



Isolation and Identification of Bacteria from Kava Extract

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Abstract

Kava (*Piper methysticum*) is a member of the *Piperaceae* family. Cultivated for over 3,000 years, kava is used by Pacific islanders to make the traditional drink. It consists of active ingredients called kava lactones that have been used in herbal medicine for stress, anxiety and depression. There have not been any studies done on microbial profile of kava extract. The objective of this study was to isolate and identify bacteria in kava extract. Three kava samples were used. Middle part of the kava stump was taken to reduce the soil contamination as much as possible. Kava extract was made, serially diluted and plated on nutrient agar. Isolated colonies were identified by using polymerase chain reaction (PCR) with 16S rDNA based universal primers. When needed, species-specific primers targeting the *tuf* gene of *Staphylococcus* were used for further confirmation. *Bacillus*, *Staphylococcus*, *Cellulomonas*, *Kocuria*, *Enterococcus*, *Pectobacterium*, *Paenibacillus*, *Microbacterium*, *Pseudomonas*, *Enterobacter*, *Lactococcus*, *Acinetobacter*, *Klebsiella*, *Pantoea*, *Citrobacter* and *Erwinia* species were identified. *Pectobacterium*, *Bacillus*, *Enterococcus*, *Staphylococcus* and *Cellulomonas* were found in all kava extracts tested. Kava stump contains large amounts of starch and fiber. Organisms like *Paenibacillus stellifer* and *Bacillus megaterium* may be involved in starch degradation. *Pectobacterium* and *Erwinia* are the plant pathogens, which produce extracellular pectic enzymes and cause soft rot in a wide range of plants. *Bacillus cereus* group has been known to potentially produce enterotoxins or emetic toxins. The results of this study provide fundamental information that may enhance microbial safety and quality of kava beverage.

Introduction

- Piper methysticum (kava) is a perennial shrub cultivated for over 3,000 years
- Used to make non-alcoholic drink by Pacific islanders in religious ceremonies, courtship rituals and social gatherings
- Kava beverage contains kava-lactones, also known as kava-pyrones, which is the main active constituent
- Used as a muscle relaxant for relief of spasms, cramps, remedy for nervousness, urinary problems, asthma, whooping cough, stomach ache, headache and fungal infections
- Shelf life of the fresh kava beverage is 3 days at 4°C
- The microbial profile of kava extract is unknown



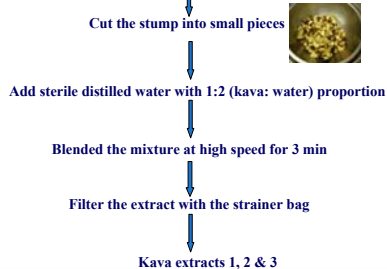
Objective

To isolate and identify bacterial species in the aqueous extract of kava stump

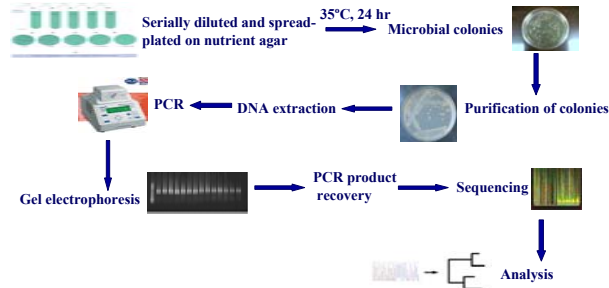
Sample Preparation



Kava sample 1 (Oahu, HI) → Pealed off the outer layer (0.5 cm)
Kava sample 2&3 (Big Island, HI)



Experimental Methods



Results

Table 1. Source, Bacterial Count and pH of the Kava Extracts

Kava Extract	Source	Bacterial Count (log ₁₀ cfu/ml)	pH
Extract 1	Oahu, HI	3.7	6.74
Extract 2	Big Island, HI	5.1	6.50
Extract 3	Big Island, HI	5.0	6.57

Table 2. Primers Used for Amplification and Sequencing: Primer Name (F/R, Forward or Reverse), Position (*E. coli* Numbering) of Primer, Primer Sequence, PCR Conditions and References

