Electronic Journal of Biotechnology ISSN: 0717-3458
Vol.12 No.3, Issue of July 15, 2009
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Received June 3, 2008 / Accepted March 25, 2009
DOI: 10.2225/vol12-issue3-fulltext-12
RESEARCH ARTICLE

Microbial succession in a fermenting of wild forest noni (*Morinda coreia* Ham) fruit plus molasses and its role in producing a liquid fertilizer

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Financial support: Graduate School, Prince of Songkla University, Hat-Yai, Thailand.

Keywords: *Lactobacillus plantarum*, *Morinda coreia* Ham, physicochemical property, *Pichia* spp., plant nutrients, wild forest noni fermentation.

Abbreviations: C/N ratio: total carbon/total nitrogen ratio
EC: electrical conductivity
FPEs: fermented plant extracts
FWE: fermented wild forest noni extract
GA_3: gibberellic acid
GAs: gibberellins
GI: germination index
IAA: indole -3-acetic acid
IP: integrated production
LAB: lactic acid bacteria
MRS: de man rogosa shape
PCA: plate count agar
PDA: potato dextrose agar
TBC: total bacterial count

The numbers of lactic acid bacteria (LAB) and yeasts that were present during a wild forest noni (*Morinda coreia* Ham) fermentation, the changes in its physico-chemical properties and levels of plant nutrients were investigated. LAB increased rapidly during the first 7 days and were the dominant population until after day 21 when the LAB were declining and the yeasts began to dominate. Identification of the LAB and yeasts to species level showed that the dominant LAB throughout was *Lactobacillus plantarum* while *Lactobacillus pentosus* was found but only at day 21. *Saccharomyces cerevisiae* was the most dominant species of yeast throughout but was slowly replaced by *Pichia membranifaciens* and then *Pichia anomala. Rhodotolura mucilaginosa*, an aerobic yeast, was only detected at the beginning of the fermentation process. It is suggested that the *Pichia* spp. were responsible for consuming lactic acid. After 56 days, the values of pH, acetic acid, ethanol and electrical conductivity in the fermented product were 3.66, 3.34 g L^{-1}, 16.98 g L^{-1} and 14.47 mS

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cm^{-1}, respectively. Increased amounts of plant nutrients were present at day 56 mostly derived from the degradation of plant material. At day 56 the amounts were as follows (in mg L^{-1}): N 633, P 1210, K 4356, Ca 693, Mg 536, Mn 7, B 51, Zn 169, and total carbon/total nitrogen ratio (C/N ratio) 18. Based on the seed germination index (GI) of cherry tomato (*Lycopersicon esculentum* Mill), the extract diluted 256-fold gave the best GI of 157%.

Nowadays, food safety is considered to be a major factor that can help to improve and promote human health because it can ensure that food contains no toxic materials such as chemical residues, heavy metals and potential microbial pathogens. Agricultural products are a main source of food and recently many people in many countries believe that the use of inorganic fertilizers poisons the soil and results in a low quality product. Accumulation of heavy metals in soil due to excessive addition of chemically synthesized fertilizers for crop production, particularly phosphate fertilizers has caused deterioration in the quality of farming land (Wu et al. 2007). It is well recognized that phosphate fertilizers can be a source of heavy metals such as arsenic (As) and cadmium (Cd). Applications of fertilizers are also one of the main causes for the eutrophication of natural water resources (De Jonge et al. 2002). Another problem that arises from applications of inorganic fertilizers, for example potassium (K) and phosphorus (P), is that they are presently produced in ways that cannot be continued indefinitely.

An awareness of these problems worldwide, including in Thailand, has resulted in the development and classification of two systems of production, organic agriculture and integrated production (IP). However, in Thailand, 4 standard kinds of farming are now recognized: natural, organic, integrated and conventional. Thai farmers are increasingly converting to one of these standards, although conventional farming is still perhaps the most practiced. The use of synthetic mineral fertilizers is not allowed in organic farming, especially with vegetables. Farmers using the IP standard are allowed to use synthetic mineral fertilizer, provided that the nutrient balance on the farm is in equilibrium (Lienert et al. 2003). Hence, natural fertilizers are becoming an attractive alternative way to avoid the use of synthetic chemical fertilizers.

