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Microbial Population in Fermented Cooked Taro Skins

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Abstract

Piglets suffering post-weaning stress and showing unthrifty hair coat, loss in weight, and diarrhea were observed to recover from gastrointestinal illness when fed fermented taro skins, a by-product of poi production. Lactic acid bacteria (LAB), which are potential probiotics, have been found to be dominant within different poi brands in previous studies. Therefore, it was hypothesized that LAB might also be present in the fermented taro skins and might be responsible for the piglets' recovery. Three samples of cooked taro skins were collected from a local commercial poi manufacturer and two samples of home pressure pot cooked dry-land taro skins were obtained. Bacteria in the samples were enumerated via plate count at 0, 24, and 34 hours. At 34 hours, samples from both sources showed a dominance of LAB in the microbial population. DNA from 37 isolates were subjected to polymerase chain reaction (PCR) amplification of the 16S rRNA gene and sequencing. Thirty-five obtained gene sequences were run through the BLASTn 16S rRNA database. LAB constituted 91% of the sequenced isolates. *Leuconostoc mesenteroides* was the dominant species (75%), with *Lactococcus* and *Weissella* genera also present. Supernatant from the *Leuconostoc mesenteriods* culture showed the ability to inhibit *Salmonella* and *Listeria* bacteria. This study confirms the presence of beneficial LAB in taro skins and suggests that *Leuconostoc mesenteroides* could potentially be utilized as a probiotic in swine production.

Key words: Lactic acid bacteria (LAB), poi, piglet, diarrhea, probiotic, taro.

Introduction

Historical perspective: Taro, *Colocasia esulenta* (L), is a root crop used as a major starchy food in many cultures, especially among Pacific Islanders. Taro, or kalo, as it is called in Hawaiian, holds a special place in Hawaiian culture. According to Hawaiian legend, the first child of the deities was stillborn and was buried. On that very site sprouted kalo, which provided sustenance to the subsequent descendants, the Hawaiian people (Bishop Museum 2014).

In general, taro is harvested when mature, washed, boiled, and peeled, and the corm is pounded into a paste called poi. The benefits of poi consumption have been documented (Roth et al. 1977, Glaser et al. 1965). Glaser et al. (1965) advocated the use of poi for treatment of gastrointestinal disorders and highlighted the non-allogenic properties of poi. The presence of potential probiotic-type bacteria has been reported (Huang et al. 1994, Brown and Valiere 2004).

Field observations: In a commercial permaculture farm, it was observed that piglets after weaning had high incidence of diarrhea. When the piglets were fed fermented taro skins/peels, diarrhea ceased and the piglet recovered. Given the beneficial health advantages of poi consumption, it was postulated that the fermented cooked taro skins might have probiotic bacteria that aided piglets confronting the stress of early weaning. Hence, a small study to examine the microbial profile of fermented taro skin was conducted as part of an undergraduate research project. The objectives were to a) identify the bacteria population in fermented taro peel and b) to conduct

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simple tests on its ability to inhibit growth of harmful bacteria.

Materials and Methods

***Taro skin samples*:** Cooked taro skins samples (n=3) were obtained from a commercial poi manufacturer. The taro for this outlet was from wetland culture. The taro was cooked above 100°C at under pressure for >30 minutes prior to cooling and skin removal. Samples were collected at a) 0 hours (h), b) 24 h, and c) 34+ h. Samples for 0 h were collected in the morning of processing at the factory, 24-h samples were collected the following morning from the factory bins, and 34+ -h samples were collected from the permaculture farm site. The dry-land taro was purchased from the local market. Dry-land taro skin samples (n=2) were obtained following pressure cooking in a household pressure cooker at 10 psi for 45 minutes. Time 0-h samples were taken following cooking. The remaining cooked skins were then stored in zipper-sealed bags and placed on a table at room temperature to mimic the conditions for the wetland taro for samples at 24 h and 34+ h.

***Proximate analyses*:** Taro skin/peel samples for proximate analyses were sent to the College of Tropical Agriculture and Human Resources Agriculture Diagnostic Center for mineral analyses by AOAC-approved methods.

***pH readings*:** The pH values for all samples were measured with a laboratory pH meter after mixing 10g of sample with 10 ml distilled water by vortexing for 1 min.

***Microbial culture and isolation*:** Serial dilution of samples for 0 h, 24 h, and 34+ h were plated on three media: plate count agar (PCA); Man, Rogosa and Sharpes agar (MRS), and potato dextrose agar (PDA). Samples were incubated at 35°C for 48 hours. The media were prepared per the manufacturer's instructions.