Primer Name	Position	Sequence	PCR Conditions	References
16S rDNA-F	<i>E. coli</i> C000091	5' GGA GAG TTT GAT CCT GGC TCA G 3'	5 min at 95°C and then 30 cycles of 30 s at 95°C, 30 s at 55°C for annealing and 45 s at 72°C extension step and final step for 10 min at 72°C	1
16S rDNA-R	4-532	5' TAT TAC CGC GGC TGC TGG CAC 3'		
<i>tuf</i> -F	<i>E. coli</i> J01690	5' GGC CGT GTT GAA CGT GGT CAA ATC A 3'	4 min 95°C and then 30 cycles of 30 s at 95°C and 30 s at 65°C for annealing and extension steps and final step for 7 min at 72°C	2
<i>tuf</i> -R	422-792	5' T [*] A CCA TTT CAG TAC CTT CTG GTA A 3'		

T^{*} = nucleotide analog inosine

References

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2. Martineau F., Picard F.J., Ke D., Paradis S., Roy P.H., Ouellette M., and Bergeron M.G. 2001. Development of a PCR assay for identification of *staphylococci* at genus and species levels. *J. Clin. Microbiol.* 39:2541-2547.

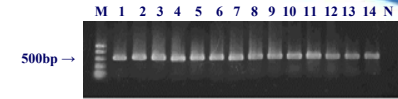


Figure 1. Gel Electrophoresis of 16S rDNA-Based PCR Products: M: DNA Marker, Isolated Bacteria: 1-14 and N: Negative Control

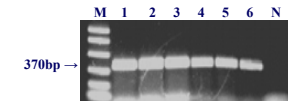


Figure 2. Gel Electrophoresis of the *tuf* Gene-Based PCR Products for *Staphylococcus* spp.: M: DNA Marker, Isolated Bacteria: 1-6 and N: Negative Control

Table 3. Organisms Identified Based on 16S rDNA Sequence from the Kava Extracts

Isolated Organism	Extracts			Closest Relative	%Identity
	1	2	3		
<i>Pseudomonas</i>	-	3	3	<i>Pseudomonas chlororaphis</i>	98
	-	1	-	<i>Pseudomonas oryzae</i>	99
	-	-	1	<i>Pseudomonas putida</i>	97
<i>Enterobacter</i>	-	1	-	<i>Enterobacter aerogenes</i>	98
<i>Lactococcus</i>	-	2	2	<i>Lactococcus lactis</i>	98
<i>Acinetobacter</i>	-	1	-	<i>Acinetobacter lwoffii</i>	97
<i>Bacillus</i>	3	3	2	<i>Bacillus cereus</i> group spp.	99
	1	1	2	<i>Bacillus megaterium</i>	99
	1	-	-	<i>Bacillus fusiformis</i>	99
<i>Erwinia</i>	-	1	3	<i>Erwinia amylovora</i>	96
<i>Pectobacterium</i>	2	1	2	<i>Pectobacterium carotovorum</i>	98
<i>Citrobacter</i>	-	1	2	<i>Citrobacter freundii</i>	99
<i>Cellulomonas</i>	2	1	2	<i>Cellulomonas denverensis</i>	99
<i>Enterococcus</i>	-	1	1	<i>Enterococcus saccharolyticus</i>	100
	1	2	1	<i>Enterococcus casseliflavus</i>	100
<i>Klebsiella</i>	-	2	1	<i>Klebsiella pneumoniae</i>	98
<i>Pantoea</i>	-	3	2	<i>Pantoea agglomerans</i>	98
<i>Kocuria</i>	2	-	-	<i>Kocuria kristinae</i>	100
<i>Paenibacillus</i>	1	-	-	<i>Paenibacillus stellifer</i>	98
<i>Microbacterium</i>	1	-	-	<i>Microbacterium thalassium</i>	97
<i>Staphylococcus</i>	-	4	3	<i>Staphylococcus epidermidis</i>	99
	3	-	-	<i>Staphylococcus pasturi</i>	98
	3	-	-	<i>Staphylococcus</i> spp.	-

Table 4. Organism Confirmed Based on the *tuf* Gene from the Kava Extract-1

Organism	No. of Samples	%Identity
<i>Staphylococcus warneri</i>	3	95

Conclusions

- Kava samples obtained from the Big Island had higher bacterial count than the kava samples obtained from Oahu
- *Pectobacterium*, *Cellulomonas* and *Bacillus* species were found in the three kava extracts. These organisms are known to degrade polysaccharides like pectin, cellulose and starch respectively

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