

*Full Length Research Paper*

# Molecular characterization of probiotic *Enterococcus hirae* from fermenting *acalypha wilkesiana* (Irish Petticoat) and *Talinum triangulare* (Water Leaf) medicinal plants

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Research on lactic acid bacteria (LAB) continues to be relevant in the development of nutraceuticals. In this study, edible medicinal *Talinum triangulare* and *Acalypha wilkesiana* were collected and subjected to spontaneous fermentation for 24 h. Samples were collected at 6 h intervals for determination of pH and microbial analysis. Determination of LAB percentage frequency occurrence, probiotic properties, and safety were done. Molecular characterization of probiotic and safe LAB was carried out. During spontaneous fermentation of *T. triangulare*, pH decreased from 7.7 at 0 h to 5.8 at 24 h. Same trend was observed during *A. wilkesiana* fermentation. Microbial loads increased from  $1.4 \times 10^3$  cfu/ml at 6 h to  $4.68 \times 10^7$  cfu/ml at 24 h during fermentation of water leaf and from  $2.56 \times 10^2$  cfu/ml at 6 h to  $5.85 \times 10^7$  cfu/ml at 24 h during Irish petticoat fermentation. Forty-three LAB (30 and 13 LAB from fermenting Water leaf and Irish petticoat, respectively) were isolated in this study. They were identified as: *Enterococcus hirae* (43.3%), *Streptococcus thermophilus* (20.9%), *Enterococcus durans* (18.6%), and *Lactococcus lactis* (14.0%). However, *Enterococcus hirae* had the highest probiotic properties followed by *Streptococcus thermophilus* but the result of their molecular characterization revealed *Enterococcus hirae* and *Weissella confusa*, respectively. Considering this confusion and uncertainty about *W. confusa*, its use as a probiotic should be approached with caution. *E. hirae* is suggested to be the best probiotic potential starter for the controlled fermentation of water leaf and Irish petticoat medicinal plants. This study therefore confirms the possibility of isolating probiotic *E. hirae* from non-dairy source and suggests its incorporation into plant-based starter fermented foods.

**Key words:** *Enterococcus hirae*, *Weissella confusa*, probiotic, water leaf, Irish petticoat.

## INTRODUCTION

Biotherapy in form of utilization of plant-based products and microorganisms in food and medicine continue to increase throughout the world due to carcinogenic related problems associated with the usage of chemical products. A medicinal plant is defined by WHO, as any plant which,

in one or more of its organs, contains substances that can be used for or which is a precursor for the synthesis of useful drugs (Gopal et al., 2014).

Therapeutic properties of medicinal plants are very useful in the treatment of various diseases and the

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advantage of these plants is 100% natural with little or no side effects (Igbayilola et al., 2017). Some herbal plants like water leaf and Irish petticoat are edible hence used in the production of herbal tea because of their nutritional and therapeutic properties (Agudo, 2005; Kuete, 2017).

*Talinum triangulare* is a medicinal plant which belongs to the family Potulaceae and performs dual functions as food and therapeutics. It can be cooked as soup and used as medication to inhibit proliferation of cancerous cells, shrink tumors, regulate hypertension and diabetes, produce anti-inflammatory and diuretic effect, fight insomnia and enhance brain activities. According to Yilni and Naanma (2020), *T. triangulare* had numerous biological uses and functions which include suppression of oxidative damage of liver cells (Liang et al., 2011), helps to enhance liver function (Ezekwe et al., 2013), enhances cerebral, reduces oxidative stress, supports neurons of cerebrum, and enhances cerebral function (Ofusori et al., 2008), helps to treat mild laxative problem and constipation (Joshua et al., 2012), essential for managing diabetes mellitus and its component helps to slow down digestion and conversion of starch to simple sugar. *Alcalypha wikesiana* is another herbal plant with therapeutic properties. It belongs to the family Euphorbiaceae. It is frequently used in traditional medicine, exclusively or as a major constituent of many herbal preparations for the management or treatment of hypertension. It is used in the treatment of malaria, dermatological and neonatal jaundice, as well as gastrointestinal disorders (Iyekowa et al., 2016).