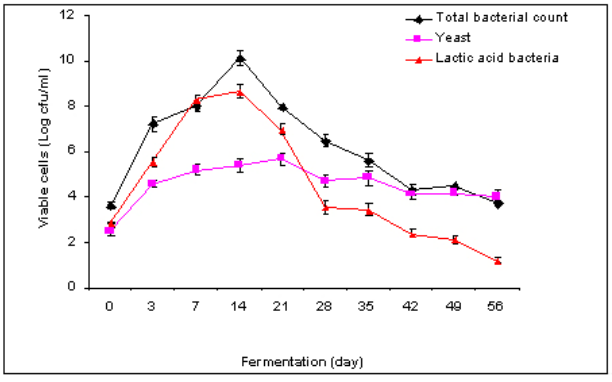

Figure 1. Microbial populations during fermentation of wild forest noni (*Morinda coreia* Ham) at room temperature, means of triplicate measurements ± standard deviations are presented.

In recent years, fermented plant extracts (FPEs) or fermented plant juices have been extensively used as natural liquid fertilizers by Thai farmers because they are easily produced from agricultural products or agricultural waste. These FPEs can promote plant growth and act as bio-control agents depending on the type of plants being used (Kantachote and Charernjiratrakul, 2008). Since FPEs are useful in eliminating problems associated with the use of chemical fertilizers and pesticides, they are now being widely applied in natural farming, organic agriculture and IP farming. However, there is very little scientific information to support the use of FPEs. FPEs are produced by lactic acid fermentation and most of the available scientific information is concerned with the production and use of them as beverages (Kantachote and Charernjiratrakul, 2008; Prachyakij et al. 2008). Lactic acid bacteria (LAB) and yeasts are normally found in fermented plant products because their habitats and some of their physiological properties are similar (Oboh, 2006; Okada et al. 2006; Olstorpe et al. 2008).

The wild forest noni tree (*Morinda coreia* Ham) is widely distributed in all parts of Thailand and has been extensively used in Thai traditional medicine. In general, the core of wild forest noni has been used to treat menstrual disorders, as a tonic for stomach and blood stasis (Palasuwan et al. 2005) while its fruit is rarely used because it has an unpleasant smell. However, in our previous work we found that the beverage produced by fermenting fruits of wild forest noni was able to inhibit enteropathogenic bacteria and also had a high amount of potassium (Kantachote et al. 2008; Kantachote and Charernjiratrakul, 2008). We therefore thought of the possibility that an FPE from wild forest noni fruit might be useful as a potential liquid fertilizer and could assist farmers because it would be easy and cheap to prepare and make use of what is at present a common but non utilized resource. Hence, the aims of this study were to investigate microbiological successions and the roles of LAB and yeasts in the production of a fermented wild forest noni extract that could be used to supply plant nutrients.

MATERIALS AND METHODS

Wild forest noni fermentation

Ripe fruits of wild forest noni (*Morinda coreia* Ham) and molasses were purchased from local markets. The proportion of raw materials used; ripe fruits, molasses and potable water, was 3: 1: 10 (w/w/v) and in this study started with whole fruit 6 kg, 2 kg and 20 liters, respectively. The mixture of raw materials was placed in a plastic bucket only

Table 1. Identification of lactic acid bacteria using API 50 CHL test and Program computer API Web Stand Alone V.1.1.0.

Fermentation day	Number of isolate	Representative isolate	Genus and species	% Identity
		L. plantarum TISTR 862	*L. plantarum*	99.9
0	10	WF01	*L. plantarum*	99.5
7	10	WF71	*L. plantarum*	99.9
14	2	WF141	*L. plantarum*	99.5
	11	WF143	*L. plantarum*	99.5
21	12	WF211	*L. plantarum*	99.5
	3	WF216	*L. pentosus*	99.9
28	12	WF281	*L. plantarum*	99.9
35	3	WF351	*L. plantarum*	99.5
	7	WF354	*L. plantarum*	99.9
42	12	WF421	*L. plantarum*	99.9
49	8	WF491	*L. plantarum*	99.5
	2	WF499	*L. plantarum*	99.5
56	10	WF561	*L. plantarum*	99.9

4/5 full and the space above the raw materials was covered with a water filled plastic bag to help to produce anaerobic conditions (Kantachote and Charernjiratrakul, 2008). The fermenting plastic bucket had a tap at a 10 cm height from the bottom for sampling and it was incubated at room temperature (28 ± 3ºC) throughout the experiment. The experiments were conducted in triplicate and sampling performed at various times for 8 weeks to monitor microbiological succession, the characteristics of the fermented wild forest noni extract (FWE) and its potential for use as a liquid fertilizer.

