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African Journal of Biotechnology

Full Length Research Paper

Evaluation of the physical properties of leathers tanned with *Plectranthus barbatus* Andrews extracts

Dennis Kimaiga Obiero[1]*, Jackson Nyarongi Ombui[1], Arthur Steven Onyuka[2] and Alvin Asava Sasia[2]

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This study was designed to assess the potential use of *Plectranthus barbatus* Andrews, a plant growing locally in Kenya as a vegetable tanning agent. The aim was a deliberate attempt to adopt green chemistry and provide cheap locally available eco-friendly tanning materials. Anova statistical test ($\alpha<0.05$) was used to compare the physical properties of leather tanned with three kinds of liquors made from the plant and the following mean values were recorded: Shrinkage temperature of 61.5 to 67.5°C; Grain crack range of 7.5 to 8.7 mm; Grain burst range of 7.9 to 9.1 mm; tear strength of 41.8 to 78.2 N with percentage (%) elongation of 68.4 to 75.9%; tensile strength of 17.6 to 40.8 N/mm^2 with % elongation of 15.9 to 42.2% and thickness of 0.7 to 1.2 mm. The study concluded that *P. barbatus* Andrews leaves have adequate tannin content of hydrolysable type required for tanning. Furthermore, the brown colour of the leather produced is unlikely to undergo saddening as usually observed in *Mimosa* because of relative stability of hydrolysable tannins to light.

Key words: Shrinkage temperature, distension, pelts, tannins, vegetable tanning, pyrogallol.

INTRODUCTION

Conversion of hides and skins into leather involves modification and partial purification of collagen in the beamhouse followed by stabilization through introduction of tanning agents that cross-link its fibres to prevent decomposition and confer versatility. Although chrome tanning is popular in industrial production of leather, the residual chrome in tannery waste pollutes the environment with oxidation of chrome (iii) to carcinogenic chrome (vi) in leather articles (Graf, 2001).

Consequently, there is growing concern on human health and environmental risk posed by accumulation of severely high levels of toxic chrome in underground and surface water due to seepage and direct flow from tannery effluent (Hossain and Bhuiyan, 2010). Vegetable

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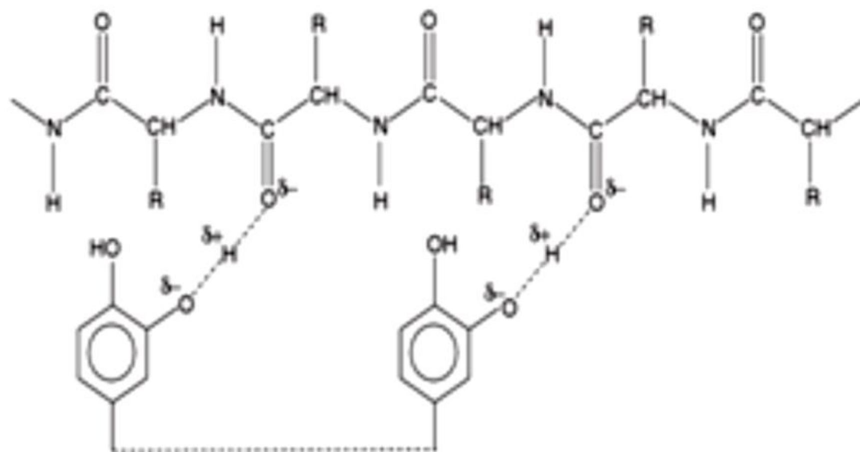

Figure 1. Proposed bonding in vegetable tanning (Source: Covington, 2011).

tanning has been identified as an alternative to chrome (Covington, 2011) which has move towards green tanning chemistry and eco-labeling. However, vegetable tanning is not as widely used as chrome in Kenya due to the high cost of *Mimosa*, which is the only available vegetable tanning agent for commercial tanning. There is also limited knowledge and information about indigenous plants that may have potential use as vegetable tanning agents in Kenya.

Plectranthus barbatus Andrews belongs to the largest family Lamiaceae of the order Lamiales, which has herbs and shrubs with distinct four sided stems and blue raceme inflorescence (Mariya et al., 2013). The entire plant is aromatic with leaves and roots having different odours (Lukhoba et al., 2006). It is an indigenous plant growing in many parts of Kenya mainly used for herbal medicine and establishment of land demarcations. The plant was identified as a potential candidate for study due to its resistance to fire, a salient characteristic of plants that are rich in tannins (Katie et al., 2006). The tannin content of *P. barbatus* Andrews crude extracts were determined using the hide powder method which was found to be 20 and 8% in leaves and stems respectively (D. Obiero, University of Nairobi, Kenya, unpublished results). Plants have since prehistoric times been utilized in organic tanning molecules and the pelt collagen react to form leather. The leather produced has improved physical characteristics performance for instance tear and tensile strength, shrinkage temperature, distension, flexing endurance, feel, handle and opacity (Covington, 2011). These alterations are thought to occur when polyhydroxyl groups in tannin molecules form bonds with the functional groups on the polypeptide chain especially –COOH (Covington, 2011). The proposed bonding in vegetable tanning is either through hydrogen bonds, ionic bonds or covalent bonds (Covington, 2011). Separately, Haslam (2007) contemplated of preferential binding in gap region of the quarter stagger structure of skin collagen. Figure 1 illustrates theoretical cross-linking of vegetable tannins with polypeptide chain.

Generally, leather tests are divided into three main classes: physical/ mechanical tests of strength, moisture related tests and chemical analysis (Thorstensen, 1993). These properties have variations depending on the various factors but most importantly the type of tanning agent. In contemporary leather science shrinkage temperature has been used to measure tanning activity although, some studies argue that the shrinking and tanning reactions are independent (Covington, 2011).

Furthermore, in vegetable tanning, shrinkage temperature is not of commercial importance (Thorstensen, 1993), rather the suitability and performance in intended use. For instance, pyrogallol tanning is coupled with deposition of a pale-colored sediment called 'bloom' (ellagic acid) which when deposited in the leather contributes to its colour and improves its solidarity, wearing properties and resistance to water (Covington, 2011). Leather by nature is an inconsistent material (Thorstensen, 1993) hence independent leather testing and analysis becomes important before use use (Sterlacci, 2010) especially when a new tanning material is proposed.

MATERIALS AND METHODS

Duplicates of bated goat pelts were tanned in separate drums for each category of liquor prepared by dissolving ground dried leaves, stems and a mixture of leaves and stems of *Plectranthus barbatus* Andrews sourced from Nyamira County in Kenya. Control pair of pelts was tanned with *Mimosa* for purposes of comparison. All tannery chemicals and reagents were of recommended standards and high quality. Standardized equipment and machinery were used in the measurement of leather physical properties.

Ground *P. barbatus* Andrews leaves, stems combined with leaves, stems and *Mimosa* were dissolved in warm water in separate containers to yield 5% extracts before 150% floats of resultant liquors in regards to the weight of the bated pelts that

Table 1. Summary table for physical properties of *Plectranthus barbatus* Andrews and *Mimosa* tanned leathers.