Probiotics are “live microorganisms, which when used in adequate amounts confer a health benefit on the host” (FAO, 2011). Consumption of a large number of probiotic live microorganisms together with a food, fundamentally promotes the health of the consumers through: prevention and treatment of infectious diseases (Rolfe, 2000), curing of irritable bowel syndrome, alleviation of allergies, digestion of lactose and lowering of serum cholesterol levels (Shah, 2015) to the prevention of cancer. Probiotics are used in microbial food supplements, because they are of different human health medical benefits. As a result, probiotics have found a place in pharmaceutical plans and are provided as pharma products (Bhattacharyya, 2009). In the pharmaceutical industries, probiotics are produced as either dietary enhancement or medication. The combinations of probiotic strains give probiotic products. These probiotic products are accessible as capsule, tablets, powder, drops, bottle caps, chewing gum, etc., in the market, but mostly probiotics are available in the form of capsules (Saxelin, 2008). Most probiotics available today belong to the lactic acid bacteria (LAB) group.

Enterococci LAB are vital in food due to fermentation as probiotics in human and animals (Kamni and Ramesh, 2020). They are Gram-positive cocci, catalase-negative, non-spore formers, and occur in vegetables, plant materials, and various dairy products. Enterococci directly

contribute to the distinctive flavor and taste of traditional dairy products. They also protect many fermented foods, such as sausages, cheese, and dairy products (Moreno et al., 2006). Enterococci are commonly associated with cow, goat, sheep, and buffalo milk fermentation (Quigley et al., 2013). Several species of *Enterococcus* have been commonly used as probiotics in humans and animals for the cure of gastroenteritis (Hu et al., 2015).

*Enterococcus hirae* is a lactic acid bacterium with reported probiotic properties such as: ability to tolerate bile, NaCl concentrations, acidic pH and phenol as well as capability to inhibit intestinal pathogens (Kamni and Ramesh, 2020).

Despite the abundance of research on the use of probiotic LAB isolated from dairy products as adjunct starter in herbal tea production, the incorporation of indigenous probiotic LAB which are thought to be better adapted to the substrate has not found the same acceptance for use in this non-dairy based functional food. This study therefore aimed to investigate the species of probiotic LAB with starter potentials for herbal tea production from fermenting water leaf and Irish petticoat.

## MATERIALS AND METHODS

### Sample collection

Leaf samples of water leaf and Irish petticoat were aseptically collected in triplicate from three different sources (IITA, University of Ibadan botanical garden and The Polytechnic of Ibadan botanical garden) and transported to the research laboratory of The Polytechnic, Ibadan for analysis.

### Spontaneous fermentation of water leaf and Irish petticoat

Spontaneous fermentation of the leaves of water leaf and Irish petticoat were carried out according to the method of Saguibo et al. (2019) with slight modification. Twentyfive grams of the washed leaves was incorporated into 500 mL of sterile brine (containing 5% of NaCl w/v) in a glass jar with metal lid. This was incubated for 24 h at ambient conditions. Microbial analysis, pH and total titratable acidity were determined at 6 h interval for 24 h.

### Isolation and purification of LAB from spontaneous fermented water leaf and Irish petticoat

Ten grams of the cut fermented leaves were De Mann Rogosa and Sharpe (MRS) agar containing 1% CaCO<sub>3</sub> using the pour plate method. The plates were incubated in an air-tight candle canister at 37°C for 48 h. Presumptive LAB colonies with clearing zones were purified by repeated streak plating using the same agar medium until pure colonies were obtained. Gram staining was carried out for preliminary identification of LAB. Further identification tests (catalase, oxidase, indole, motility and spore staining test) were carried out on the isolates.

### Determination of pH

The changes in pH of fermenting samples were monitored at 6 h

intervals for 48 h using a ROHS pH meter (HANNAH instrument, Italy) (Eremosele et al., 2017).

