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Research Paper

DYNAMICS OF LACTIC ACID BACTERIA ISOLATED PALM WINE DURING THE EXPLOITATION OF TWO VARIETIES OF OIL PALM FOR THE PRODUCTION OF NEWS BEVERAGES

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Abstract

In order to Identify of lactic acid bacteria species predominant during production and storage of palm wine of two palms varieties for the production of news beverages, Palm wine samples were collected every three days during using of oil palm trees (*Tenera* and *Dura*). The grouping of different strains gave 5 genera of lactic acid bacteria. Among five (5) genera of lactic acid bacteria, *Lactobacillus* is dominant with a proportion of 52.86%, followed by representing 31.43% *Pediococcus*. During the operation of palm wine, *P. pentosaceus* is dominant with a share of 9.29% while during storage of palm wine, *L. delbrueckii bulgaricus* sp is the dominant strain with a proportion of 14.29%. These results showed that the species *Pediococcus pentosaceus* and *Lactobacillus delbrueckii sp Bulgaricus* were predominant the exploitation and storage of palm wine. Thus, the use of these lactic acid bacteria as starters for making lactic beverage from palm sap could have a benefic effect on consumer health and thus increasing the interest of these beverages.

Key words: *Beverage, palm wine, lactic acid bacteria, dynamics*.

INTRODUCTION

Natural palm sap is clean sweet colorless syrup containing about 10-20% of sugar that exudes from the trunk of the various species of palm tree during tapping operations [10]. It is a cloudy whitish beverage with a sweet alcoholic taste and has a very short shelf life of only one day. The drink is particularly common in parts of Africa, South

India, Mayanmar and Mexico [6]. In Africa, the sap is most often taken from wild date palms such as *Phoenix sylvestris* (the palmyra) and *Caryota urens*, from oil palms such as *Elaeis guineensis*, or from *Raphia*, kithul or nipa palms. In various traditional African societies, palm wine plays significant role in customary practices. The sap of the palm tree (*Elaeis guinneesis*) serves as a rich substrate for various microorganisms to grow. In various African countries and beyond, the sap of the palm tree is tapped and allowed to undergo spontaneous fermentation, which allows the proliferation of yeast species to convert the sweet substrate into an alcoholic beverage [1, 23, 30]. The alcoholic content increases as the palm wine stays more days. The microorganisms in the overnight palm wine have the effect on the palm wine and this effect is due to the increase in their population as well as their activities [27]. However, these effects are on the physical and biochemical properties of the beverage such as color, sugar concentration, temperature, water, flavor and oxygen concentration [11, 6]. Palm wine appears as a white liquid due to the increased microbial suspension arising from the prolific growth of the fermenting organisms. The palm is a unique habitat which harbors microbes which are mostly the fungi and bacteria. The *Sacchromyces spp,* lactic acid bacteria and acetic acid bacteria involved with the fermentation of palm wine and with other bacteria contribute to the physical, chemical and microbial features of the palm wine. Many researchers have investigated the adding value to sap: [34] proposed the transformation of sap collected from oil palm (*Elaeis guineensis*) and wine palm trees (*Raphia hookeri*) for caramel production. [14] carried out palm sugar production from *Arenga pinnata* palm sap tree. Others researchers focused on the valorization of palm sap, oil palm and palm wine by adding industrial culture for producing ethanol, lactic acid and biomass [15]. Some authors have suggested the use of autochthonous sap microbiota (mainly yeasts) as inoculum for the fermentation of coconut palm sap (*Cocos nucifera*) to palm wine [37]. However, to the best of our knowledge no studies focused in the use of authchtonous lactic acid bacteria as starters for making lactic beverage from palm sap since that autochthonous strains always have better performances than allochthonous strains [8]. In fact, lactic acid bacteria showed important roles in the fermentation and preservation of a great variety of food products [32, 20, 13]. In this way, the purpose of this work was to identify of lactic acid bacteria during production and storage of palm wine of two palms varieties and to use these lactic acid bacteria as starters for the production of news beverages.