LAB were isolated from the 34+ h samples on MRS agar. Ten colonies were selected per sample of wetland taro skins and five colonies were selected per sample of dry-land taro skins. In total, 40 selected colonies were streaked and incubated at 35°C for 48 hours. Three plates showed no growth. The remaining 37 colonies were grown in 5mL of MRS broth at 35°C for 24 hours. From each tube, 1 µL of sample was mixed with 24 µL of PCR master mix containing 16S F primer (5'-GGA GAG TTT GAT CCT GGC TCA G-3') and 16S R primer (5'-TAT TAC CGC GGC TGC TGG CAC -3'), and then subjected to polymerase chain reaction (PCR) for 30 cycles. The resulting amplicons were loaded into 1.5% agarose gel, electrophoresed at 100 volts for 35 minutes, and then stained with GelGreen™ for 20 minutes. PCR Marker from Promega was used as the DNA ladder. Remaining amplicons were cleaned of excess primers and dNTPs

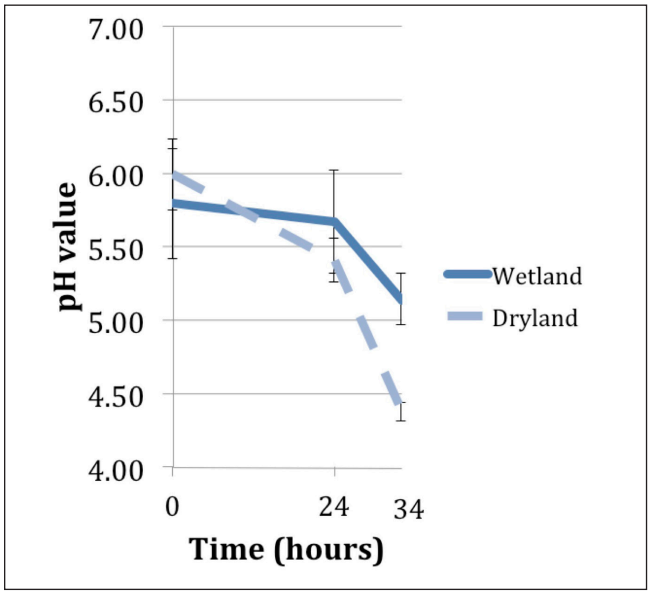

Fig. 1. The mean (± SE) pH values of cooked and fermented taro skins over time.

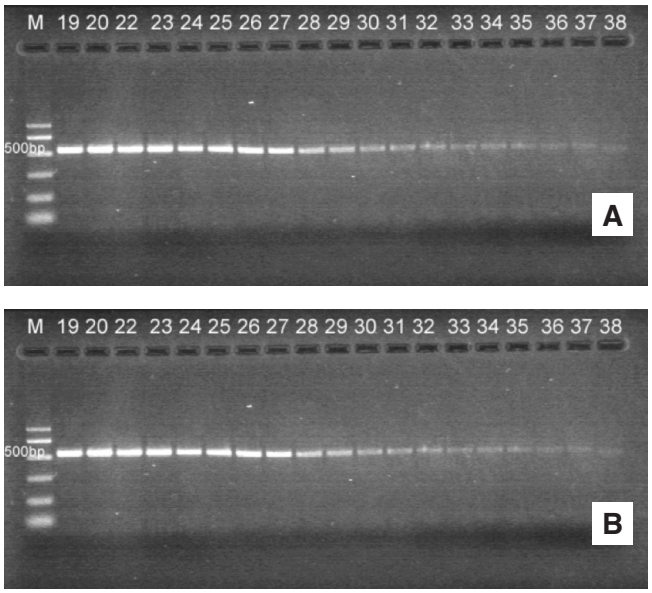

Fig. 2. Gel electrophoresis of A) isolated bacterial colonies and B) bacterial colonies.

Table 1. Proximate analyses (mineral composition) of cooked, fermented taro skins (n=5).

	CP	P	K	Ca	Mg	Na	Fe	Mn	Zn
Average	6.14	0.15	0.86	0.43	0.24	0.09	12305.6	283.2	117
SD	1.31	0.03	0.31	0.13	0.06	0.02	4660.4	180.2	58.9

Table 2. Bacterial population in PCA and MRS culture media for respective sampling time: 0 h, 24 h and 34+h after cooked taro skin was left to ferment in ambient temperature.