#### Determination of total titratable acidity (TTA)

TTA was determined using the method described by Eremosele et al. (2017) by titrating 25 ml of supernatant from fermenting unripe plantain with 0.1 N NaOH using phenolphthalein as indicator. Triplicate determinations were carried out. TTA was determined and expressed as (Eremosele et al., 2017):

$$\% \text{ Lactic acid} = A \times 0.009 \times 100/V$$

where A=ml of 0.1 N NaOH, V=ml of sample taken for test (=25 ml).

#### Identification of LAB using culture dependent methods

##### Morphological, physiological and biochemical tests

LAB isolates were identified according to their morphological, physiological, and biochemical characteristics in line with standard techniques and this identity was confirmed using Bergey's manual of determinative bacteriology (Mulaw et al., 2019).

**Probiotic characterization of LAB:** Antibacterial activity of the isolates, resistance to some conventional antibiotics, tolerance to gastric acidity and resistance to bile salts were conducted according to the method described by Iyer et al. (2010).

##### Antibacterial activity

Antibacterial activity of the isolates was determined against *Staphylococcus aureus*, *Escherichia coli*, *Shigella* species and *Bacillus cereus*. LAB isolates were incubated for 48 h at 37°C. After incubation, cells were removed by centrifugation and pH of supernatants was set at 6.5 and it was filtered through 0.22 µm filter to obtain cell free supernatants (CFS). This CFS was used as antimicrobial agent using agar well diffusion method. Antimicrobial activity was evaluated by measuring zone of inhibition against the test organism.

##### Resistance to some conventional antibiotics

This was carried out according to the method of Shaikh and Gaurav (2013). The susceptibility to ten antibiotics of the LAB was investigated by the disc diffusion method on Mueller Hinton agar. After incubation at 37°C for 24 h, the diameters of inhibition zones were measured.

##### Tolerance to gastric acidity and resistance to bile salts

Each sample was used in triplicate to investigate the ability of the LAB to withstand gastric acidity. The bacterial pellet of young culture obtained after centrifugation (13000 rpm for 4 min) was suspended in 10 ml of MRS broth at three different pHs (2, 2.5 and 6.5). The OD from each culture obtained at 660 nm was recorded respectively at the start of the experimentation ( $T_{0h}$ ) and 2 h after incubation at 37°C ( $T_{2h}$ ). The number of viable cells was determined and survival rate was calculated using the following formula:

$$\text{Survival rate (\%)} = [\log_{10} \text{UFC} (T_{2h}) / \log_{10} \text{UFC} (T_{0h})] \times 100$$

The ability of the pure lactic bacteria to resist bile salt was

determined. This methodology was similar to the test of tolerance to acidity described earlier except that MRS broth was supplemented with 0.3% bile salts.

#### Safety assessment of probiotic LAB

Safety tests such as hemolysis, gelatinase and DNase were carried out according to the method described by Nabil et al. (2004).

##### DNase test

This was carried out according to the method of Nabil et al. (2004). Isolates without halos-formation or clear zone formation around the colonies were negative and were selected for further studies.

##### β-hemolysis test

This was carried out according to the method of Nabil et al. (2004). Zones of clearing around colonies indicated β-hemolysis production. Isolates without clearance around were selected for further studies.

#### Molecular characterization of probiotic safe LAB

Genomic DNA extraction, PCR amplification of 16S rDNA, DNA electrophoresis, sequencing of the PCR products and phylogenetic analysis were carried out at Genoscientific Laboratory, Samonda, Ibadan with voucher number DS10031147.