MATERIAL AND METHODS

Materials and sampling

Palm wine samples were obtained during the taping of 20 palm trees (*E. guineensis)* of two varieties (*Dura* and *Tenera*) over a period of 5 months. Collection of palm wine during tapping was normally done twice a day by the tapper, but in this study samples were collected at three day intervals each morning of sampling day at 7:00 AM in stomacher bags from the beginning until the end of tapping. These samples were immediately transported to the laboratory for analyses, carried out in replicates.

Isolation and biochemical identification of Lactic acid bacteria

The palm wine samples were shaken by hand in the stomacher bag and tenfold serial dilutions were prepared and spread-plated for determination of micro-organism counts. After dilutions, Lactic acid bacteria (gram positive catalase negative rods, cocci and coccoids) were enumerated by pour plate on DeMan, Rogosa and Sharpe Agar (MRS, Merck 10660; Merck KGaA, Darmstadt, Germany) containing 10 mg ml^{-1} cycloheximide (ICN 100183 Biomedical Inc., Aurora, OH, USA) to suppress yeast growth after incubation at 30°C for 3 days in an anaerobic jar with anaerocult A (Merck). Presumptive LAB was phenotypically characterized by Gram staining, determination of morphology by phase-contrast microscopy, catalase activity. Only Gram-positive, catalase negative, no motility rod and cocci isolates strains were selected. The presence of catalase activity was assessed by the formation of gaz bubles after the suspension of bacterial cells in a droplet of 3% hydrogen peroxide on MRS. Stock cultures of the isolates were stored in MRS broth containing 15% glycerol (Merck) at -80°C. Carbohydrate fermentation pattern of lactic acid bacteria used for sap fermentation were determined at least in duplicate using API 50CH®system (API system, BioMérieux, Marcy l'Etoile, France) according to the manufacturer's instructions [1]. The results were recorded after 24 and 48 h of incubation at 30°C. The ultimate identification was ensured by API Labplus software provided by Bio-Mérieux

RESULTS AND DISCUSSION

Figure 1 shows Changes in pH and total titratable acidity content during the tapping of different palm wine samples. The decrease of pH allow acidification palm wine provided by the population of lactic acid bacteria founded in the palm wine samples, which was

very little during the semi-continuous fermentations. Indeed, microorganisms such as lactic acid bacteria produce organic acids including lactic allowing increased titratable acidity and therefore lower pH. Normally, the sap of the palm has an approximately neutral pH according to the work done by [18]. Therefore, high titratable acidity and the low pH of palm wine obtained indicate that initial fermentation of the sap was done before collection (Figure 1). As the results of pH, titratable acidity and lactic acid confirm the presence of lactic acid bacteria in palm wine. Similar observations were reported by [1], who confirm that the lactic acid bacteria were considered to be responsible for the rapid acidification of the product as the acetic acid bacteria were not isolated in the palm wine samples on the first day of tapping. The increase in acidity indicates production of acid in palm wine as had previously indicated work done by [28] and those of [26]. 140 strains of lactic acid bacteria, isolated from fresh palm sap, were chosen according to the difference in cell morphology to study the acidifying activity. These strains were identified using API galleries. The strains are divided in five (5) groups (Figure 2). In the present study, an identification system based on the morphological characteristics, the fermentative types and the biochemical characteristics of the lactic bacteria founded 5 genera. Among the 5 genera of lactic bacteria, the genus Lactobacillus is dominant with a proportion of 52.86% followed by *Pediococcus* which represents 31.43%. The high proportion of *Lactobacillus* and *Pediococcus* in palm wine indicates high production of organic acids. The production of organic acids in palm wine causes the decrease in pH. This explains the low pH in the various palm wines harvested. These results are in agreement with those of [2] and [25], which showed that palm wine contains bacteria consisting mainly of *Zymomonas* and *Lactobaccilus*. In addition, lactic acid bacteria are generally not pathogenic microorganisms, but have interesting biological properties that are widely exploited in many industrial processes [7, 32, 5]. They have, in fact, inhibitory properties against the pathogenic flora of foodstuffs and therefore make it possible to increase the shelf life of these foods. They also improve the organoleptic and nutritional qualities of foods and have a beneficial effect on the health of humans and animals [32, 29, 5]. During the exploitation of palm wine, the species *Pediococcus pentosaceus* is dominant with a proportion of 9.29%. The second dominant species is *Lactobacillus pentosus* with a proportion of 8.57%. In addition to these species, minority species have also been identified notably *Carnobacterium divergens*, *Weissella viridescens*, *Pediococcus*