Enumeration and isolation of microorganisms

Microbial populations were counted using standard methods according to FDA (2001) at days 0, 3, 7, 14, 21, 28, 35, 42, 49 and 56. Ten fold dilutions beginning with 25 ml of a sample were added to 225 ml of normal saline solution (0.85% NaCl) to obtain a 10^{-1} dilution and then appropriate dilutions were used for the pour plate counting of LAB and a total bacterial count (TBC), whereas the spread plate technique was used to count molds and yeasts. The media used were; de Man Rogosa Shape (MRS), Plate Count Agar (PCA) and Potato Dextrose Agar (PDA) for counting LAB, TBC and molds and yeasts, respectively. Because the fermentation buckets were kept at room temperature (28 ± 3ºC), all plates were also incubated at room temperature. At each sampling time, representative colonies of LAB and yeasts based on their distinct morphologies were isolated from the MRS and PDA plates. All microbial isolates were purified and kept at 4ºC for further identification.

Identification of lactic acid bacteria

A total of 102 representative isolates of LAB that were Gram positive, non spore forming and catalase negative, were obtained from the 10 different sampling times. All isolates were identified according to their morphology, cell arrangements and physiological properties as described in LAB (Axelsson, 2004). The following 1% single sugar substrates; amygdalin, arabinose, cellobiose, esculin, gluconate, mannitol, melezitose, melibiose, raffinose, ribose, sorbitol, sucrose and xylose were used to investigate the ability of each isolate to metabolize sugars. This information was used to identify the LAB to the genus and

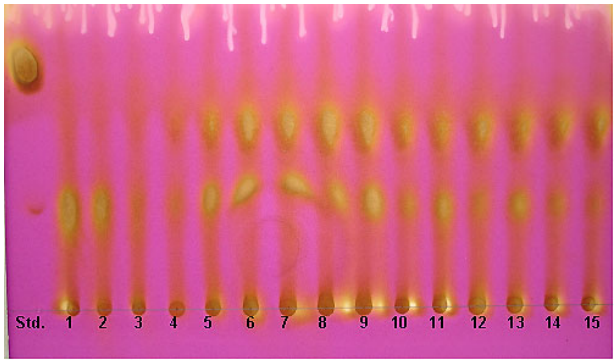

Figure 2. Plant hormone (gibberellins) of wild forest noni (*Morinda coreia* Ham) extracts during fermentation time; Std. = standard hormone (GA_3); spots 1-3 = day 0; 4-6 = day 14; 7-9 = day 28; 10-12 = day 42; 13-15 = day 56.

species levels by traditional methods; 14 representative isolates (Table 1) were confirmed to their species level using the API 50 CHL test kit (Bio Merieux, Lyon, Fance). To investigate their ability to ferment the various sugars each LAB isolate was grown in 10 ml of MRS broth at 30ºC for 24 hrs. The cells were collected by centrifugation at 4ºC at 5000 g for 10 min, and washed twice with phosphate buffer saline, pH 7. Cell pellets were suspended in a 2 ml of suspension medium (Bio Merieux) and then inoculated into the API CHL strips. The strips were incubated at 30ºC for 48 hrs and the observed changes in the color of the bromocresol purple indicator used as an indicator of acid formation. *Lactobacillus plantarum* TISTR 862 was used as a reference strain. Results were keyed using the computer software, API Web Stand Alone V.1.1.0.