##### Genomic DNA extraction

Genomic DNA was extracted from pure cultures of probiotic LAB. Separately, 1 ml of each pure liquid culture was centrifuged for 3 min at 10000 rpm. Supernatant was removed and the cells were suspended in 300 µl buffer (10 mM Tris-HCl, pH 8.0; 50 mM glucose, and 10 mM EDTA). To the suspension, 3 µl lysozyme (10 mg/ml) was added, and cells lysed at 37°C for 60 min under occasional stirring of the tube content by inversion. Lysing buffer (20 mM Tris-HCl, pH 8.0; 75 mM NaCl; 1% SDS; 10 mM EDTA) of 300 µl and 3 µl RNase (10 mg/ml) were added to the mixture. Mixture was incubated at 37°C for 30 min and then cooled on ice for 1 min. Ammonium acetate (7.5 M; 100 µl) was added to the mixture, mixed on a vortex for 20 s and centrifuged at 13000 rpm for 5 min). Supernatant was transferred into clean 1.5 ml tubes, and 300 µl isopropanol was added. The mixture was mixed by inversion for 1 min and stored at -20°C for 30 min. Mixture was centrifuged at 13000 rpm for 5 min. The supernatant obtained was decanted, and the tubes were placed overturned on a clean filter. Four hundred microliters of 70% ethanol were added and mixed several times by overturning to wash the DNA sediment. Finally, the sediment was dried at 37°C for 15 min till ethanol drops disappeared completely. Dried sediment was dissolved in 30 µl TE buffer.

##### PCR amplification of 16S rDNA

For the amplification of the 16S rDNA gene, the specific primers AMP\_F 5'- GAG AGT TTG ATY CTG GCT CAG -3' and AMP\_R 5'- AAG GAG GTG ATC CAR CCG CA -3' were used. PCR reaction mixture was prepared by mixing 25 µl of the Taq 2x Mastermix (buffer, polymerase and dNTPs), forward primer 1 µl, reverse primer 1 µl and ultrapure water (22 µl) mixture (49 µl) was added to a sterile PCR tube, and 1 µl of the gDNA was used as a template;

**Table 1.** pH and total titratable acidity (TTA) of fermenting *Talinum triangulare* and *Acalypha wilkesiana*

Time (h)	Fermenting <i>T. triangulare</i>		Fermenting <i>A. wilkesiana</i>	
	pH	TTA	pH	TTA
0	7.4	0.078	7.2	0.079
6	6.1	0.084	6.8	0.088
12	6.0	0.144	6.5	0.094
18	5.5	0.151	6.2	0.116
24	5.2	0.224	5.8	0.124

**Table 2.** Total viable count of LAB isolated during spontaneous fermentation of *T. triangulare* and *A. wilkesiana*.

Time (h)	Total viable count of LAB (cfu/ml)	
	Fermenting <i>T. triangulare</i>	Fermenting <i>A. wilkesiana</i>
0	-	-
6	$1.40 \times 10^3$	$2.56 \times 10^3$
12	$2.60 \times 10^5$	$4.0 \times 10^4$
18	$3.20 \times 10^6$	$4.52 \times 10^6$
24	$4.68 \times 10^7$	$5.85 \times 10^7$

the amplification reaction was carried out in a thermal cycler (Bio-Rad Mycycler).

#### DNA electrophoresis

The PCR products was separated in a 1% agarose gel and stained with ethidium bromide followed by examination on a UV illuminator.

#### Sequencing of the PCR Products

A 16S rRNA PCR amplification and sequencing were performed by Eurofins, Novogene (Hong Kong). V4 hypervariable region of the 16S rRNA was amplified using specific primers 515F and 806R. All the PCR reactions were carried out with Phusion® High-Fidelity PCR Master Mix (New England Biolabs). Libraries generated with TruSeq DNA PCR-Free Sample Preparation Kit were sequenced using paired-end Illumina sequencing (2 × 250 bp) on the HiSeq2500 platform (Illumina, USA).

#### Phylogenetic analysis

Forward and reverse sequences were assembled and edited using BioEdit Sequence Alignment Editor Version 5.0.9. Sequence similarity was estimated by searching the homology in the Genbank DNA database using BLAST. Finally, the isolates were identified based upon the sequence. Evolutionary history was inferred using the neighbor joining method. Percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) was shown next to the branches. Tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Evolutionary distances was computed using the maximum composite likelihood method and are in the units of the number of

base substitutions per site. The position containing gap or missing data was eliminated. Evolutionary analysis was conducted in MEGA7.