damnosus 2, *Leuconostoc mesenteroides / dextranicum 2*, *Lactobacillus plantarum 1*, *Lactobacillus curvatus subsp curvatus*, *Lactobacillus delbrueckii ssp delbrueckii*, *Pediococcus damnosus 1*, *Carnobacterium maltaromaticum, Lactobacillus delbrueckii ssp bulgaricus*, *Lactobacillus delbrueckii ssp lactis 1*. The physiological study of Pediococcus pentosaceus randomly reveals that all strains are homofermentatives and that the *Lactobacillus pentosus* isolates are composed of 83.33% of the homofermentatives strains and 16.67% of the heterofermentatives strains. The species of *Pediococcus pentosaceus* and *Lactobacillus pentosus* are responsible for the acidification of palm wine during exploitation. In addition, minority species such as *Lactobacillus brevis* and *Lactobacillus acidilactici* isolated from palm wine would also be responsible for the sour taste and milky appearance of palm wine, respectively. The results obtained does not correspond to those of [1]. According to their work, the dominant species of palm wines are *Lactobacillus plantarum* and *Leuconostoc mesenteriodes*; they are therefore responsible for the rapid acidification of the sour taste of palm wine from palm trees slaughtered in Ghana. In addition, [35] isolated *Leuconoctoc mesenteroides*, *Leuconostoc dextranicum* and *Lactobacillus sp* from palm wine samples in Nigeria. [4] reported that *Lactobacillus plantarum* and *Leuconostoc mesenteriodes* were responsible for the sour taste of palm wine extracted on the living palm. *Lactobacillus brevis 1* and *Lactobacillus plantarum 1* detected in palm wines are often cited as agents of alteration of the market quality of modern and traditional beers. *Lactobacillus plantarum* strains identified in this study are homofermentatives. The homofermentatives and optional heterofermentatives *Lactobacillus* strains have been isolated by several authors [20, 17]. This species is very important in several fermented foods; but in the present study it does not appear to be much involved in the production of the sour taste of palm wine. Indeed, Lactobacillus plantarum represents 4.29% of the lactic bacteria identified in the palm wine during the exploitation. Lactic acid bacteria strains isolated from palm wine during exploitation show a great diversity of phenotypic character. During storage of palm wine, *Lactobacillus delbrueckii sp*. *bulgaricus* is the dominant strain with a proportion of 14.29%. In addition to this species, minority species have also been identified. These include: *Carnobacterium divergens*, *Weissella viridescens*, *Pediococcus pentosaceus*, *Pediococcus damnosus 2*, *Pediococcus acidilactici*, *Carnobacterium maltaromaticum* and *Lactobacillus delbrueckii sp lactis 1*. The presence of these microorganisms in palm wine may be due to microorganisms colonizing palm oil, the

collection container and those used for the harvesting of palm wine. The work done by [3] indicate the presence of 17 species of yeasts and 7 genera of lactic acid bacteria in the spontaneous fermentation of palm wine. *Lactobacillus lactis* species is responsible for the milky appearance of the palm wine. These results are in agreement with those of [18]. According to them, *Lactobacillus lactis* species are responsible for the whitish color of palm wine during storage by the production of dextrans in the beverage. Indeed, this species becomes dominant after 32 hours of storage. The production of organic acids in palm wine thus permits the acidification of palm wine. Moreover, fresh palm wine is rich and predominantly made up of sugars. However, lactic acid bacteria in wine convert sugars into organic acids and alcohol [23]. Of the 140 isolates tested with the API 50 CHL gallery, none starch fermented. This clearly demonstrates that they are not capable of producing amylase. This inability can be explained by the nature of the sugar contained in the palm wine. Indeed, palm wine already contains fermentable sugars that can be used directly by microorganisms [19, 31].

