Research Article

Microbial Profile of Fresh Beef Sold in the Markets of Ngaoundéré, Cameroon, and Antiadhesive Activity of a Biosurfactant against Selected Bacterial Pathogens

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Owing to its composition, meat is recognized as one of the best media for microbial growth leading to meat spoilage and food-borne illness. The ability of microorganisms to adhere to surfaces where meat is deposited during selling is a nonnegligible cause of meat contamination. This work was performed to assess the microbial profile of fresh beef sold in the markets of Ngaoundéré town and evaluate the antiadhesive activity of a biosurfactant derived from *Lactobacillus paracasei* subsp. *tolerans* N2 against selected pathogenic strains isolated in fresh beef. All fresh beef samples analysed were contaminated with both pathogenic and spoilage microorganisms at levels higher than the microbiological criteria set by the European Commission. A total of 151 strains belonging to 12 species (*Pseudomonas putida*, *Pseudomonas aeruginosa*, *Pseudomonas* sp., *Escherichia coli* 1, *Escherichia coli*, *Salmonella enteritidis*, *Salmonella* sp., *Staphylococcus epidermidis*, *Staphylococcus xylosum*, *Staphylococcus aureus*, *Candida albicans*, and *Candida* sp.) were isolated and identified. A specific relationship between the microbial diversity of fresh beef and the sampling sites was observed. Biosurfactant displayed antiadhesive activity against all the tested strains and the complete inhibition (100%) of *Bacillus* sp. BC1, *S. aureus* STP1, and *S. xylosum* STP2 was noticed at biosurfactant concentration of 10 mg/mL. This study indicates the microbial diversity of fresh beef sold in Ngaoundéré markets and suggests the potential use of biosurfactant as an antiadhesive agent in the meat industry.

1. Introduction

Meat is a regular part of the human diet worldwide. It contains proteins (with all essential amino acids), vitamins (A, B12, B6, D, and E), and minerals (iron and zinc) which are very important for human growth and well-being [1]. Due to its nutrient content, meat is a highly perishable food as it represents good media for the proliferation of microorganisms [1, 2]. These microorganisms are essentially spoilage organisms (*Brochothrix thermosphacta*, *Lactobacillus* spp., *Clostridium* spp., *Leuconostoc* spp., *Carnobacterium* spp., *Pseudomonas* spp., *Candida* spp.,

Penicillium, *Mucor*, *Aspergillus*, *Rhizopus*, and *Enterobacteriaceae*) responsible for off-odors, discoloration, development of slime, and production of toxic compounds and pathogens (*Salmonella* spp., *E. coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Campylobacter* spp.) responsible for food-borne diseases [3]. Generally, meat is consumed cooked, but according to some food habits and traditional recipes, meat may be consumed raw or partially cooked [4]. Under these conditions, the consumption of meat was many times associated with outbreaks of food-borne diseases [5]. According to the World Health Organization, 30% of inhabitants in industrialized countries

suffer every year of food-borne diseases [6] with most of the cases attributable to the consumption of meat [7]. In sub-Saharan Africa, food-borne diseases due to the consumption of meat are not uncommon. Because of the weakness of bacteriological diagnose resources associated with the unawareness and nonenforcement of laws [8], the estimation of food-borne diseases as well as resulting losses is underevaluated [9].

Nowadays, as the demand and consumption of raw meat have increased in many countries [10], regulations which specified the microbiological criteria of raw meat have been established in order to protect consumers' health and to facilitate its commercialization. To this end, the microbial contamination of meat is becoming a public health concern worldwide and particularly in Cameroon [11]. It has been reported in the literature that microorganisms contaminate meat during the production, processing, transportation, and distribution processes [12]. The first step in the control of the microbiological quality of meat consists of evaluating their initial contamination levels and identifying the different microorganisms involved. The studies assessing the microbiological quality of fresh beef have been carried out in several countries such as Turkey [13], Ethiopia [1], Nigeria [14], Sudan [15], Ghana [16], Rwanda [17], and India [18]. It therefore appears interesting to perform such kind of study in some Cameroonian metropolises where fresh beef is highly consumed by the whole class of the population.