Parameter		*Plectranthus barbatus* Leaves	*Plectranthus barbatus* Stems	*Plectranthus barbatus* leaves+stems	*Mimosa*	Minimum recommended value
Shrinkage temperature (°C)		67	68	62	76	60
Thickness (mm)		→ 0.7 ↑ 0.9	→ 1.2 ↑ 1.2	→ 0.9 ↑ 0.8	→ 1.0 ↑ 1.0	≥0.5
Tensile strength (N/mm^2)		→ 31.2 ↑ 27.9	→ 40.8 ↑ 26.5	→ 17.6 ↑ 21.6	→ 23.9 ↑ 26.1	≥12
Elongation at break (%)		→ 46.3 ↑ 40.3	→ 21.3 ↑ 17.6	→ 35.4 ↑ 26.2	→ 34.3 ↑ 21.0	≥40
Tear strength (N)		→ 70.8 ↑ 58.7	→ 75.6 ↑ 78.7	→ 41.8 ↑ 51.3	→ 70.6 ↑ 77.7	≥20
Ball burst extension (mm)	Grain crack	8.7	7.5	8.1	7.7	6.5
	Grain burst	9.1	7.9	8.5	8.2	7.0
Flexing endurance		→No damage @ 100000 ↑ No damage @ 100000	→Creasing @ 100000 ↑ creasing @ 100000	→No damage @ 100000 ↑ No damage @ 100000	→No damage @ 100000 ↑ No damage @ 100000	No damage @ 100000 flexes

↑ Parallel to the backbone, → Perpendicular to the backbone.

were put into drums. The pairs of bated pelts were then immersed in the respective floats and drums run at an initial uniform speed of 8 rev/min for 6 h. Tanning progress was monitored by checking change of liquor colour and penetration through the pelt cross-section3% addition of the extracts was made carefully then run for 1 h before the pelts were left to stand overnight, completely immersed in the liquor.

Penetration of the extracts was inspected by cutting a small piece in the neck region of the pelt and colour uniformity through the pelt cross-section was used to establish completion of tanning. Additions of subsequent reduced percentages of extract concentrations were made and drums run until each tannage was complete and thereafter fixation was done with 1% formic acid. All the *P. barbatus* Andrews tanned leathers were further re-tanned in the same respective 5% extracts liquors after they had been horsed up for one day and later hang dried in the air for one day in order to improve colour, handle and weight. The dried leathers were then fatliquored with 4% vegetable oil followed by toggle drying.

Each processed leather sample was labeled, then sampling and measurement of the following physical parameters was done according to the official methods of International Union of Leather Technologists and Chemists Societies (IULTCS) (2001): Shrinkage temperature was measured on SATRA STD 114 (IUP/16), flexing endurance on Bally Flexometer Model 2184 (IUP/20), thickness (IUP/4), tear strength and tensile strength on Instron 1011(IUP/8; IUP/6) and distension test on Lastometer STD 104- SATRA test equipment (IUP/9). Data analysis was done using ANOVA statistical test ($\alpha<0.05$).

RESULTS AND DISCUSSION

P. barbatus Andrews leaves, stems and a combination of leaves and stems tannages produced brown, black and brown coloured leathers respectively. All other results are recorded in Table 1. The thickness of the leathers was a significantly different (p=0.0) among the four types of leathers with *Mimosa* tanned leathers having the highest mean value of 1.1 mm followed by *P. barbatus* Andrews stem extract tanned leathers at 1.0 mm while leaves extract tanned leather was the thinnest at 0.7 mm with an overall coefficient of variation of 38.6%. Earlier work has reported *Acacia nilotica* tanned leathers of West African dwarf goats and Sahelian goats with values above 0.98 and 0.87 mm respectively which were also reported for Lori goat breed of Iran (Salehi et al., 2013).

The mean shrinkage temperature of raw goat skins used in tanning was 55°C therefore *P. barbatus* Andrews leaves tannage recorded a rise

in 12°C and stems tannage managed an increase in 13°C which was the highest. The mixture of leaves and stems showed a minimum elevation of 7°C in regard to this parameter and this was thought to have been due to unfavorable interactions between soluble compounds in the two components. Comparatively natural valonia tanned kidskins leather reported a mean shrinkage temperature of 65.66°C (Afsar and Sekeroglu, 2008) and hence the observations made in this study were not inconsistent with earlier studies on hydrolysable tanning materials. Other previous studies show that shrinkage temperature of leather tanned with condensed tannins (*Mimosa*) is between 80 to 85°C and for hydrolysable tannins (sumac) the shrinkage temperature is <80°C (Covington, 2011). Divi-divi and myrobalan tannages (hydrolysable) both have a shrinkage temperature of 68°C and quebracho and wattle tannages (condensed) produce leather with a shrinkage temperatures of 76 and 78°C respectively (Covington, 2011).

However, it is assumed that the shrinking reaction is independent of the stabilizing effect because the enthalpy of denaturation is independent of stabilizing chemistry (Covington et al., 1989). Hydrothermal stability depends on the chemistry of bonding with hydrogen bonding conferring only moderate rise in shrinkage temperature; this may be assumed to be the case with *P. barbatus* Andrews tannage. No similar work has been done with *P. barbatus* Andrews in previous studies and consequently the comparison is only strictly based on materials with similar type of tannins (hydrolysable) though not exactly the same.

The higher the grain bursting height, the greater the softness achieved in the leather (Jianzhong et al., 2003) and results of tested leathers indicated good softness with no significant difference (p=0.18) among the three forms of *P. barbatus* Andrews tanned leathers. Leaves tannage gave the best outcome of 9.1 mm and comparatively De-Britol et al. (2002) also recorded that, the minimum recommended bursting height of good leather is 7.0 mm.

Leathers tanned with *P. barbatus* Andrews stems had the highest tear strength of 80.0 N that was 0.5 N higher than the strength tested in *Mimosa* tanned leather. Leaves and stem mixture tanned leather showed the lowest tear strength of 39.0 N whereas leather tanned with *P. barbatus* Andrews leaves liquor had a measured tear strength value of 47.3 N. There was a significant difference (p=0.0) between the tear strength means of the four tannages however, the difference is between *P. barbatus* Andrews leaves combined with stems and *Mimosa,* combined leaves and stems and stems (p=0.0), stems and *Mimosa* (p=0.0), leaves and *Mimosa* (p=0.0) and leaves compared with stems (p=0.0) tannages. There was no significant difference (p=0.12) between *P. barbatus* Andrews leaves and combined leaves and stem tanned leathers. Related studies show that *Acacia nilotica* tanned leathers have tear strength of 40 N, semi-chrome 47 N and Bureau of Indian Standards (BIS) which gives a value of 30 N (Musa and Gasmelseed, 2013).

No significant difference (p=0.6) was seen among *P. barbatus* Andrews tannages that is; leaves, leaves combined with stems and stems alone in regard to tensile strength. Stem tannage showed the highest tensile strength of 48.5 N/mm^2 while leaves tannage recorded the lowest value of 43.2 N/mm^2. When compared with *Mimosa* which had a mean tensile strength of 25.0 N/mm^2 there was no significant difference (p=0.2) among the four tannages. Previous research on *A. nilotica* tanned goat skins recorded a tensile strength of 23.5 N/mm^2 and BIS sets the value at 19.6N/mm^2 (Musa and Gasmelseed, 2013).

The percentage elongation at break values, among the *P. barbatus* Andrews tanned leathers revealed no significant difference (p=0.5) with a mean of 22% and a maximum value of 50% posted by both *P. barbatus* Andrews leaves and leaves combined with stems tannages. When this property was compared with *Mimosa* which had a highest value of 53% and a mean % elongation value of 28%; which was the highest among the four types of leathers, the statistical analysis showed no significant difference (p=0.5) among them. Comparatively leathers produced by tanning goat skins with *Acacia nilotica* pods by Sudan rural tanners had a percentage elongation value of 41% and semi-chrome giving a value of 58% with BIS range between 40 to 65% (Musa and Gasmelseed, 2013).