Identification of yeasts

Eighty four isolates of yeasts were first identified to the genera level using their morphology and physiological properties according to methods as described in Standard Taxonomic Manuals (Barnett et al. 2000; Middelhoven, 2002). Assimilation of various carbohydrate compounds was determined in a yeast nitrogen base medium containing 5% of the following single compounds: galactose, inulin, lactose, maltose, raffinose and sucrose; while fermentation of the following compounds was also examined using 6% of the following single compounds: glucose, cellobiose, galactose, lactose, maltose, raffinose, sucrose, starch, trehalose and xylose also in a basal medium. Utilization of 1.5% ethanol, 0.5% methanol, 2% urea and 0.5% citrate were also investigated. Assimilation of nitrogen compounds was tested in a yeast carbon base by adding 40 mM of each of the single inorganic nitrogen compounds as follows: ammonium sulfate, potassium nitrate and sodium nitrite. In addition, the ability to grow in a basic yeast extract malt extract medium (YM) with added 0.01 or 0.10% cycloheximide, 10 or 16% NaCl was tested. 26S rRNA sequencing genes (600 base pairs) were used to identify yeast to the species level using standard methods. In brief, genomic DNA was extracted using the standard method and then 26S rRNA was amplified by the GeneAmpPCR system 9600 according to Vasdinyei and Deak (2003). PCR products were purified by the PCR purification kit (QIAGEN, Inc.) and then sequenced by the ABI PRISM310 sequencer (Applied Biosystems, Foster City, USA). The data output was analyzed by the DNASIS V3.7 program and sequence output files were identified by a database similarity search in the GENBANK collection using the BLASTN software (http://www.ncbi.nlm.nih.gov/BLAST/). Yeast identification was assumed if the query sequence showed more than 99% identity with the DNA sequences from strains in the collection.

Characteristics of fermented wild forest noni extract (FWE)

The following parameters were measured at days 0, 14, 28, 42 and 56. Electrical conductivity (EC) was measured using an EC meter, while pH was measured using a pH meter. Total sugar (TS) as glucose was measured by the phenol sulfuric method, total acidity was determined as lactic acid by titration (AOAC, 2002). Organic acids (acetic acid and lactic acid) and ethanol were measured using gas chromatography following the method as described by Yang and Choong (2001). Plant nutrients (primary: N, P, K; secondary: Ca, Mg and micronutrients: Mn, B, Zn) were also determined as follows. Total carbon (C) and organic matter were determined using the Walkley-Black method (Mikhailova et al. 2003), while total nitrogen (N) in the form of nitrate was measured using a test kit (Spectroquant Picco) according to the manufacturers instructions (Merck company), and then total carbon/total nitrogen ratio(C/N) ratios were calculated. The amounts of the following elements (P, K, Ca, Mg, Mn, B and Zn) were determined by inductively coupled plasma atomic emission spectroscopy

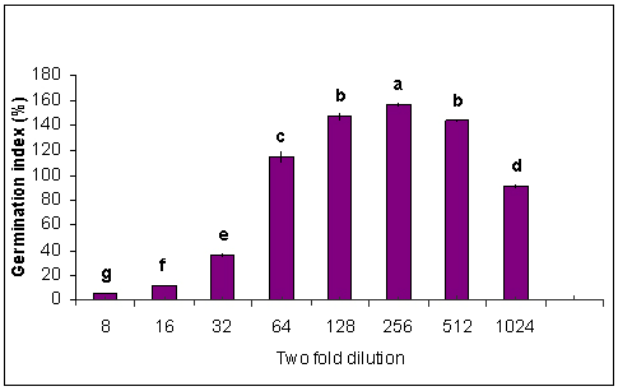

Figure 3. Percentages of GI of cherry tomato seeds by wild forest noni extract (liquid fertilizer). Letters above bars, when different, indicate significant differences between means ($p < 0.05$), means of triplicate ± standard error of means are presented.

Table 2. Microbial succession during fermentation of wild forest noni (*Morinda coreia* Ham) at room temperature.

Fermentation day	Lactic acid bacteria		Yeast	
	Number of isolate	Genus and species	Number of isolate	Genus and species
0	10	*L. plantarum*	8	*S. cerevisiae*
			2	*R. mucilaginosa*
7	10	*L. plantarum*	5	*S. cerevisiae*
			6	*P. membranifaciens*
14	13	*L. plantarum*	5	*S. cerevisiae*
			5	*P. membranifaciens*
			2	*P. anomala*
21	12	*L. plantarum*	4	*S. cerevisiae*
	3	*L. pentosus*	5	*P. membranifaciens*
			4	*P. anomala*
28	12	*L. plantarum*	3	*S. cerevisiae*
			4	*P. membranifaciens*
			5	*P. anomala*
35	10	*L. plantarum*	3	*S. cerevisiae*
			4	*P. membranifaciens*
			5	*P. anomala*
42	12	*L. plantarum*	2	*S. cerevisiae*
			3	*P. anomala*
49	10	*L. plantarum*	2	*S. cerevisiae*
			4	*P. anomala*
56	10	*L. plantarum*	3	*P. anomala*
Total	102		84	

L. plantarum = *Lactobacillus plantarum*; *S. cerevisiae* = *Saccharomyces cerevisiae*; *Rhodotorula mucilaginosa* = *R. mucilaginosa*; *P. membranifaciens* = *Pichia membranifaciens*; *P. anomala* = *Pichia anomala*

(ICP-AES) according to the instructions for the instrument.