Another important step in the control of the microbiological quality of meat is the cleaning process of surfaces where meat is processed [19]. A study conducted by Zerabruk et al. [1] showed the presence at high levels of different groups of microorganisms on cutting tables in butcher shops of Addis Ababa. In fact, microorganisms display the ability of adhering to the surface and forming structured communities encapsulated within a self-developed polymeric matrix known as biofilms which are resistant to the conventional cleaning processes. Therefore, inhibiting the initial adhesion of microorganisms to surfaces is a challenging task in the meat industry. Utilization of biosurfactants which deserve antiadhesive properties [20–22] appears as an alternative. Biosurfactants are amphiphilic compounds produced by a wide variety of microorganisms such as bacteria, yeasts, and moulds that can have some influence on interfaces. Opposite to their synthetic homologues, these compounds have unique characteristics such as being nontoxic, biodegradable, environmentally friendly, and selective, having structural diversity, being effective under extreme conditions of pH, temperature, and salinity, and having unique surface-active properties [23]. To the limit of our knowledge, the antiadhesive properties of biosurfactants were not yet tested against strains isolated from fresh beef despite the ability of these strains of adhering to cutting meat surfaces. Hence, the objective of the present work is to establish the microbial profile of fresh beef sold in Ngaoundéré, Cameroon, and evaluate the antiadhesive properties of biosurfactants against selected pathogenic strains isolated in fresh beef.

2. Materials and Methods

2.1. Study Area. The study was conducted in the city of Ngaoundéré, Adamawa region of Cameroon, from February 2015 to May 2016. The six main markets of the town where fresh beef is daily sold were chosen. These markets were Bantail market (MBT), Bamnyanga market (MBG), Dang market (MD), “Grand Marché” (GM), “Petit Marché” (PM), and “Centre Commercial” (CC).

2.2. Fresh Beef Samples. The normalized method ISO 17604: 2015 [24] which specifies the sampling methods for the detection and enumeration of microorganisms on the surface of carcasses or part of carcasses of slaughtered meat was used for the sampling procedure. 10 samples of 500 g each were collected in the morning (between 9:30 and 10:30 AM) from butcher shops in each market using a systematic random sampling technique. Briefly, in each market, butcher shops were numbered and the corresponding number was noted on a paper. The papers bearing each number were folded, introduced into a closed container, and thoroughly mixed. One paper was selected from the container and its number was recorded. The paper was reintroduced into the container and the container was thoroughly mixed again. In the case that the same number was drawn twice, the second drawing was not considered and the number was returned to the container. The operation was performed until 10 butcher shops were selected. The collected samples were transferred into a sterile bag. After being labelled, the samples were stored in an icebox and transported to the lab for analyses.

2.3. Biosurfactant. The biosurfactant used in this study was previously produced by *Lactobacillus paracasei* subsp. *tolerans* N2 with sugar cane molasses as substrate and characterized [25]. Biosurfactant was dissolved in methanol and centrifuged at 15000g, 4°C for 10 min. The supernatant was filtered (0.22 µm) and evaporated to dryness under nitrogen.

2.4. Chemicals. Glacial acetic acid, ethanol, and methanol of HPLC-grade were purchased from Sigma-Aldrich Co., Germany. Plate Count Agar (PCA), Eosin Methylene Bleu (EMB) agar, Bile Azide Esculin (BEA) agar, Mannitol Salt Agar (MSA), Mossel agar, Cetrimide agar and Sabouraud agar supplemented with chloramphenicol, Tryptone Sulfite Neomycin (TSN) agar, *Salmonella* and *Shigella* (SS) agar, Selenite cystine broth, Xylose Lysine Decarboxylate (XLD) agar, Triple Iron Sugar (TSI) agar, Brain Heart Infusion (BHI) broth, and Nutrient Agar (NA) were purchased from Scharlau (Germany). All other reagents used in this study were of analytical grade.

2.5. Cultures. The following reference cultures: *E. coli* MTCC 118, *Staphylococcus aureus* MTCC 1430, *Micrococcus luteus* MTCC 106, and *Listeria monocytogenes* MTCC 839 were used for the antiadhesive test. These Microbial Type Culture Collection (MTCC) strains were kindly provided by the Department of Microbiology and Fermentation

Technology, CSIR-Central Food Technological Research Institute, Mysore, India.

2.6. Microbiological Analysis of Fresh Beef Samples

2.6.1. Sample Preparation. Samples were processed according to the method ISO 6887-2:2017 [26]. In the procedure, 500 g of the different samples was weighed and ground and 25 g was introduced into an Erlenmeyer containing 225 mL of sterile peptone water. The mixture was homogenized for 2 min with a vortex (IKA, Vortex Genius 3, UK) and allowed to stand for 30 min at room temperature. Serial decimal dilutions (10^{-1} to 10^{-6}) were prepared.