The leather tanned with *P. barbatus* Andrews stems was brittle but when it was re-tanned with additional 20% offer, it showed improvement in the flexing endurance since it completed the 100,000 dry flexes with minimum creasing and change in colour. All other categories had good feel and recommended flexibility but it is important also to remember that several factors such as breed, age at slaughter, nutrition and environment also influence the physical properties of leather (Yusuff et al., 2013). For this reason it is therefore expected that these properties will vary considering studies done in different parts of the world. Results of flexing endurance of *Acacia nilotica* tanned goats skins in Nigeria from three breeds: West African dwarf, Sahelian and Sokoto red goats showed that 5.83% of the samples subjected to flexometer test showed emergence of cracks however there was no significant difference (p>0.05) among them (Yusuff et al., 2013).

The colours produced by the three *P. barbatus* Andrews tannages were notably different with leaves tannage and the mixture of leaves and stems yielding brown leathers while the stems tannage gave rise to black leathers. Undyed vegetable tanned leather will show varying shades of brown, yellowish brown or reddish brown (Mongkholrattanasit et al., 2011). Catechols produce red, pink and dark brown leathers while pyrogallols produce paler tans (Covington, 2011). The brown colour of the leather produced is unlikely to

suffer saddening as usually observed in *Mimosa* because of relative stability of hydrolysable tannins to light (Covington, 2011).

This study concluded that *P. barbatus* Andrews leaves have adequate tannin content required for tanning and this plant can produce leather with quality comparable with conventional *Mimosa* tanned leathers. It was recommended that leaf extracts from *P. barbatus* Andrews can be used for tanning light leathers and also re-tanning. Leaves sourced from Nyamira County in Kenya can be used in rural tanning however, commercial production of the plant should be, encouraged through intercropping especially in small holder farming communities in order to boost their income.

Conflict of interests

The authors have not declared any conflict of interests.

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MiR-124 involvement of apoptosis, immunity and regulator of diseases

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Most microRNAs (miRNAs) are noncoding, conserved RNA molecules in vertebrates, and their roles are similar and very important. MiRNAs usually have tissue-specific expression, and abnormal levels of miRNAs have been associated with diseases and have been used as disease biomarkers. MiRNAs are widely involved in biological processes by regulating target mRNAs. MiR-124 is one of the best studied miRNAs in organisms. By targeting different mRNAs, miR-124 plays important roles in the central nervous system, cellular infiltration, pathophysiological processes of cardiovascular diseases, inflammation, immunity and tolerance, etc. This review mainly focuses on tissue-specific or abnormal expression of miR-124 as a biomarker and on the ways miR-124 for the treatment of serious diseases such as cancers.

Key words: miR-124, apoptosis, immune responses, diseases.

INTRODUCTION

MiRNAs are a group of noncoding, small, single-stranded RNA molecules that are approximately 19-25 nucleotides long and usually repress the expression of their target genes in multicellular organisms (Sharma, 2017; Hu and Zhang, 2019). MiRNAs are widely involved in biological processes by regulating target mRNAs and are also used as biomarkers of a number of diseases (Zhang et al., 2016; Komal et al., 2019; Mohammadi et al., 2019).

The first miRNA, lin-4, was discovered in 1993, and its roles were revealed to be involved in the larval development programs of the nematode *Caenorhabditis elegans* (Bartel, 2004; Sharma, 2017). Subsequently, a number of miRNAs have been found, and their roles have been characterized (Ramakrishna and Muddashetty, 2019; Shirjang et al., 2019). MiRNAs in humans and mice have been well studied, while the studies of miRNAs in lower vertebrates, such as fish, are just beginning. MiRNAs have been found in different fish, and their potent roles by targeting genes have been analyzed (Yang and He, 2014). It is well known that miRNAs are widely involved in biological functions through the regulation of target mRNAs (Ni et al., 2018; Zhang et al., 2016). The miRNA sequence and the binding site sequence on the 3' untranslated region (3′-UTR) of the mRNA are usually complementary (Li et al., 2013). Imperfect base pairing can lead to translational inhibition at the level of translation initiation and elongation of the target mRNA. However, it is demonstrated that miR-124

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can directly regulate multidrug resistance protein 4 (MRP4, ABCC4), and polymorphisms in the ABCC4 3'-UTR have no significant effect on miRNA regulation (Markova and Kroetz, 2014). It has been demonstrated that one miRNA usually has multiple target sites to target different genes in tissues to regulate different processes *in vivo*, and the miRNA can promote the degradation of the target mRNA or block its translation into protein, meanwhile one mRNA can also be targeted by multiple miRNAs (Sharma, 2017; Shirjang et al., 2019). In addition, long noncoding RNAs can also target miRNAs and regulate their expression (Shu et al., 2019).

MiRNAs are very important regulators of cellular mechanisms and physiological processes, such as cell cycle progression, cell division, apoptosis and necroptosis (Shirjang et al., 2019). To date, numbers of miRNAs have been discovered in different organisms, and diverse roles of these miRNAs have been described in physiological or pathological conditions (Mohammadi et al., 2019). It has been reported that humans have approximately 1000 miRNAs that can interfere with approximately 30% of gene expression, mostly as gene suppressors (Rassi et al., 2017). MiRNAs play crucial roles in the development and progression of human cancers, such as hepatocellular carcinoma (Lang and Ling, 2012). In brief, miRNAs can regulate nearly all cell signaling pathways from early development to cancer formation: differentiation, metabolism, proliferation, development, apoptotic cell death, viral infection and tumorigenesis (Ahir et al., 2017). Furthermore, miRNAs could be used to treat viral infections, such as hepatitis C virus infection (Thibault and Wilson, 2013).

A number of miRNAs are ubiquitously expressed in tissues, while they usually have tissue-specific expression. The functions of miRNAs and the relationships between miRNAs and mRNA were shown in Figure 1. MiR-124 is a member of the miRNA family and has common characteristics of miRNAs. Three pre-miR-124 variants (miR-124a or miR-124-1, miR-124-2 and miR-124-3) produce the same mature miRNA (He et al., 2016). MiR-124 is an evolutionarily conserved, noncoding microRNA in organisms. In humans and rats, miR-124 is most abundant in the brain, and many diseases of the brain are associated with abnormal levels of miR-124 (He et al., 2016; Taniguchi et al., 2015). Over the years, many studies have focused on the role of miR-124 in the nervous system (Wang et al., 2014; Sun et al., 2015), cardiovascular diseases (Bao et al., 2017), apoptosis (Han et al., 2019). The function of miR-124 in immune and inflammatory responses has been explored (Jin et al., 2017a, b). Studies have shown that miR-124 plays multiple roles in behavior, growth, immunity, signaling. MiR-124 can target neuronal genes to control behavior, immune genes to control inflammatory processes, and tumor-associated genes to affect tumors. This review mainly focuses on tissue-specific or abnormal expression of miR-124 as a biomarker and miR-124 for the treatment of serious diseases such as cancers.

MATERIALS AND METHODS

The information of this review was from journal articles published in Pumbed central database. Reviews "microRNA/miRNA", "miR-124", "miR-124, apoptosis", "miR-124, immune responses" and "miR-124, diseases" keywords in possible database were conducted in humans and animals.

RESULTS AND DISCUSSION

MiR-124 is a novel biomarker

The roles of miR-124 are usually associated with abundant expression in tissues. In humans, miR-124 is enriched in tissues including brain, liver, spinal cord, and neurons (Wang et al., 2014; Taniguchi et al., 2015; Zhao et al., 2015; Shaw et al., 2018), and its expression level can affect tumorigenesis, such as colorectal cancer (Taniguchi et al., 2015). Abnormal expression of miR-124 occurs in some diseases (Zeng et al., 2012). Therefore, miR-124 is known as a promising, novel biomarker of early diagnosis of diseases, especially cancers.