## RESULTS

Table 1 shows the result of pH and total titratable acidity of the extracts of fermenting water leaf and Irish petticoat. The pH decreased from 7.4 at 0 h to 5.2 at 24 h while total titratable acidity increased from 0.078 to 0.224 at 48 h during fermentation of water leaf. Similar trend was observed during fermentation of Irish petticoat.

Table 2 shows the total viable count of LAB during fermentation of water leaf and Irish petticoat. The viable count increased in both fermentations from  $10^3$  to  $10^7$ .

Morphological and biochemical characteristics of LAB isolated from water leaf and Irish petticoat are shown in Table 3. Fortythree LAB were isolated. They were identified as: *Lactococcus lactis*, *lactis*, *E. hirae*, *Enterococcus durans* and *Streptococcus thermophilus*.

Table 4 shows percentage frequency of occurrence of LAB isolated from fermenting water leaf and Irish petticoat. *E. hirae* had the highest frequency of occurrence (43.3%) while *L. lactis* had the least (18.6%).

The antagonistic activity of LAB isolated from fermenting water leaf and Irish petticoat is presented shown in Table 5. *E. hirae* had the highest antimicrobial activity against all the tested pathogens followed by *S. thermophilus* while the safety and probiotic properties of LAB in this study are shown in Tables 6 and 7. Both *E. hirae* and *S. thermophilus* were sensitive to all the

**Table 3.** Morphological and biochemical characteristics of LAB isolated from *T. triangulare* and *A. wilkesiana*.

Isolate code	Morphology	Gram reaction	Catalase lase	Oxidase	Indole	motility	Endospore staining	Glucose	Fructose	Galactose	Maltose	Lactose	Mannitol	Sorbitols	Sucrose	Probable organisms
1A	Small, cream, shine, convex, circular and smooth	+ cocci	-	-	-	-	-	+	+	+	+	+	-	+	+	<i>Lactococcus lactis</i>
12A	Small, cream, shine, round, convex and smooth	+ cocci	-	-	-	-	-	+	-	-	+	+	-	+	+	<i>Streptococcus thermophilus</i>
18A	Small, cream, circular, smooth and convex	+ cocci	-	-	-	-	-	+	+	+	+	+	-	-	+	<i>Enterococcus durans</i>
43A	Medium, circular, smooth, white and convex	+ Cocci	-	-	-	-	-	+	+	+	+	+	+	+	+	<i>Enterococcus hirae</i>

antibiotics while *L. lactis* and *E. durans* were resistance to all. Also, *E. hirae* and *S. thermophilus* had the highest tolerance of bile salt (3%) while *L. lactis* and *E. durans* had the least tolerance (2%). Hemolysis and gelatinase tests as presented in Table 6 were used to assess the safety of the LAB isolates. *E. hirae* and *S. thermophilus* were negative for both tests while *L. lactis* and *E. durans* were positive to hemolysis and gelatinase tests, respectively.

Summary of molecular characterization of safe probiotic LAB and their corresponding phylogenetic tree is shown in Tables 8 and Figure 1, respectively. *E. hirae* and *S. thermophilus* were observed to be the safe probiotic LAB in this study. While the isolate 43A1 was confirmed as *E. hirae* by molecular methods, isolate 12A1 which was tentatively identified as *S. thermophilus* using conventional methods, was confirmed as *Weissella confusa* using molecular techniques.

## DISCUSSION

The decrease in pH and its corresponding

increase in total titratable acidity observed in this study confirm that the fermentation of *T. triangulare* and *A. wilkesiana* were carried out by acid producing isolates of which LAB is a major example among bacteria. This was in line with the findings of Eromosele et al. (2017). The increase in LAB count observed, further gives credence to the claim that the fermentation was based on LAB (Omemu et al., 2007; Onilude et al., 2008). The increase in microbial population can be attributed to their ability to utilize the available nutrient in the fermentation medium, favorable environmental condition, and absence of growth inhibitors (Augustine et al., 2019). The conventional identification of isolates which identified them as *L. lactis*, *Enterococcus hirae*, *E. durans* and *S. thermophiles* was in conformity with the reports of Adeleke and Olaniyi (2018) which stated that LAB are generally ubiquitous. They are found everywhere including air, soil and on most food substrates at low pH. The dominance of *E. hirae* in this work is supported by the reports of Adeleke et al. (2017), Ojokoh et al. (2016) and Achi and Akubor (2000). This dominance depicts the ability of *E. hirae* to produce more inhibitory substances

such as acid than other fermenting organisms which tend to favor its growth and inhibit other organisms.