CONCLUSION

To conclude in this study, of the 140 strains analyzed per gallery API 50 CHL, five (5) genera gave 15 species of Lactobacillus. During the exploitation of palm wine the predominant species are *Pediococcus pentosaceus* and *Lactobacillus pentosus,* while during storage the species of *Lactobacillus delbrueckii sp bulgaricus* are dominant. These analyzes have showed that during the exploitation of palm wine acidification is ensured by the species *Pediococcus pentosaceus* and *Lactobacillus pentosus* whereas during storage it is the species of *Lactobacillus delbrueckii sp. Bulgaricus* which ensured the acidification. These strains can be used as mixed starter cultures for the fermentation of various local products for the production of new beverages.

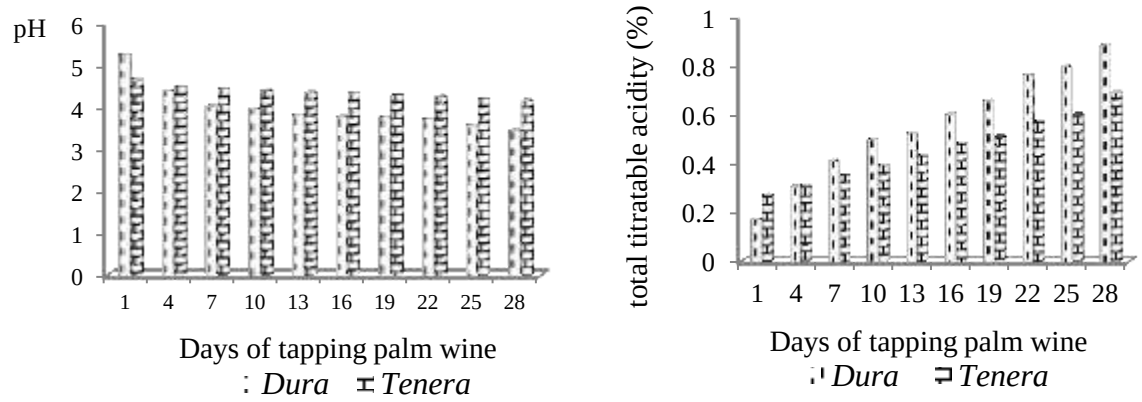

Figure 1 : Changes in pH and total titratable acidity content during the tapping of different palm wine samples.

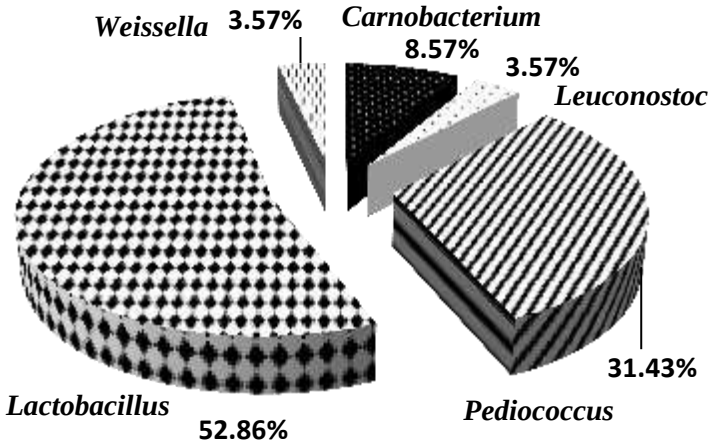

Figure 2 : Différents genres de bactéries lactiques isolés du vin de palme.