MiR-124 is involved in apoptosis and cell death

Recent studies have demonstrated that miR-124 is associated with apoptosis/cell death (Liang et al., 2017; Song et al., 2019). In rats, miR-124, which can be a biomarker of myocardial injury and infarction, regulates oxidative stress, cardiomyocyte apoptosis and myocardial infarction by targeting the gene Dhcr24 (Han et al., 2019). In cholangiocarcinoma cells, miR-124 can induce apoptotic cell death by targeting EZH2–STAT3 signaling (Ma et al., 2018). MiR-124 can silence polypyrimidine tract-binding protein 1 (PTB1) to cause drastic apoptosis of colon cancer cells (Taniguchi et al., 2015). The miR-124/AMPK/mTOR pathway can affect cell apoptosis (Gong et al., 2016). MiR-124 in glioma cells can inhibit cell growth and promote apoptosis (Wang et al., 2018). MiR-124 targeting Hic-5 can affect cell apoptosis after hypoxia damage in H9C2 cells (Jiang et al., 2018).

Roles of miR-124 in immune responses

Inflammation is a complicated cascade of reactions of the response of organisms to infections/injuries and is closely associated with various diseases (Lawrence et al., 2002). Classic inflammatory responses are induced by recognition receptors, such as Toll-like receptors (TLRs), which combine with the respective ligands to activate the innate immune system to release proinflammatory cytokines, including IL-1, IL-6, TNF-α, etc., resulting in the development of diverse inflammatory and autoimmune diseases (O'Shea and Murray, 2008; Wang and Xu 2017a, Wang et al., 2017b). However, detailed signaling pathways and regulatory mechanisms are not completely clear.

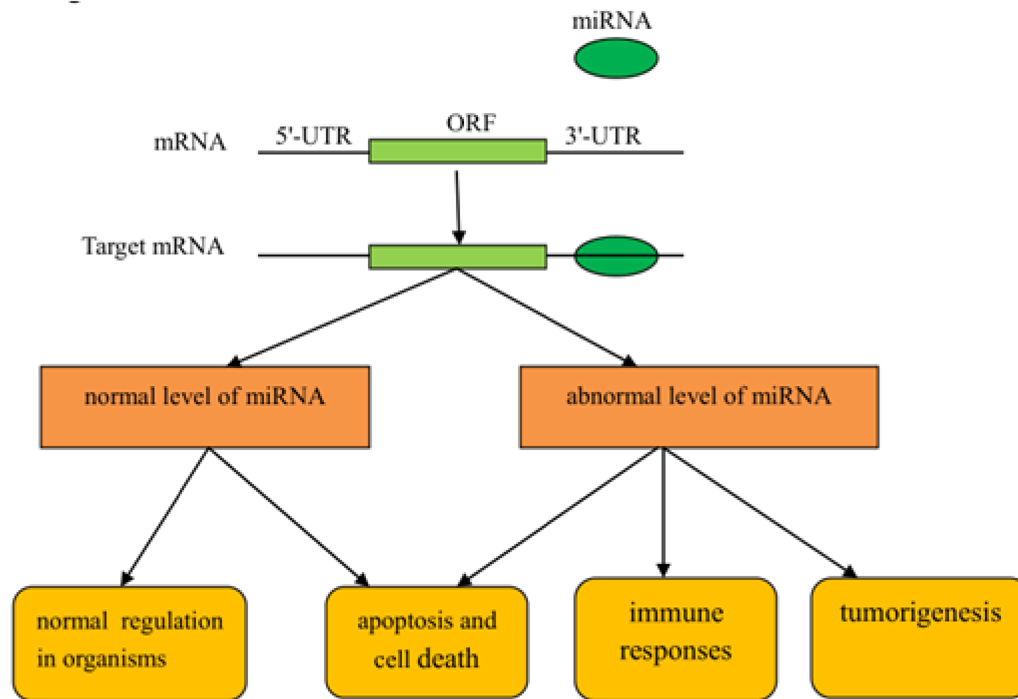

Figure 1. Relationship and the roles between miRNAs and mRNAs.

MiR-124 has been found to regulate various inflammatory processes. In mesenchymal stem cells, overexpressed miR-124 can upregulate IL-6 and STAT3 to improve the immunomodulatory capacity of the cells (Zhao et al., 2018). By inhibiting the expression of proinflammatory cytokines, miR-124 can mediate the cholinergic anti-inflammatory pathway (Sun et al., 2013). By targeting PPARγ, miR-124 can affect the production of proinflammatory cytokines in mice (Wang et al., 2017c). MiR-124 can reduce the activation of NF-κB (Li et al., 2013), and activated NF-κB can downregulate miR-124 (Wang et al., 2015). MiR-124 inhibits the mTOR signaling pathway and inhibits neuronal inflammation (Huang et al., 2018). MiR-124 in keratinocytes inhibits innate immune responses to all eviatechronic skin inflammation in atopic eczema (Yang et al., 2017). MiR-124 can activate peroxisome proliferator-activated receptor gamma (PPARγ) to regulate proinflammatory cytokine levels (Wang et al., 2017c). The level of miR-124 is decreased in lesion tissue of patients with atopic eczema and in keratinocytes response to inflammation factors that control chronic inflammatory processes, which suggests that miR-124 can alleviate chronic skin inflammation in atopic eczema by suppressing innate immune responses in keratinocytes (Yang et al., 2017). The miR-124-STAT3 pathway can partially regulate the immunomodulatory capacity of mesenchymal stem cells (Zhao et al., 2018). The expression of miR-124 can be induced during *Mycobacterium bovis* Bacillus Calmette–Guerin (BCG) infection in rats, and miR-124 is also able to regulate Toll-like receptor (TLR) signaling activity in RAW264.7 cells in response to BCG infection (Ma et al., 2014). MiR-124 downregulates the TLR signaling pathway during mycobacterial infection (Ma et al., 2014). Inhibition of miR-124 can activate the JNK and p38 pathways, which participate in the MAPK response to various stresses (Gong et al., 2019). MiR-124 in alveolar macrophages plays a role in the response to mycobacterial infection by negatively regulating TLR signaling genes (Ma et al., 2014). MiR-124 in human cells can induce mitochondrial apoptosis (Jin et al., 2017b).

MiR-124 is associated with some serious diseases

Various target genes of miR-124 have been associated with diverse diseases, including cancers. Abnormal expression of miR-124 in diverse cells usually occurs in cancers such as breast cancer and prostate cancer (Gu et al., 2016; Zhang et al., 2016; Liang et al., 2017), and miR-124 usually suppresses tumor formation, and its expression is downregulated in cancer patients (Zhang et al., 2015). Cancer is a malignant tumor, in which cells become unresponsive to inhibitory cellular growth signals and intrinsic cell replication limits, evade apoptotic signals, leading to tumorigenesis (Ahir et al., 2017).

As shown in Table 1, in humans, miR-124 targeting the PIK3CA gene can suppress cell proliferation in hepatocellular carcinoma (Lang and Ling, 2012), targeting HIPK3 can affect oncogenic properties of lung cancer

Table 1. Studies of miR-124 in some serious diseases.