The high probiotic properties of *E. hirae* followed by that of *S. thermophilus* was depicted from their respective high antagonistic activity, pH, bile tolerance and lowest resistance to antibiotic as well as their safety for consumption; hence, the reason for molecular characterization of these two LAB in order to confirm species with potential starters for the fermentation of *T. triangulare* and *A. wilkesiana*.

Surprisingly, the 16s RNA molecular characterization results yielded *E. hirae* MH259885.1 and *W. confusa* DQ321751.1. *W. confusa* and other members of the genus have been known to have similar characteristics with and difficult to differentiate from other heterofermentative *lactobacilli* based simply on morphological characteristics (Fusco et al., 2011). Many of its morphological and biochemical characteristics are similar to those possessed by the homofermentative *Streptococcus* shown in Table 3. Many commercial systems that are based on conventional techniques are not enough

**Table 4.** Percentage frequency of occurrence of LAB isolated from fermenting *T. triangulare* and *A. wilkesiana*.

Isolate	No. of LAB isolates	Percentage frequency of occurrence (%)
<i>Enterococcus hirae</i>	20	43.3
<i>Lactococcus lactis</i>	6	14.0
<i>Enterococcus durans</i>	8	18.6
<i>Streptococcus thermophilus</i>	9	20.9

**Table 5.** Antagonistic activity of LAB isolated from fermenting *T. triangulare* and *A. wilkesiana*.

LAB isolates	Susceptibility of pathogens (mm)			
	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Shigella spp.</i>	<i>Bacillus cereus</i>
<i>Enterococcus hirae</i>	12	10	11	9
<i>Lactococcus lactis</i>	6	5	6	5
<i>Enterococcus durans</i>	4	5	4	3
<i>Streptococcus thermophilus</i>	10	9	8	8

**Table 6.** Antibiotic susceptibility of LAB Isolated from fermenting *T. triangulare* and *A. wilkesiana*.

Isolate	Zones of Inhibition of microbial growth by various antibiotics (mm)									
	APX	CN	PEF	E	SXT	S	CPX	AM	Z	R
<i>Enterococcus hirae</i>	1	0	0	0	1	0	0	0	0	0
<i>Streptococcus thermophilus</i>	0	0	2	0	0	0	1	0	0	0
<i>Lactococcus lactis</i>	17	12	10	14	12	10	10	15	10	12
<i>Enterococcus durans</i>	14	12	9	10	14	12	0	12	10	0

