, the medium which is Macconkey agar provide enough growth condition which perhaps contributed in bacteria growth.

D. Phylogenetic Tree Construction

The sequence retrieved from Gene Bank data base available at the NCBI website (http://www.ncbi.nlm.nih.gov/) from blast result was retrieved with fasta format and aligned using Clustal W (1.6) using Clustal analysis and the construct test neighbour joining tree analysis were performed using mega 7.0.26. Phylogenetic trees contain a lot of information about the inferred evolutionary relationships between a set of species (McLennan, 2010). The taxa joined together in the tree are implicit to have descended from a common ancestor. In which by constructing phylogenetic tree bacteria species or group can be specified and evolutionary relationship can be studied between the bacterial isolates with other bacterial species that might be obtained from the same ancestor based on their genetic properties. Results of phylogenetic tree for colony from fermented *Garcinia Mangostana* pericarp was shown on Figure 3.

The evaluator lineages can be determine from the horizontal line generated from the phylogenetic tree which changing over time, the shorter the horizontal branch the amount of change is less. Meanwhile, the vertical lines connected to the horizontal lines represent how long they are represent. In the tree shown on figure 3, the phylum of p*roteobacteria* has the highest species obtain from the pure culture. 13 species of found. Meanwhile the bacteria species of *Komagataeibacter saccharivorans* is the out group in *proteobacteria*. It is possible the out group has significant genomic differences suggesting they are a different group of bacteria species.

For the phylum of Formicates, 11 bacteria genera of bacillus and lactobacillus were found. Previous tudies have attributed the fermentation of tempoyak to different LAB species and these studies conclude that *Lb. plantarum* is the predominant organism responsible for the fermentation (26; 27; 28). In this study, the *Lb. plantarum* is found of the mid fermentation time. This species commonly found on fermented vegetable. Thus it is possible that this species is the predominant species on this fermentation. The existence of large species of *Bacillus cereus* is unfavorable due to pathogenic characteristic. However, this spontaneous fermentation is due to indigenous microflora and not due to pure culture (starter culture), the type of microorganism responsible is not well characterised. From the phylogenetic tree, the only LAB species that found is *Lactobacillus plantarum* and *Lactobacillus brevis*. This is due to poor sequence result and few sampling was damaged during the experiment.

As for the fungi kingdom, 3 genera of fungi was found which is *Pichia, Saccharamycetes* and *Meyerozyma*. The existence of fungi group is concurrent with the LAB where the The yeasts in provide growth factors like amino acids and vitamins for the growth of the LAB.

From the fermentation, 13 major genera was found which is *Klebslella, Enterobector, Msngovibactor, Escherichia, Cronobactor, Komagataeibacter, Lactobacillus, Bacillus, Meyerozyma, Saccharomycete, Hanseniaaspora, Mycobacterium* and *Pichia* including the out group.

E. Pure isolates

Commonly the spontaneous fermentation is associated with lactic acid fermentation. The rapid decline in pH and increase in lactic, acetic and propionic acids content can be attributed to LAB dominance. Microbiological studies on fermented products of Western Europe, Africa and Asia which were derived from various raw materials such as milks, meats, cereals, fruits and vegetables highlighted the predominance of lactic acid bacteria (LAB) which were believed to benefit sensory quality, digestibility, detoxification, microbiological safety, health-promoting and shelf stability of the spontaneous fermentation products [29]. From identify dominant lactic acid bacteria in the fermentation, 2 species of LAB was found which is of *Lb. plantarum*, and *Lb. brevis*. These two species play the important role in in to indigenous microflora diversity of microorganism towards the end of fermentation.

Various studies have reported on the role of *Lb. plantarum*, and Lb. brevis in fermented vegetable products such as cucumber, sauerkraut, tempoyak and kimchi [28;30; 31], hence the association of these LAB species with *Garcinia Mangostana* Pericarps is not surprising.. As reported by Breidt [31] the dominance of heterofermentative *Leu. mesenteroides* at during the early stages of vegetable fermentation is well recognised. They produce a significant amount of acetic acid in addition to lactic acid, as well as carbon dioxide which rapidly lower the pH and creating an anaerobic condition, which favours the growth of more acidtolerant homofermentative lactobacilli such as *Lb. plantarum* . In this study the *Lb. plantarum* was found during the early stage of fermentation and *Lb. brevis* later than found on the end of fermentation. The correlation between this two species due to diverse consortia of LAB microflora. However, other species fail to identify due the poor genetic sequence result and sample loss.

As *Garcinia Mangostana* Pericarps fermentation is due to indigenous microflora and not due to pure culture (starter culture),

the type of microorganism responsible to the fermentation is not well characterized. Antibiotic-resistant organisms may be presented in fermented *Garcinia Mangostana* Pericars during fermentation. Previous study of fermentation of *Tempoyak* [28], the *Lb. plantarum* isolated from tempoyak were resistant to multiple antibiotics of various classes. This study were in agreement with the study reported by Nawaz et al. [32] who also reported on multi drug resistant *Lb. plantarum* isolated from fermented foods. The LAB could serve as a reservoir of antibiotic resistance genes with the potential to be transferred to humans, animals and pathogenic microbes via the food chain though many LAB strains are not pathogenic [33]. Numerous studies have reported on a wide range of antibiotic resistance detected in *lactobacilli* as being intrinsic, hence, safety issue is not of the worry (Ashraf and Shah, 2011). Li-Oon [28] also reported that most of *Lb. plantarum* isolates from fermentation of *Tempoyak* is resistant to tetracycline, there is a potential risk for the horizontal transfer of antibiotic resistance genes as Tet-genes are plasmidmediated.

IV. Conclusion

In conclusion, the present study reports that diverse LAB species and other yeast species were involved in the fermentation. The increase in yeasts and LAB could be due to a symbiotic relationship in which LAB provide an acidic environment which enables the proliferation of yeasts. The *Garcinia Mangostana* Pericarps fermentation is fermented by indigenous microorganisms, namely species belonging to LAB and yeast, which influences to the sensory characteristics of the final product. It is of great concern that LAB and yeast isolated from foods are resistant to multiple antibiotics as they pose a threat to food safety and human health. Hence, it is of utmost importance that antibiotic resistant determinants present in the LAB and yeast isolates are determined for foods produced by spontaneous fermentation. This study showed that the pathogens survive up to 21 days if there was contamination at any stage of fermentation. The diverse indigenous LAB microflora provides a prospective consortium for product development in future. Even though this study do not anticipate the selection of starter culture for *Garcinia Mangostana* Pericarps fermentation, further research on investigating the potential probiotic properties of LAB microflora is ought to be undertaken.

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