Target	Diseases	References
PIK3CA	hepatocellular carcinoma	Lang and Ling, (2012)
HIPK3	lung cancer	Yu et al. (2018)
EphA2	glioma	Wu et al. (2018)
ITGA3	colorectal cancer	Sa et al. (2018)
RLIP76	malignant melanoma cells	Zhang et al. (2016)
Fra-2	glioma cells	Luo et al. (2018)
Toll-like receptor signaling	neuropathic pain	Grace et al. (2018)
K-ras mutation and NNK	lung tumorigenesis	Jin et al., (2017a)
PTPN1 signaling	Alzheimer's disease	Wang et al. (2018)
C/EBPα	hepatocellular carcinoma	Hu et al. (2019)
ZEB2	breast cancer	Ji et al. (2019)
DDX6/c-Myc/PTB1	colon cancer	Taniguchi et al. (2015)
Calpatn/CDK5 pathway proteins	Parkinson's disease	Kanagaraj et al. (2014)
SMYD3	Hepatitis C Virus	Zeng et al. (2012)
KITENIN	Colorectal Cancer	Park et al. (2014)
BACE1/-secretase	Alzheimer's disease	Fang et al. (2012)
STAT3	hepatocellular carcinoma	Lu et al. (2013)
ITGB3	endometriosis	Liu et al. (2019)

(Yu et al., 2018), targeting EphA2 can inhibit cell growth and motility in glioma (Wu et al., 2018), targeting integrin subunit alpha 3 (ITGA3) can be a potential target for the treatment of colorectal cancer (Sa et al., 2018), targeting gene RLIP76 can affect proliferation and invasion of malignant melanoma cells (Zhang et al., 2016), targeting Fra-2 suppresses glioma aggressiveness in glioma cells (Luo et al., 2018), and targeting Toll-like receptor signaling can be a valid strategy for reversing neuropathic pain (Grace et al., 2018). MiR-124 also inhibits lung tumorigenesis caused by K-ras mutation and NNK (Jin et al., 2017a). MiR-124 is involved in major depressive disorder (He et al., 2016; Fang et al., 2018). MiR-124 can suppress tumorigenesis in mice by silencing gene PTB1 (Taniguchi et al., 2015). MiR-124-PTPN1 signaling is a mediator that affects the synaptic and memory deficits in Alzheimer's disease (Wang et al., 2018). MiR-124 can improve brain repair in Parkinson's disease (Saraiva et al., 2016) etc. In addition, miR-124 has been associated with pancreatic cancer (Wang et al., 2014b), cervical cancer (Wilting et al., 2010), hematopoietic malignancies (Wong et al., 2011), leukemia (Chen et al., 2014), breast cancer (Ji et al., 2019), prostate cancer (Gu et al., 2016), etc.

Other functions

In addition to its involvement in apoptosis/cell death, immunity, and cancer, miR-124 is associated with fat metabolism, triglyceride homeostasis, stress response, and drug resistance (Fang et al., 2018; Shaw et al., 2018). In sheep, miR-124 is a crucial factor for adipogenesis (Pan et al., 2018). MiR-124 is also associated with neurite outgrowth in mammals (Yu et al., 2008; Su et al., 2019), miR-124 can also control drosophila behavior and neural development (Wang et al., 2014). In mice, miR-124 regulates the survival and differentiation of neural stem cells by regulating PAX3 (Wei et al., 2018). miR-124 is important in the response to various stresses, such as oxidative stress (Feng et al., 2017). MiR-124 is involved in the regulation of fatty acid and triglyceride homeostasis (Shaw et al., 2018). MiR-124 plays some roles in multidrug resistance (Popović et al., 2016; Liang et al., 2017).

In conclusion, MiRNAs, which are highly conserved and have a complex regulatory network in organisms, are important for regulating various biological responses to diverse environments. Abnormal levels of miRNAs would cause various diseases. MiR-124 is abundant in tissues and plays crucial roles in organisms. MiR-124 works *in vivo* by targeting various mRNAs and has multiple roles.MiR-124 can be involved in apoptosis, immunity and regulator of disease. More data are necessary to clarify the potential role and mechanisms of miR-124.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Association of probiotic potential of strains of *Pichia kudriavzevii* isolated from "*ogi*" with the number of open reading frame (ORF) in the nucleotide sequences

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In this study, a total of 9 morphologically and biochemically different yeast species were isolated from Nigerian fermented food product, '*ogi*'. These yeast species were screened for probiotic properties such as antibacterial activities, acid and bile tolerance. Six species out of the yeast isolates that survived pH 2 were grown in 0.3% bile salts which were monitored by spectrophotometer for 5 h. Three yeast species with the highest growth performance measured by increment in optical density were identified as *Pichia kudriavzevii* by sequencing the conserved (D1/D2) region of 16S rDNA. Among the three selected strains, isolate MH458240 (M9) showed the highest acid tolerance of 5.80±0.20 log cfu/ml while isolate MH458239 (M5) manifested the least acid tolerance of 3.80±0.20 log cfu/ml. At 0.3% bile salts concentration, isolate MH458239 (M5) demonstrated the highest growth rate of 1.110±011. The antibacterial activities of the three selected yeast strains were also determined. While isolates MH458240 (M9) and MH458239 (M5) inhibited the growth of the tested pathogens, isolate MH458238 (M4) could not inhibit the growth of any of the pathogens used. Out of the three strains of *P. kudriavzevii* used, isolate MH458240 (M9) which demonstrated the best overall probiotic potential was found to contain only three open reading frames (ORF) while the two other isolates contained five. Isolate MH458240 (M9) was found to be in a complete different cluster from the two other strains.

Key words: Yeasts, fermented food, *Pichia kudriavzevii*, probiotic properties, open reading frames (ORF).

INTRODUCTION

'*Ogi*', a fermented cereal gruel considered as a beverage and also a weaning food in Nigeria, could be processed from maize, although sorghum or millet can also be employed as the substrate for fermentation. Fermentation is one of the oldest technologies used for food processing as well as preservation. It has been found to enhance food flavor, produce useful enzymes, vitamins, amino acids and anti-microbial products (Vlasova et al., 2016). Integral parts of fermentation process are non-pathogenic, useful microorganisms commonly referred to as probiotics (Corgan et al., 2007; Kalui et al., 2008). Researchers in the field of science have changed their

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view of the role of food as being just an energy giver and body builder to having an important role of possessing active substances/ingredients that affect the health of consumers positively. Such foods that are beneficial to health are referred to as functional foods since they go beyond keeping the consumers' health to reducing the risk of chronic diseases (Lei et al., 2008; Vasiljevic and shah, 2008).

The two major types of microorganisms associated with traditional fermented foods and beverages are yeasts and bacteria. Yeasts are found in different classes of foods ranging from plant to animal origins where they have a notable impact on food safety and organoleptic properties (FAO and WHO, 2001; Chelliah et al., 2016). Various studies have reported the occurrence of different yeast genera such as *Saccharomyces*, *Candida*, *Debaryomyces*, *Kluyveromyces*, and *Pichia* species, in African traditional fermented food (Kumura et al., 2004; Mahasneh and Abbas, 2010).

Probiotics are live microorganisms, which when administered in adequate amount confer a health benefit on the host (Sanchez et al., 2017). This definition suggests that there must be a measurable physiologic benefit to the host who used the probiotic product (Vandenplas et al., 2014). A joint FAO/WHO working group in 2002 released guidelines for the evaluation of probiotics in food. The basic requirements needed include assessment of strain identity, resistance to gastrointestinal acidity, digestive enzymes, bile acids, antimicrobial activity against potentially pathogenic bacteria and *in vivo* studies for substantial health effects in the target host. However, the ultimate criterion to select a successful probiotic strain is the ability to confer to consumers a health benefit (Łukaszewicz, 2012). The commonly used probiotics include Lactic Acid Bacteria especially those of the genera *Lactobacillus, Bifidobacteria* and yeast of the genus *Saccharomyces* (Vlasova et al., 2016). Recently, it was reported that all probiotics are not equally effective (Szajewska et al., 2016). Both *in vitro* and *in vivo* experiments suggested that probiotic efficiency differs from strain to strain among tested probiotic strains. Different strains of potential probiotics have different mechanism of action and the ability to elicit immune response (McFarland, 2014; Goldstein et al., 2015).