Plant hormones, gibberellins and auxins, were examined after hormones were extracted by using the method of Karadeniz et al. (2006). The gibberellins were investigated using silica gel thin layer chromatography. Samples were spotted on a silica TLC (20 x 20cm) plate and separated with the solvent (isopropanol/water/ammonia = 8:1:1) was

run for 15 cm at room temperature and gibberellic acid (GA_3) was used as a standard. Silica gel thin layer chromatography was also used to detect auxins using the solvent (chloroform/methanol/water = 84:14:1) and indole-3-acetic acid (IAA) as a standard. GA_3 and IAA spots and R_f values of the FWE samples were visualized under 254 nm ultraviolet light.

Phytotoxicity test

A seed germination assay is one of the most common techniques used to assess phytotoxicity (Kapanen and Itavaara, 2001). A cherry tomato (*Lycopersicon esculentum* Mill) seed germination test was used to investigate the toxicity of the wild forest noni extract fermented for 8 weeks following the methods of Wong et al. (2001). Briefly, 20 ml of the FWE was centrifuged at 8000 rpm for 10 min followed by filtration through a sterile 0.45 µm cellulose filter. 5 ml of filtrate was diluted by two fold dilutions in the range of 8-1024 then each poured into a 9 cm diameter Petri dish containing a Whatman no. 1 filter paper. Ten tomato seeds were distributed evenly on the filter paper and the Petri dishes were incubated in the dark at room temperature for 72 hrs. Distilled water was used as a control set for the testing of seed germination (Hoekstra et al. 2002; Fuentes et al. 2004). This experiment was conducted in three replicates. The seed germination bioassay was evaluated by computing the germination index (GI), a factor derived from the relative seed germination and the relative root elongation. The % GI was calculated according to the formula of Hoekstra et al. (2002). One-way analysis of variance was used followed by the Duncan' multiple comparison test to analyze significant differences between treatments using the SPSS version 10 for Windows.

RESULTS AND DISCUSSION

Microbial succession

During the first week of the fermentation gas production was observed by the movement of a lid on the top of a plastic bucket. This was a sign that a fermentation was occurring. At the start (t = 0) of the FWE, the TBC was 4.1 x 10^3 cfu ml^{-1} and this increased to a maximum count of 1.5 x 10^{10} cfu ml^{-1} at day 14 (Figure 1). The LAB also increased from 1.3 x 10^3 cfu ml^{-1} at zero time to a maximum of 5.2 x 10^8 cfu ml^{-1} at day 14. In contrast, the numbers of yeast increased from 3.0 x 10^2 cfu ml^{-1} at zero time and reached a maximum of 8.1 x 10^5 cfu ml^{-1} at day 21 while fungi were found only at zero time. At day 7, LAB became the predominant group of microbes in the FWE presumably because the anaerobic conditions and high sugar content were ideal for the proliferation of the microaerophilic LAB (Kantachote and Charernjiratrakul, 2008; Olstorpe et al. 2008). Although these conditions are also probably suitable for yeast their natural specific growth rates are much lower than those of the LAB (Olstorpe et al. 2008) so the LAB population outcompeted the yeast population, especially while the sugar concentration was high.

Identifications of the LAB isolated throughout the fermentation process showed that initially 99 of the isolates (97% of LAB) were *Lactobacillus plantarum* and the rest were *Lactobacillus pentosus* (Table 1). This finding was slightly different from our previous observations (Kantachote and Charernjiratrakul, 2008) when *Leuconostoc mesenteroides* was found during days 1-3 and later *Lactobacillus* spp. such as *L. plantarum*, *L. brevis*, *L. fermentum* and *L. coryniformis* were detected. However, even at that time *L. plantarum* was the most abundant lactobacillus species detected during the fermentation. As the microbial population originated from raw materials the source and type of the raw materials used will affect the initial microbial population. In this study we used molasses and ripe fruit instead of sugar and unripe fruit. As *L. plantarum* and *L. pentosus* both normally originate from plants, and are all closely related, with similar phenotypes (Torriani et al. 2001), it is possible that they proliferate on the fruit as it ripens and then become the predominant population in the inoculum for the fermentation.