2.6.2. Inoculation and Culture Conditions. The total mesophilic aerobic count was determined using the pour plate method [27]. Briefly, 1 mL of each dilution was introduced into a sterile Petri dish followed with the addition of 20 mL of sterile PCA. The plates were incubated at 37°C for 48 h. Spread plate method was used for the enumeration of total and faecal coliforms [28], faecal *Streptococci* [29], *Staphylococcus* spp. [30], *Bacillus* spp. [31], *Pseudomonas* spp. [32], yeasts, and moulds [33]. In the procedure, 0.1 mL of the different dilutions was surface-inoculated on Petri plates containing EMB agar, BEA agar, MSA agar, Mossel agar, Cetrimide agar, and Sabouraud agar supplemented with chloramphenicol, respectively, for total and faecal coliforms, faecal *Streptococci*, *Staphylococcus* spp., *Bacillus* spp., *Pseudomonas* spp., and yeasts, and moulds. The plates were incubated at 37°C for 48 h for total coliforms, faecal *Streptococci*, *Staphylococcus* spp., *Bacillus* spp., and *Pseudomonas* spp., at 44°C for 48 h for faecal coliforms, and at 25°C for 3–5 days for yeasts and moulds.

The presence of *Salmonella* spp. in samples was assessed according to the method ISO 6579-1:2017 [34]. After homogenization of 25 g of sample in 225 mL of sterile peptone water, the suspension was incubated for 16 h at 37°C for preenrichment. Then, 1 mL of the suspension was transferred into a tube containing 10 mL of sterile selenite cystine broth and incubated for 24 h at 37°C for enrichment. Thereafter, one loopful of each enrichment broth was streaked onto SS and XLD agar and incubated at 37°C for 24 h. Uncolored colonies with black centers on SS agar and red colonies with black centers on XLD agar were considered as *Salmonella*.

The enumeration of sulphite-reducing *Clostridium* was done according to the method ISO 7937:2004 [35]. 2 mL of dilution (10^{-2}) was introduced into a tube containing 15 mL of sterile TSN agar, homogenized, and heated at 80°C for 10 min. Then, the tubes were rapidly cooled and 1 mL of sterile paraffin oil was introduced into each tube followed with incubation at 37°C for 48 h. After incubation, black colonies were considered as sulphite-reducing *Clostridium*.

2.6.3. Plates Reading. The colony-forming units (CFU) appearing on the Petri dishes after the incubation period were counted. Only plates with colony-forming units between 30 and 300 were considered. All experiments were

performed with three replications and the results were expressed as colony-forming units per gram of fresh beef (CFU/g).

2.7. Identification of Microorganisms Isolated from Fresh Beef Samples. From each Petri plate, individual colonies were streaked twice on nutrient agar and characterized through macroscopic (colony shape and color), microscopic (Gram staining), cultural (growth at different temperatures and NaCl concentrations), and standard biochemical tests (catalase, motility, coagulase, oxidase, urease, citrate utilization, indole, gelatin hydrolysis, and TSI test) according to Bergey's Manual of Determinative Bacteriology [36]. Further characterization of the isolates was performed through their Analytical Profile Index (API). The following galleries were used according to the manufacturer's instructions: API 20 E, API 20 non-E, API 20 Strep, API 20 Staph, and API 20 AUX (BioMérieux SA, France). The specificity of these galleries was at least 92%. The results were recorded and the identification process was performed with Apident 2.0 (BioMérieux, France). Identity of isolates was confirmed using online API web services (<https://apiweb.biomerieux.com>) and Bergey's Manual of Determinative Bacteriology for comparison of fermentation profiles.

2.8. Antiadhesive Activity of Biosurfactants. The antiadhesive activity of biosurfactant was assessed against reference cultures and selected cultures isolated from fresh beef following the method of Gudiña et al. [37] with slight modifications. In the procedure, the different strains were cultured in BHI broth for 16 h at 37°C. The culture was centrifuged (10000g, 10 min, 4°C) and cells were washed three times with phosphate-buffered saline (PBS, pH 7.2). Cells were suspended in PBS and their concentrations were adjusted to 10^8 CFU/mL. Solutions of biosurfactant at concentrations ranging from 0.01 to 10 mg/mL were prepared in millipore water. For the test, 200 µL of each solution of biosurfactant was introduced into the wells of a sterile 96-well flat-bottomed plastic tissue culture plate (Tarsons, India) and the plates were incubated for 1 h at 4°C. Afterwards, the plates were washed twice with PBS and 200 µL of the bacterial suspension was added to each well followed with incubation at 4°C for 24 h. Then, the plates were washed twice with PBS and adherent cells were fixed with 200 µL of methanol 99%. After incubation at room temperature for 15 min, methanol was removed and plates were air-dried. Adherent cells were stained for 5 min with 200 µL of 33% crystal violet. The plates were washed three times with PBS and air-dried and 200 µL of 33% (v/v) glacial acetic acid was introduced in each well to resolubilize adherent cells. The optical density of the plates was read at 595 nm using a Spark® 10M Multimode Microplate Reader (Tecan, Switzerland). Wells filled with PBS without biosurfactants were used as control. The inhibition of microbial adhesion was calculated using

$$\text{Microbial inhibition (\%)} = \left(\frac{A_0 - A_i}{A_0} \right) \times 100, \quad (1)$$

where A_0 is the absorbance of the control and A_i is the absorbance of the sample at biosurfactant concentration i . For each tested microorganism and biosurfactant concentration, essays were performed in triplicate.