Many characteristic attributes distinguished yeasts as probiotic such as their robust size, ability to tolerate low pH, oxygen, water activity, high osmotic pressure, nutritional flexibility, enzymatic activity and ability to produce several useful metabolites (Fredlund et al., 2002; Lukaszewics, 2012). However, very limited numbers of yeasts are considered as probiotics with proven clinical efficiency in double blind studies (Foligne et al., 2010; Riaz et al., 2012; Lohith and Appaiah, 2014).

Pichia kudriavzevii (the teleomorph of *Candida krusei*) is one of the microflora predominant in the fermentation of maize for '*ogi*' production. It has also been implicated in the fermentation of fruit juices and cocoa beans where it has been found helpful in amplifying the aroma of chocolate (Oberoi et al., 2012). *P. kudriavzevii* can be found in the soil as well as on the surfaces of fruits and vegetables (Chan, 2012). Although this organism was present on plant produce, it was not considered as a species that causes food spoilage (Fleet, 2011).

P. kudriavzevii has the potential of producing toxin that is able to kill several pathogenic microorganisms thereby contributing to food preservation (Bajaj et al., 2013). This organism has also been considered a potential probiotics for its ability to assimilate cholesterol (Psomas et al., 2003). In this study therefore, the probiotic potentials of three strains of *P. kudriavzevii* isolated from a Nigerian traditional fermented food were evaluated.

MATERIALS AND METHODS

Sample collection

Samples of *ogi* were randomly collected from retailers within Ogbomoso Township, Oyo State, Nigeria. All samples were collected in sterile, glass screw-capped bottles and transported immediately to the laboratory for analysis.

Isolation of yeasts from collected samples

Ten-fold serial dilutions (10^{-1} to 10^{-9}) of the samples were made using sterile peptone water. 1 mL aliquot of 10^{-4}, 10^{-6} and 10^{-8} dilutions were pour plated in duplicates on yeast peptone dextrose (YPD) agar (per Litre; 10 g yeast extract, 20 g peptone, 20 g glucose and 20 g agar) (LAB M, UK) supplemented with chloramphenicol (100 mg/l) and incubated at 25°C for 48 h.

Colonies with distinct morphological differences such as colour, shape and size were streaked out as representative colony and were then purified by subculturing on Yeast Extract Peptone (YPD) agar to obtain pure culture of the isolates. The purified isolates were maintained on YPD agar slants at 4°C until required for identification (Kurtzman et al., 2011).

Screening for probiotic properties

Tolerance to pH 2.0

The isolates were subjected to a pH 2.0 assay in order to select the resistant isolates for further studies. Yeasts cells were cultivated in YPD broth for 24 h. The culture was centrifuged (4000 g (rpm) for 10 min at 24°C) and washed two times in 0.1% w/v peptone water pH 7.0. The cell cultures (optical density of 0.2 at 600 nm) in peptone water corresponding to approximately 10^8 cell/ml were centrifuged and resuspended in YPD broth with pH adjusted to 2.0 using 1 N HCl were incubated for 3 h at 37°C. Samples (10 µL) were obtained at time 0 and at 3 h and inoculated in YPD agar plates. Duplicate plates were obtained and tolerance to pH 2.0 was indicated by subsequent growth on agar plates after 48 h of incubation at 37°C as described by Ramos et al. (2013).

Determination of bile tolerance

The method described by Guo et al. (2009) was modified and used to determine the effect of bile on the growth rate of acid tolerant

isolates. Growth performance was expressed as the difference in optical density at 600 nm between the initial and 5 h of incubation 0.3% bile salts concentration (SIGMA ALDRICH, Co, St Louis, USA). The experiment was performed in duplicate.

Molecular identification of yeast isolates

Three acid and bile tolerant yeast species were selected for molecular identification. Genomic DNA of the yeast isolates were extracted using phenol-chloroform extraction method (Green and Sambrook 2017). The D1/D2 region of large subunit of 26S rDNA of selected yeast species was amplified with universal primers, 5' -GCATATCAA TAAGCGGAGGAAAAG-3' and 5' -GGTCCGTGTTTCAA GACGG-3' for forward and reverse, respectively (Kurtzman and Robnett, 1998) in a thermocycler (MJ RESEARCH, INC, programmable thermal controller, BIO-RAD). The amplicon was sequenced using the Sanger's sequencing method (Beckman coulter, USA) and the nucleotide sequences were aligned in genbank using the BLAST program (Website: www.ncbi.nlm.nih.gov/blast). Phylogenetic tree was constructed using MEGA 4.1 software programme (Tamura et al., 2007).

Comparative genomic and phylogenetic analysis

The comparative genomic analysis was carried out on the nucleotide sequences, the exploitation of evolutionary information to draw powerful and robust inferences about phylogenetic history using webPRANK (https://www.ebi.ac.uk/goldman-srv/webprank/). The phylogenetic analysis was carried out through multiple sequence alignment of the nucleotide sequences using MEGA 7.0. Approximately-maximum-likelihood tree was created with MEGA (version 7.0).

Antibacterial activity against enteric pathogens

The double layer method of Maia et al. (2001) was adopted in the determination of the inhibitory effects of yeast isolates against some enteric pathogens. The yeasts were tested against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas* species, *Klebsiella* species and *Proteus* species. A 24 h old culture of yeast isolates were prepared in YPD broth and was individually inoculated onto YPD agar plates by swabbing an inch by 1.5 inch area in the centre of each plate. The plates were incubated at 37°C for 48 h. These plates were overlaid individually with 10 mL of molten nutrient agar that has been previously inoculated with 1 mL of the pathogen culture. The agar was allowed to solidify and the plates were incubated aerobically at 37°C for 24 h. Plates were examined for growth of the yeast strains with their ability to inhibit the growth of the pathogens.

Statistical analysis

Results were expressed as mean+/- standard error. Multiple comparisons were performed by one-way analysis of variance (ANOVA) followed by least significance difference (LSD) test and statistical significance was accepted at values of $P<0.05$ using SPSS.

RESULTS AND DISCUSSION

Tolerance to pH 2.0

Ability of the yeast isolates to tolerate acidic pH is shown in Table 1. The highest survival rate was observed in isolate MH458240 (M9) with initial count of 2.80±0.40 and 5.80±0.20 cfu/ml after 3 h of incubation. The least survival was observed in isolate M6 with initial count of 3.55±0.55 cfu/ml and declined after 3 h of incubation to 2.05±0.05 cfu/ml. A slight decline was observed in isolate MH458239 (M5) from initial viable count of 3.93±0.28 to 3.80±0.20. Isolates M3 and M2 also declined significantly ($P<0.05$) after 3 h of incubation, which suggested that they could not be successful candidates for probiotic use.

Bile tolerance test

Without the addition of bile salt an overall increase was observed in the rate of growth at 5 h of incubation (result not shown). At 0.3% bile salt concentration, isolate MH458239 (M5) manifested the highest significant increase in its rate of growth with absorbance of 0.926±0.1, 1.047±0.11 and 1.11±0.11 at 0, 3 and 5 h of incubation, respectively (Table 2). Isolates MH458238 (M4) and MH458240 (M9) also showed significant high growth rate with initial absorbance reading of 0.939±0.08 and 0.878±0.07, respectively which increased to 1.073±0.06 and 1.099±0.09 at the end of 5 h of incubation.