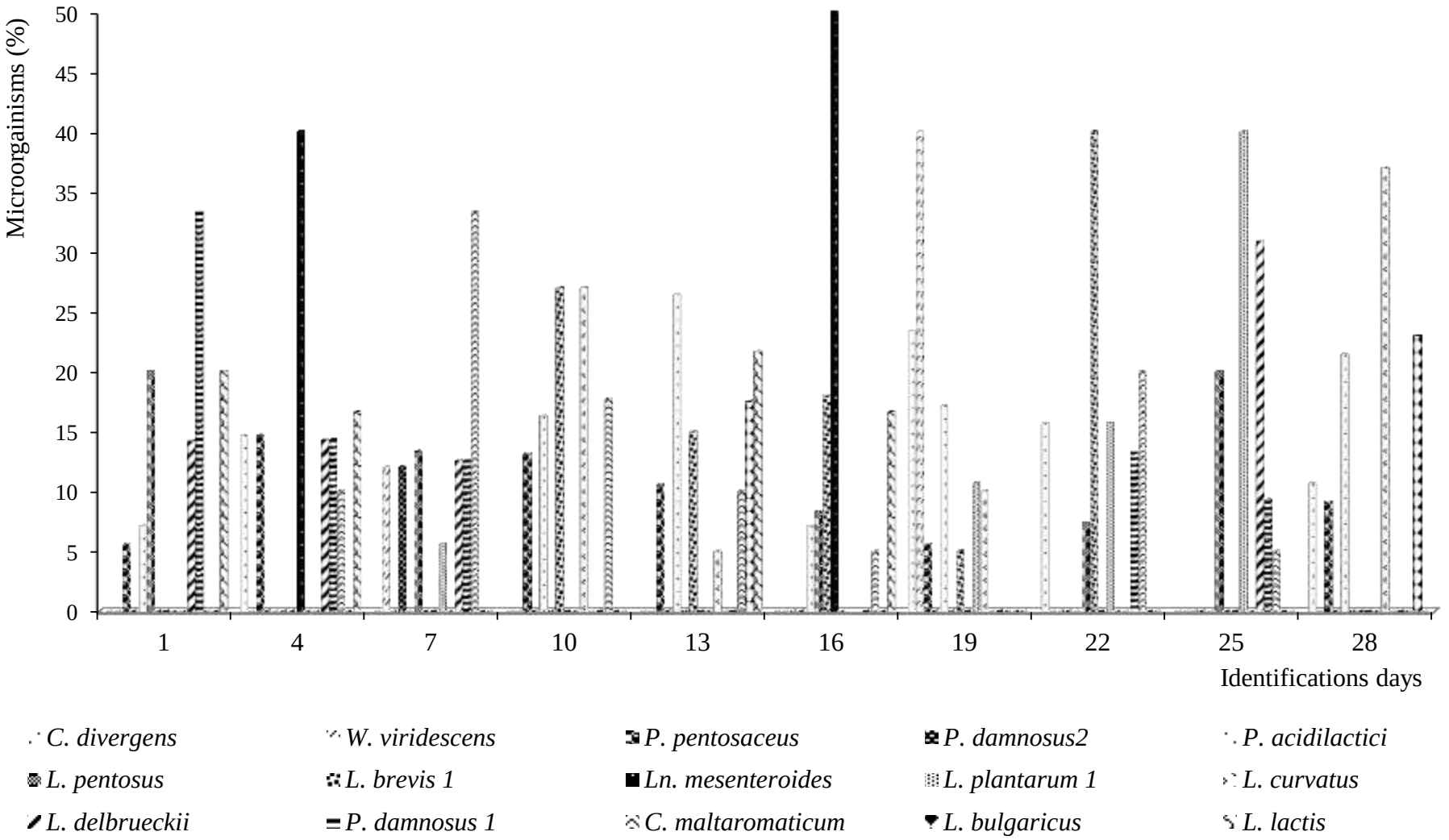

Figure 3: Succession and proportion of different species of lactic acid bacteria isolated during the operation of palm wine