	Wetland Taro (n=3)		Dry-Land Taro (n=2)	
Time	PCA	MRS	PCA	MRS
0	3.0±0.87	3.1±0.21	5.1±0.0	4.5±0.0
24	8.5±0.39	8.2±0.17	7.9±0.0	7.6±0.0
34+	8.7±0.09	8.7±0.04	8.7±0.07	8.8±0.02

with ExoSAP-IT®. Gene sequencing was performed by the Pacific Biosciences Research Center.

***Testing for antimicrobial effect of LAB from fermented taro skins*:** LAB isolates from fermented taro skins were cultured in MRS broth. An aliquot of the medium was then subjected to centrifugation at 13,000 rpm for 2 min. The supernatant was filtered through 0.22 µm membrane; and 50 μl of each filtrate was used to determine the ability of the LAB supernatants to suppress bacterial growth via agar well diffusion tests on two pathogenic bacteria, *Salmonella typhimurium* and *Listeria monocytogenes*.

Results and Discussion

Table 1 shows the proximate analyses of cooked fermented taro skins, in which high levels of Ca++ and Fe^{+} were found. Calcium's role in signaling the activation of defense mechanisms and the initiation of immune responses has been reported (Baba et al. 2008, Feske 2007). More recent studies have demonstrated how a flash (high dose) of calcium is necessary in wound repair (Razell et al. 2013). The findings of higher levels of Ca^{++} in the taro skin warrants future research to determine its benefits in piglets after weaning.

In modern animal husbandry, it is the norm to provide the fast-growing piglet with an injection of iron in the first week of life to prevent anemia. In light of larger litter sizes and faster-growing pigs, some researchers are suggesting the need to examine the role of iron supplementation prior to weaning (Estrup 2012). It remains unclear if there are any advantage for additional supplementation of iron to piglets after weaning. Some reports point to positive benefits (Bach et al. 2006), especially in immune-compromised pigs [Postweaning Multisystemic Wasting Syndrome (PMWS), herds] while others showed no improvement in growth performance (Bruininx et al. 2000). Iron in soil is considered an effective route of providing iron to piglets (Miller and Ullrey 2007). The high levels of iron in the taro skins were possibly due to the volcanic soils in Hawai'i. It is uncertain if this iron is biologically available.

The pH of the cooked fermented taro skins decreased over time. Both taro sources had pH close to 5.8–6.0 but by 34+ h, the pH had decreased to 5.1 for wetland taro and 4.4 for dry-land taro. Huang, et al. (1994) reported a pH 4.0–4.5 after poi was left at 20°C for 2–3 days.

The bacterial populations on different agar media (PCA and MRS) for wetland and dry-land taro skins are detailed in Table 2. By 34+ h, the majority of the bacteria found in cooked fermented taro skins were LAB.

Subsequent gel electrophoresis demonstration of the presence of these bacteria is presented in Figure 2a and 2b. Thirty-five isolates were subject to DNA sequencing,

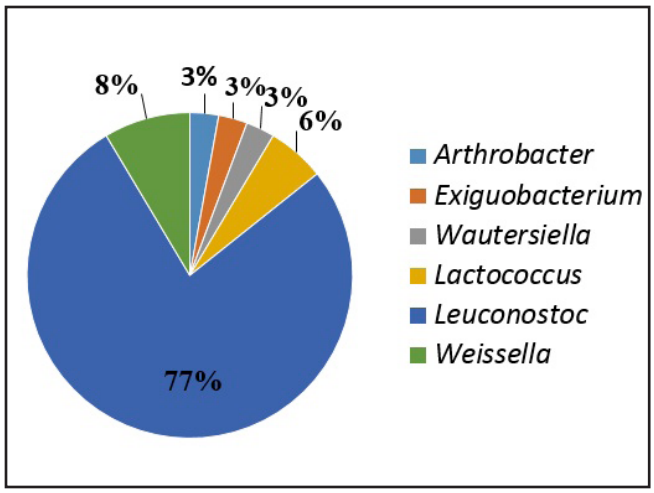

Fig. 3. Bacterial identified from the cooked fermented taro skins following DNA sequencing.

and 91% of the bacteria found were of the lactic acid bacteria strains. Over 75% of the LAB were identified to be *Leuconostoc mesenteroides*. Other LAB were also identified as shown in Figure 3.