Antibacterial activity

The result of the antagonistic effect of the three strains of selected yeasts against *E. coli, Pseudomonas* spp., *S. aureus, Klebsiella* spp. and *Proteus* spp. is shown in Table 3. Yeast isolates MH458239 (M5) and MH458240 (M9) inhibited the growth of the test pathogens which was indicated by outgrowth of the yeast culture, although isolate MH458239 (M5) could not outgrow *Proteus* species. Isolate MH458238 (M4) did not express any antagonistic effect against any of the bacterial enteric pathogens and therefore, no growth of the yeast was observed on the plate.

Comparative genomic and phylogenetic analysis

The comparative genomics of the three strains of *P. kudriavzevii* isolates obtained is shown in Table 4. This result was generated using webPRANK for the sequence alignment of the three strain nucleotide sequences. From the alignment of the sequences, isolate MH458240 (M9) had several missing gaps when compared with the other two isolates.

The various open reading frame (ORF) positions in the nucleotide sequences of the selected three strains are shown in Table 5. While isolate MH458240 (M9) had three ORF; isolates MH458238 (M4) and MH45839 (M5) each had five. From the phylogeny shown in Figure 1, it

Table 1. Viable cell counts of yeast isolates during incubation at pH 2 (log_{10} cfu/ml).

Isolate	Initial	3 h
M1	4.90±0.10	5.50±0.50
M2	4.75±0.75	4.40±0.40
M3	4.35±0.85	2.30±1.70
M4 (MH458238)	3.05±0.15	4.10±0.10
M5 (MH458239)	3.93±0.28	3.80±0.20
M6	3.55±0.55	2.05±0.05
M9 (MH458240)	2.80±0.40	5.80±0.20
M10	1.00±0.50	4.85±0.35
S3	1.025±0.83	5.50±0.50

Data are means with standard deviation of duplicate values. Cultures were incubated for 3 h with the pH adjusted to 2 using 1 N HCL.

Table 2. Optical density reading of acid-tolerant yeasts showing their growth rate at 0.3% bile salts concentration.

Isolate	Initial	3 h	5 h
M1	0.834±0.07	1.015±0.08	1.023±0.07
M2	0.859±0.09	0.977±0.09	0.945±0.08
M4 (MH458238)	0.939±0.08	1.069±0.09	1.073±0.06
M9 (MH458240)	0.878±0.07	1.083±0.09	1.099±0.09
M10	0.916±0.08	1.085±0.09	1.046±0.08
M5 (MH458239)	0.926±0.1	1.047±0.11	1.110±0.11
S3	0.900±0.09	1.018±0.10	0.979±0.09

Data are means with standard deviation of duplicate values. Optical density of cultures was measured at the different time point at 600 nm wavelength.

Table 3. Antibacterial activities of the three selected potential probiotic yeast isolates.

Isolate	M5 (MH458239)	M9 (MH458240)	M4 (MH458238)
E. coli	+	++	-
S. aureus	++	++	-
Pseudomonas spp.	+	+	-
Klebsiella spp.	+	+	-
Proteus spp.	-	++	-

(-) No growth; (+) moderate growth; (++) luxuriant growth.

was observed that isolates MH458238 (M4) and MH458239 (M5) were more closely related as shown in the same cluster while isolate MH458240 (M9) was in a complete different cluster .

DISCUSSION

Passage through the stomach is the first line of barrier to be overcome by probiotic organisms when administered through the oral route. In this present study, yeasts were isolated from a Nigerian traditional fermented food, *ogi*. It was observed that majority of the tested yeast isolates could thrive at pH 2, the average pH of the gastric juice. After about 3 h of ingestion, the stomach would empty its contents into the intestine where some digestive enzymes act on it. One of those important enzymes is the bile salts. A successful potential probiotic candidate should be able to grow and replicate significantly in the presence of bile salts so that it can produce its

Table 4. Comparative genomic analysis of the three stains of *Pichia kudriavzevii*.

Sequence ascension identity/name/isolate	Sequence annotation using webPRANK analysis
>MH458240.1_Pichia_kudriavzevii_ M9	CGGAGGAAAAG-AACCAACAGGGATTGCCTCAGTA-CGGCGAGTGAA- CGGCAAGAGCTCAGATTTGAAATCGTKCTTTGCGGCACGAGTTGTA GATTGCAGGTTGGAGTSTGTGTGGAAGGCGGTGTCCAAGTCCCTTGS AACAGGGCGCCCAGGAGGGTGAGAGCCCCGTGGGAT- CCGGCGGAAGCAGTGAGGCCCTT-TG--GAGTYGAG TTGTTTGGGAATGCA-CTC--AACGGGTGGTAAATTCCAT-TAAG- CTAAAT-CTGGCGAGAG-CCGATA-CGAACAAGT— CGTGAAGGAAAGATGAAAAGCACTTTGAAAAGAGAGTG AAACAGCACGTGAAATTGTTGAAAGGGAAGGGTATTGCRCCC GACATGGGGATTGCGCRCCSCTGCCTYTCGTGGGCGGCGCTCT GGGCTTTCCCTGGGCCAGCATCGGTTCTTGCTGCA GGAGAAGGGGTTCTGGAACGTGGCTCTTCGGAGTGTTATAGCCA- GGCCAGAT-CTGCGTGCGGGGA-CGAGGACTGCG-CCGTGTAGTC
>MH458239.1_Pichia_kudriavzevii_ M5	AAAAGCGGAGGAAAAGAAACCAACAGGGATTGCCTCAGTAGC GGCGAGTGAAGCGGCAAGAGCTCAGATTTGAAATCGTGCTTT GCGGCACGAGTTGTAGATTGCAGGTTGGAGTCTGTG TGGAAGGCGGTGTCCAAGTCCCTTGGAACAGGGCGCCCAG GAGGGTGAGAGCCCCGTGGGATGCCGGCGGAAGCAGTGAGG CCCTTCTGACGAGTCGAGTTGTTTGGGAATGCAGCTCCAA GCGGGTGGTAAATTCCATCTAAGGCTAAATACTGGCGAGAGA CCGATAGCGAACAAGTAC TGTGAAGGAAAGATGAAAAGCACTTTGAAAAGAGAGTGAA ACAGCACGTGAAATTGTTGAAAGGGAAGGGTATTGCGCCCG ACATGGGGATTGCGCACCGCTGCCTCTCGTGGGCGGCGC TCTGGGCTTTCCCTGGGCCAGCATCGGTTCTTGCTGCAGGA GAAGGGGTTCTGGAACGT GGCTCTTCGGAGTGTTATAGCCAGGGCCAGATGCTGCGTG CGGGGACCGAGGACTGCGGCCGTGTAG
>MH458238.1_Pichia_kudriavzevii_	AAAAGCGGAGGAAAAGAAACCAACAGGGATTGCCTCAGTAG CGGCGAGTGAAGCGGCAAGAGCTCAGATTTGAAATCGTGCT TTGCGGCACGAGTTGTAGATTGCAGGTTGGAGTCTG TGTGGAAGGCGGTGTCCAAGTCCCTTGGAACAGGGCGCCCA GGAGGGTGAGAGCCCCGTGGGATGCCGGCGGAAGCAGTGAG GCCCTTCTGACGAGTCGAGTTGTTTGGGAATGCAGC TCCAAGCGGGTGGTAAATTCCATCTAAGGCTAAATACTGGCGA GAGACCGATAGCGAACAAGTACTGTGAAGGAAAGATGAAAAGC ACTTTGAAAAGAGAGTGAAACAGCACGTGAAA TTGTTGAAAGGGAAGGGTATTGCGCCCGACATGGGGATTGCGC ACCGCTGCCTCTCGTGGGCGGCGCTCTGGGCTTTCCCTGGG CCAGCATCGGTTCTTGCTGCAGGAGAAGGGGTTC TGGAACGTGGCTCTTCGGAGTGTTATAGCCAGGGCCAGATGC TGCGTGCGGGGACCGAGGACTGCGGCCGTGTAGTCACGGA

metabolites and deliver the health benefit for which it was administered. It was observed that some of the yeast isolates also grew well in medium supplemented with bile salts.