The following yeasts were identified; *Saccharomyces cerevisiae*, *Rhodotolura mucilaginosa*, *Pichia membranifaciens* and *Pichia anomala* (Table 2). Throughout the fermentation (days 0-49), approximately 38% of the yeasts were *S. cerevisiae* whereas *P. anomala* was present at roughly 31% at days 14-56 followed by *P. membranifaciens* at 28.6% during days 7-35. *R. mucilaginosa* was present at roughly 2.4% but only at zero time presumably because it is recognized as being an aerobic film yeast and would therefore not grow well. It is well recognized that the yeasts identified above are commonly found in fermented beverages and foods based on fruit and vegetable extracts (Middelhoven, 2002; Basilio et al. 2008). In one of our previous publications we reported that the yeast species detected as contaminants in 27 kinds of finished fermented plant beverages were *Rhodotorula*, *Pichia*, *Hansenula*, *Saccharomyces* and *Candida* (Prachyakij et al. 2007).

Again the fermentation substrates of molasses and the ripe fruit of wild forest noni may select some specific yeasts and this might explain why a slightly different array of yeast species were obtained from other fermented plant beverages. The main reason that *S. cerevisiae* was the most prevalent yeast species could be that this species is a fermentative yeast whereas *P. anomala* is a respiratory strain and a Crabtree-negative yeast (Veiga et al. 2003), therefore these yeasts have the ability to grow under completely anaerobic conditions when supplied with ergosterol and unsaturated fatty acids (Fredlunda et al. 2002) and this would indicate that these compounds were present in the FWE fermentation. *P. anomala* has been found in a variety of alcoholic beverages, including drinks produced from cereals and plants (Thapa and Tamang, 2004). *P. anomala* produces ethanol during growth with

anaerobic conditions and the organism may convert sugar to ethanol like *S. cerevisiae*. It is interesting that both yeast species (*P. anomala* and *P. membranifaciens*) are killer yeasts and have been implicated as possible biocontrol agents of plant pathogenic fungi (Masih and Paul, 2002; Izgü et al. 2006). Hence, currently research is in progress to verify this possibility.

Roles of microbial succession on the characteristic of FWE

The initial sugar concentration in the wild forest noni fermentation was 8% and this dramatically decreased to 1.8% at day 14 and then further to 0.8% at the end of the fermentation (56 days) (Table 3). The pH had a similar trend starting at a pH of 4.43 dropping sharply to 3.70 at day 14 then more slowly to 3.66 at day 56. On the other hand, total acidity increased regularly from 0.22% at zero time to 0.96% at day 56. The EC increased from an initial value of 12.20 mS cm^{-1} to a maximum of 15.39 mS cm^{-1} between days 14 and 28 and then decreased to 14.47 mS cm^{-1} at day 56. At the beginning the C/N ratio in the FWE was 97 and this sharply decreased to 25 between days 14-28 and finally dropped to 18 at day 56 in line with the decrease of sugar.

The negative correlation between the amounts of sugar and acidity were related to the increased numbers of LAB. The results indicated that the LAB governed the most important changes that occurred in the FWE. As only *L. plantarum* and *L. pentosus* were detected and they are facultative heterofermenters (Axelsson, 2004) that use the phosphoketolase pathway it was not surprising to find significant amounts of acetic acid (0.33% at day 56). However, high amounts of ethanol (1.7%) were also found at day 56 and no lactic acid even though the acidity had continued to increase. This indicated that the lactic acid was being utilized by the dominant yeast species found in the fermentation such as *P. membranifaciens* and *P. anomala*. It is well recognized that *Pichia* and *Candida* species are lactic acid-utilizing yeasts (Kitamoto et al. 1999). This removal of lactic acid may have provided a positive

Table 3. Changes of physicochemical properties and plant nutrients in wild forest noni (*Morinda coreia* Ham) fermentation.