Supernatant filtrates (50μl) from selected LAB isolates were then tested for their ability to inhibit the growth of *S. typhimurium* and *L. monocytogenes*. Figure 4 shows that supernatant from *Leuconostoc mesenteroides* was capable of inhibiting both these harmful pathogens. Currently, studies are being conducted to test all the various strains of LAB found in this study. The antibacterial potential of fermented taro has been reported (Muller et al. 2002). In addition, recent research showed that the *Leuconostoc mesenteroides* present in kimchi, a Korean preserved cabbage, was capable of killing H9N2 influenza virus of chicken (Seo et al. 2012). Hence, it is very possible the high levels of *Leuconostoc mesenteroides* found in the fermented taro skins helped in the recovery of the sick piglets following weaning when they were fed the taro skins. To affirm the beneficial effects of the taro skin, animal studies would be warranted. The current study sets the stage for this by providing a profile of the bacteria population in the fermented taro skins.

Implications

Since this study, the farmer, with the help of the local Extension service, has altered the way he feeds the fermented taro peels to the weaned piglets. The fermented taro skins are provided to the piglets early in the morning, prior to the normal feeding regimen, the rationale being that the piglets are hungry then and are more likely to eat a reasonable amount of the product. It was observed (>9mo.) that when this was done consistently, there was a very low incidence of diarrhea in the weaned piglets. Nevertheless, there is a need for a more defined experiment protocol that involves live animals to verify the potential use of taro skins in sustainable farming.

Summary

Based on the preliminary results, the higher calcium and iron levels in taro skins coupled with the bactericidal capabilities of some of the LAB imply that taro skins could be an important source of probiotic or a potential alternative to antibiotics in animal feed. Further investigation is warranted to unravel the possibilities.

Acknowledgement

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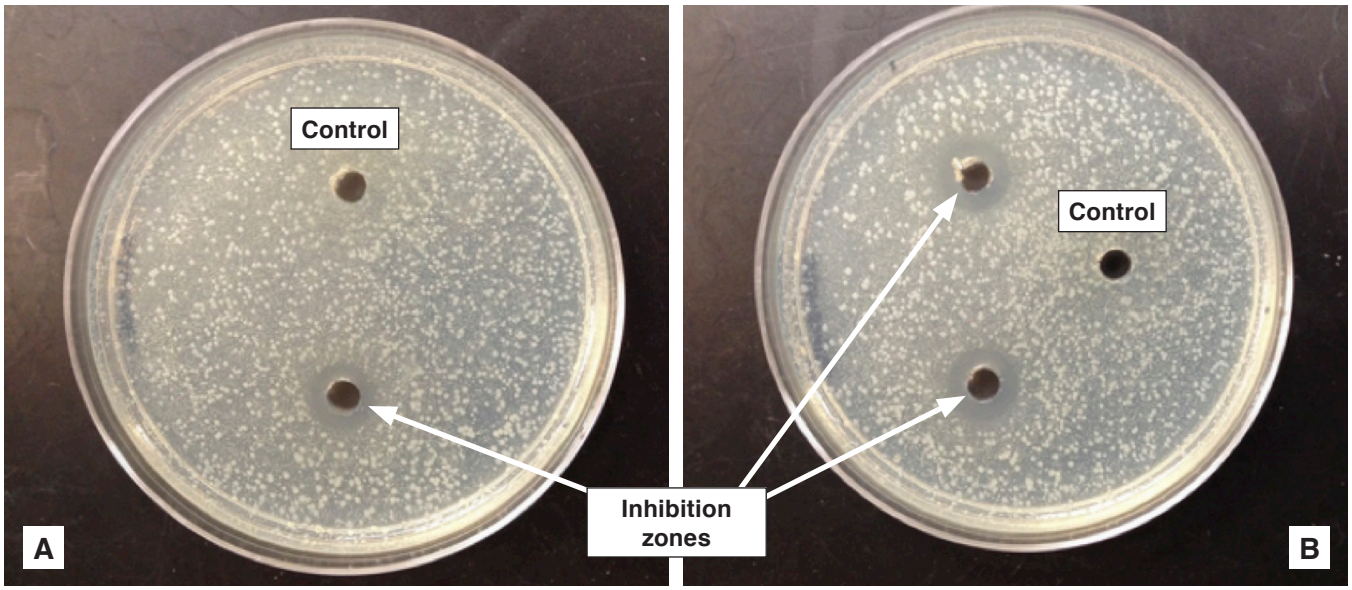

Fig. 4. Inhibition of A) *L. monocytogenes* and B) *S. typhimurium* by supernatant (50µl) from *Leuconostoc mesenteroides*.

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