The best acid and bile tolerant yeast strains were selected for molecular identification. They were isolates MH458240 (M9), MH458239 (M5) and MH458238 (M4) which were all identified as *P. kudriavzevii*. Several works

Table 5. Comparative Open Reading Frame (ORF) analysis of the three stains of *Pichia kudriavzevii*.

Sequence ascension identity/name/isolate	Label	Strand	Frame
>MH458238.1_Pichia_kudriavzevii_M4	ORF 3	-	1
	ORF 5	-	2
	ORF 4	-	2
	ORF 1	+	1
	ORF 2	+	2
>MH458239.1_Pichia_kudriavzevii_M5	ORF 5	-	3
	ORF 4	-	1
	ORF 3	-	1
	ORF 1	+	1
	ORF 2	+	2
>MH458240.1_Pichia_kudriavzevii_M9	ORF 3	-	2
	ORF 1	+	3
	ORF 2	-	1

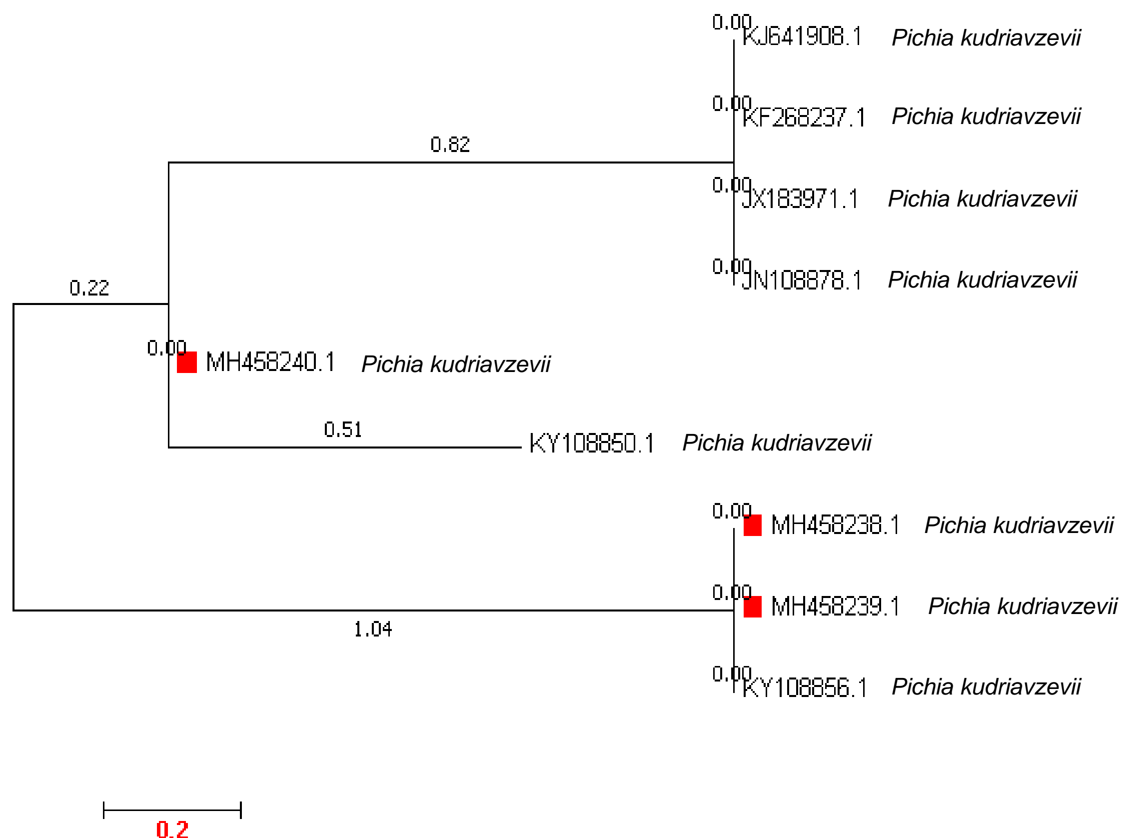

Figure 1. Molecular phylogenetic analysis by Maximum Likelihood method. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model (Bajaj et al., 2013). The tree with the highest log likelihood (-2205.27) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site (next to the branches). The analysis involved 9 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 446 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

have indicated that *P. kurdriavzevii* tolerate acid and bile well. For example, Greppi et al. (2016) showed that out of the 93 yeast strains screened for their tolerance to pH 2 and 0.3% bile salts, *P. kudriavzevii* isolates gave the best results in thriving well in these culture conditions.

Ogunremi et al. (2015) also isolated *P. kudriavzevii* from ogi, a Nigerian traditional fermented food. *P. kudriavzevii* has been implicated in all sorts of traditionally fermented food and beverages. *P. kurdriavzevii* was isolated from fermented African cassava, Ghanaian fermented cocoa bean, tea and ginger tea (Kurtzman et al., 2011; Chan et al., 2012). van Rijswijck et al. (2017) also isolated *P. kudriavzevii* from fermented Masau fruits in Zimbabwe. The yeast has also been isolated from non-fermented food origin like chicken egg, human heart blood, swine waste, sputum and human feaeces and was considered as potential producer of bioethanol and phytase (Chan et al., 2012). Ogunremi et al. (2015) used this yeast as a starter culture for the development of cereal-based functional food.

From the three strains of *P. kurdriavzevii* used in this work, isolate MH458240 (M9) showed the highest acid tolerance of 5.80±0.20 log cfu/ml while isolate MH48239 (M5) manifested the least acid tolerance of 3.80±0.20 log cfu/ml. At 0.3% bile salts concentration, isolate MH48239 (M5) demonstrated the highest growth rate of 1.110±011 while isolate MH48238 (M4) showed the least bile tolerance. While both isolates MH458240 (M9) and MH458239 (M5) inhibited the growth of the tested pathogens, isolate MH458238 (M4) could not inhibit the growth of any of the pathogens used. These differences in the probiotic potentials of the three strains of *P. kurdriavzevii* could probably be due to the three ORF found in isolate MH458240 (M9) compared to five ORF in isolates MH458238 (M4) and MH458239 (M5). These differences in the ORF number may also be the reason why isolate MH458240 (M9) was in a completely different cluster from the two other isolates.

Conclusion

It can be concluded from this work that probiotic properties are strain specific as demonstrated by these three strains of *P. kudriavzevii*.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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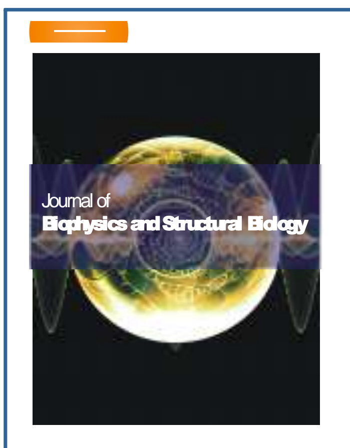

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