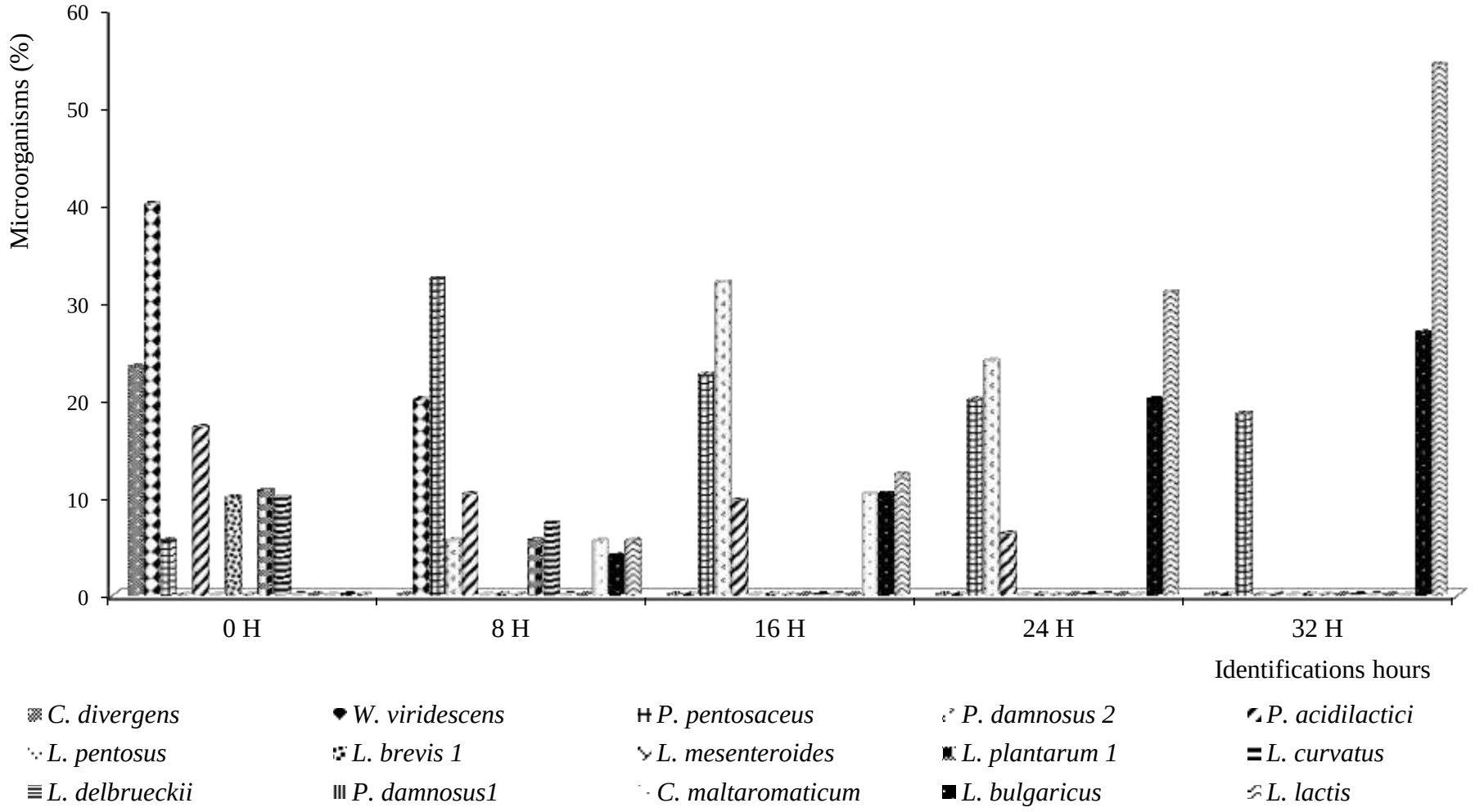

Figure 4: Succession and proportion of different species of lactic acid bacteria isolated during storage of palm wine 22nd day

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