**Table 7.** Safety and probiotic properties of LAB isolated from fermenting *T. triangulare* and *A. wilkesiana*.

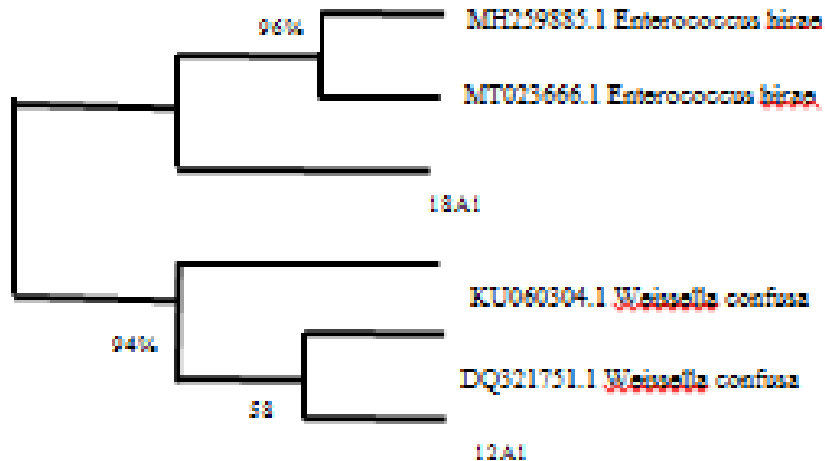
Isolate	Hemolysis	Gelatinase	pH 1	pH 2	0% bile salt	3% bile salt	5% bile salt
<i>Enterococcus hirae</i>	-	-	-	+	+	+	-
<i>Lactococcus lactis</i>	+	-	-	+	+	-	-
<i>Enterococcus durans</i>	-	+	-	+	+	-	-
<i>Streptococcus thermophilus</i>	-	-	-	+	+	+	-

**Table 8.** Summary of molecular characterization of safe probiotic LAB.

Sample ID	Matched Organism	% Identity	Accession number
12A1	<i>Weissella confusa</i> strain Inje LM S-338	99.02	DQ321751.1
43A1	<i>Enterococcus hirae</i> strain HA7	93.87	MH259885.1

to completely identify members of the genus *Weissella*. Its presence in this plant based fermenting broth is not entirely novel since members of the *Weissella* genus has been isolated from plant sources such as vegetables, fruits and their fermentation based products (Cho et al.,

2006; Miyashita et al., 2012; Yang et al., 2014). In line with result of this study, *W. confusa* was reported to be isolated from fermented food sources. Aveni et al. (2001) and Lee et al. (2012) discussed its potential as a probiotic which was also studied and confirmed in this



**Figure 1.** Phylogenetic tree of *Enterococcus hirae* MH259885.1 and *Weisella confusa* DQ321751.1.

study. The use of *W. confusa* as a probiotic should be approached with extreme caution since it has been implicated in human infections, including bacteremia.

Considering these confusions and uncertainty about *W. confusa*, *E. hirae* is recommended as a potential starter for the controlled fermentation of *T. triangulare* and *A. wilkesiana* medicinal plants. This is also supported by its dominance, probiotic properties and safety as observed in this work. The probiotic potential of *E. hirae* observed in this work was in line with the finding of Arokiyaraj et al. (2014), Kamni and Ramesh (2020), de Castro Santos Melo et al. (2021), and Rajput et al. (2022). Its antibiotic susceptibility contradicts the findings of Zaidi et al. (2022) who reported that antimicrobial susceptibility profiling of *E. hirae* isolates originating from beef production systems showed high resistance to tetracycline (65%) and erythromycin (57%) with 50.4% isolates harboring multi-drug resistance while it was in conformity with the work of Kamni and Ramesh (2020) who isolated *E. hirae* from indigenous raw goat milk and reported that it was susceptible to amikacin, carbenicillin kanamycin, ciprofloxacin, co-trimazine, nitrofurantoin, streptomycin, and tetracycline.

## Conclusion

The fermentation of *T. triangulare* and *A. wilkesiana* was with resultant dropped pH and increased population of LAB showed that it is LAB fermentation. Out of four species of probiotics obtained in this study, only isolate 12A1 and 43A1 were safe for consumption hence the reason for their molecular characterization. Isolate 12A1 which was tentatively identified as *S. thermophilus* using conventional methods, was confirmed as *W. confusa* using molecular techniques while isolate 43A1 was confirmed as *E. hirae* by both conventional and molecular

methods, considering confusion and uncertainty of *W. confusa*, *E. hirae* is therefore suggested to be a potential starter for the controlled fermentation of *T. triangulare* and *A. wilkesiana* medicinal plants. It can therefore be concluded from result of this study that *T. triangulare* and *A. wilkesiana* medicinal plants are sources of potential starter probiotic *E. hirae* MH259885.1 which met the criteria to be considered as safe probiotic for application in food fermentation.

The role of *E. hirae* in starter produced medicinal plants is recommended.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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