Parameter mg L^{-1}*	Fermentation day				
	0	14	28	42	56
Total sugar (%)	8.0 ± 0.44	1.8 ± 0.25	1.4 ± 0.44	1.2 ± 0.31	0.8 ± 0.41
Acidity (%)	0.22 ± 0.01	0.44 ± 0.03	0.52 ± 0.02	0.70 ± 0.05	0.96 ± 0.03
EC (mS cm^{-1})	12.2 ± 0.13	15.39 ± 0.01	15.39 ± 0.1	14.20 ± 0.03	14.47 ± 0.04
pH	4.43 ± 0.01	3.70 ± 0.06	3.73 ± 0.07	3.67 ± 0.06	3.66 ± 0.03
C/N ratio	97	25	25	20	18
Acetic acid (%)	0.03 ± 0.002	0.16 ± 0.002	0.21 ± 0.03	0.28 ± 0.03	0.33 ± 0.02
Lactic acid (%)	0.08 ± 0.01	0.30 ± 0.005	0.23 ± 0.004	0.12 ± 0.001	<LOQ
Ethanol (%)	0.09 ± 0.003	0.30 ± 0.009	0.39 ± 0.008	1.4 ± 0.05	1.7 ± 0.07
N	140 ± 6	513 ± 34	520 ± 87	573 ± 97	633 ± 99
P	270 ± 3.2	1360 ± 45	1086 ± 36	1190 ± 53	1210 ± 64
K	3566 ± 102	3318 ± 149	3796 ± 33	3982 ± 172	4356 ± 333
Ca	605 ± 77	736 ± 89	816 ± 164	819 ± 30	693 ± 219
Mg	399 ± 15	428 ± 52	468 ± 58	490 ± 46	536 ± 80
Mn	0.16 ± 0.01	6.34 ± 0.81	4.85 ± 0.99	5.45 ± 0.84	6.09 ± 0.95
B	0.33 ± 0.01	0.53 ± 0.05	58 ± 7.73	60 ± 4.5	51 ± 4.4
Zn	0.04 ± 0	2.02 ± 0.56	1.32 ± 0.05	1.45 ± 0.05	1.69 ± 0.08

stimulus to the proliferation of *S. cerevisiae* because it is sensitive to acetic acid and lactic acid (Narendranath et al. 2001) and agrees with the results in this study where *S. cerevisiae* was present throughout the fermentation and also correlates with the high amount of ethanol in the FWE. However, some ethanol may be produced by *P. anomala* as previously mentioned and also the lactobacilli via the phosphoketolase pathway depending on the redox potential but unfortunately this was not measured in this study.

Because a 10-fold dilution of molasses still produces a high level of carbohydrate (1.36%) but is low in protein (0.014%); the initial C/N ratio was 97 (Table 4). However, after the fermentation, the final C/N ratio of the FWE dropped to 18 because the sugar had been consumed for growth while the nitrogen had been converted to microbes. This could be part of the explanation for the increase of total N from 0.014% to 0.063% in combination with the release of nitrogen from the initial solid mass of wild forest fruits. It has been claimed by Kayhanian and Tchobanoglous (1993) that liquid fertilizer should have a C/N ratio in a range of 15-20 so that the best time to use the FWE for use as a liquid fertilizer would be at days 42 and 56 when the C/N ratios were 18-20.

Plant nutrients

At the start of the fermentation most of the nutrients in FWE originated from the molasses and the addition of the wild forest noni fruits into the molasses caused very little change (Table 4). Plant nutrients particularly B, Zn, Mn, N and P increased significantly 155, 42, 38, 4.5 and 4.5 times from the beginning in the FWE (Table 4). It seems that the fermentation of sugar by LAB produces acidic condition and this together with other products produced by the growing microbes led to the release of extra nutrients from the wild forest noni fruits and explained the positive relationship between total acidity and EC values (Table 3 and Table 4). Amongst the primary plant nutrients, especially P the acids produced by the LAB will make P products more soluble. This result is supported by one of our previous studies (Prachyakij et al. 2008), when it was reported that LAB improved the availability of elements (Cu, Zn and Fe) in a fermented seaweed beverage.

Table 4. Physicochemical properties of molasses and wild forest noni (*Morinda coreia* Ham) extract.

Parameter mg L^{-1}*	Diluted molasses (10^{-1}) prior to adding fruits	Wild forest noni extract		
		T = 0	56 d	Ratio (T56/T0)
EC (mS cm^{-1})	12.08	12.20	14.47	nd
pH	5.81	4.43	3.66	nd
Organic matter (%)	2.52	2.35	1.92	nd
Organic carbon (%)	1.46	1.36	1.11	nd
C/N ratio	115.6	97.3	17.5	nd
Total N	126	140	633	4.52
Total P	237	270	1210	4.48
Total K	3652	3566	4356	1.22
Total Ca	580	605	693	1.15
Total Mg	381	399	536	1.34
Total B	0.7	0.33	51	155
Total Mn	0.3	0.16	6.09	38
Total Zn	0.5	0.04	1.69	42.25

*Unless stated; nd = not determined; mean value and standard deviation of three determinations are presented.

A similar pattern was found for the amounts of the secondary plant nutrients (Ca and Mg) including the micronutrients (Mn and Zn) and the numbers of lactobacilli. On the other hand, the amount of B increased to a maximum at day 42 (60 mg L^{-1}) followed by no significant further change. This might indicate that further degradation of solid plant material occurred after the maximum level of LAB and pH was achieved closer to day 14. B was definitely being extracted from the wild forest noni fruit during the fermentation because the amount of B that originated from the molasses used was only 0.70 mg L^{-1}, but in the FWE it had increased to 51 mg L^{-1} at day 56 (Table 3 and Table 4). It has long been known that fruits and vegetables are a rich source of B (Bellaloui and Brown, 1998). It is interesting that the fermentation process has provided a relatively high amount of plant nutrients particularly B, therefore after an appropriate dilution the FWE may be a suitable liquid fertilizer.

Plant hormones and phytotoxicity

Gibberellic acid (GA_3) was used as a standard for determining the amounts of gibberellins (GAs) in the FWE. GA_3 had a R_f value of 0.70, but none was detected with this R_f in the FWE (Figure 2). However, 2 compounds with R_f values of 0.43 and 0.60 were found at days 14, 28, 42 and 56, whereas at zero time there was only one compound with an R_f value of 0.35. Therefore, some gibberellin-like substances may be present as Tamura et al. (1967) who used a similar method to that used in this study reported that GAs secreted by immature seeds of sword bean (*Canavalia gladiata* DC) had R_f values in a range of 0.30-0.60. Indole acetic acid was used as a standard for detecting auxins but none were detected in the FWE. It has long been recognized that GAs play an important role in the stimulation of seed germination (Chen et al. 2001), thereby the FWE may also stimulate seed. Nevertheless, the acetic acid and ethanol are both toxic to plants (Kapanen and Itavaara, 2001) and these are present in the FWE.

In this study, germinating cherry tomato seeds were used to investigate the phytotoxicity or stimulating effects of FWE. The absence of phytotoxicity can be implied if similar results of the seed GI are obtained for any test sample and distilled water (Amaral da Silva et al. 2005). The GI has been proved to be a very sensitive index of phytotoxicity (Kapanen and Itavaara, 2001). It was found that a 256 fold dilution gave the best GI (157), followed by the 128 and 512 fold dilutions (Figure 3). The GI for cherry tomato was reduced to less than 50% for the 8, 16 and 32 fold diluted FWE so these dilutions were toxic to the plant. However, dilutions of FWE between 128 and 512 provided suitable conditions for cherry tomato seeds to germinate. This could be a reflection of the dilution of acetic acid and ethanol to non toxic levels, whereas some growth stimulating substances were still at levels able to promote seed germination. Other plant nutrients particularly P, Mg, Mn and B may also be present at appropriate concentrations to stimulate seed growth (Bellaloui and Brown, 1998).

CONCLUDING REMARKS

The fermentation of wild forest noni into FWE involves a complex microbial succession between LAB and yeasts. Both groups of organisms originate from the raw materials used in a traditionally fermented FWE. LAB were the predominant microbes during the first 14 days but LAB were then slowly replaced by yeasts. *L. plantarum* was the dominant LAB species at all times whereas the yeast species changed. *R mucilaginosa* being an aerobe was only present at the start. *S. cerevisiae*, was always present, but was slowly replaced by *P. membranifaciens* and *P. anomala*. The changes were associated with the changing nature of the yeast nutrients for example from sugar to lactic acid. Results indicate that plant nutrients present in the FWE were at an appropriate level for potential use as a liquid fertilizer, particularly for the micronutrients such as B, Mn and Zn.

ACKNOWLEDGEMENTS

Dr. We are grateful to Dr. Brian Hodgson for critical reading of the manuscript.

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