2.9. Statistical Analysis. All experiments were carried out in triplicate. Microbial loads were transformed into logarithms and expressed as means $\pm$ standard deviation. Duncan's Multiple Range test was performed to compare microbial loads of samples with the microbiological criteria of the European Commission [38] and to compare the anti-adhesive activity of biosurfactant against the tested microorganisms. The statistical significance was set at $p < 0.05$ and Statgraphics centurion XVI version 16.1.18 (StatPoint Technologies, Inc., USA) was used to perform these analyses. Principal component analysis was done with XLSTAT 2018 (Addinsoft, Inc., New York, USA) in order to visualize the association between the microbial strains identified and the sampling sites.

3. Results and Discussion

3.1. Microbiological Contamination of Samples. Total aerobic count shows the wide variability of microorganisms which can be found in a product. As observed in Table 1, the total aerobic count of fresh beef samples ranges significantly ($p < 0.05$) from 5.38 ± 0.01 (GM) to 5.93 ± 0.022 Log CFU/g (PM). The high level of contamination of fresh beef samples was also reported by Obeng et al. [39] and Goja et al. [15] in their studies. They, respectively, found a total aerobic count ranging from 4.82 to 6.92 Log CFU/g of fresh beef sold in Ghanaian markets and from 4.67 to 5.53 Log CFU/g of fresh beef sold in Sudanese markets. Zerabruk et al. [1] notified in their study a total aerobic count of fresh beef sold in butcher shops of Addis Ababa, Ethiopia, which ranged from 5 to 7.12 Log CFU/g. Compared to the microbiological criteria [38], all samples analysed in this study presented a total aerobic count lower than the recommended values (7 Log CFU/g). However, it does not mean that there is no pathogen because it is not necessary to have a high concentration of the total aerobic flora to have safety issues. According to Emele et al. [40], the total aerobic count of a food product does not necessarily determine its sanitary quality; rather it is the range of its different group of microorganisms which determined if the product is safe or not. The safety criteria of fresh beef placed on the market during their shelf life deal with the presence of *Salmonella* in the samples analysed [38]. Another important criterion for fresh beef placed on the market during their shelf life is the process hygiene criteria. It concerns the presence of microorganisms like *Enterobacteria* and *E. coli* in the samples analysed [38]. In this light, pathogenic microorganisms were sought.

Faecal *Streptococci*, which are indicative of faecal contamination, were found in all samples at loads ranging from 4.33 ± 0.02 (PM) to 5.11 ± 0.14 Log CFU/g (MBT). The coliforms, another pathogenic group of microorganisms whose presence in raw meat is indicative of faecal contamination, were assessed. The results (as shown in Table 1) show that

total and faecal coliforms were present in the fresh beef samples at loads higher than the values specified by the microbiological criteria (3 and 2.7 Log CFU/g, resp.) of the European Commission [38]. The highest level of contamination was observed at the site CC for both total (5.93 ± 0.04 Log CFU/g) and faecal (5.38 ± 0.01 Log CFU/g) coliforms. The presence of coliforms in the fresh beef samples could result from the nonrespect of good slaughtering and evisceration practices. The unwholesome cleaning process of equipment and cutting meat surfaces could also explain the high contamination. Moreover, the presence of these *Enterobacteria* in fresh beef samples could originate from the deplorable levels of hygiene and sanitary practices of butchers as observed during this study. The nonrespect of hygiene during the production, distribution, and selling of meat was notified in the literature as the source of coliforms' contamination [39]. Afnabi et al. [19] have justified the presence of coliforms in beef sold in Northern Cameroon by the poor application of the principles of cleaning and disinfection and the lack of supervision of staff on hygiene.

According to safety criteria of the European Commission Regulation [38], *Salmonella* must be absent in 25 g of fresh beef intended to human consumption. However, *Salmonella* spp. were present in all samples analysed probably as the result of improper sanitary practices of butchers. Hence, the meat was consequently unsafe for consumption. *Staphylococci*, the germs which indicate the level of hygiene of fresh beef handlers, were found in all samples at loads which significantly ($p < 0.05$) vary from one site to another. The high level of *Staphylococcus* spp. was observed in the site MD (4.97 ± 0.03 Log CFU/g) while the least level was noticed in the site MBT (4.30 ± 0.13 Log CFU/g). Poor hygienic practices of meat handlers during slaughtering, evisceration, cutting, and selling (such as coughing and sneezing) could explain the presence of *Staphylococcus* spp. in the analysed samples at level higher than the 3 Log CFU/g recommended by the European Commission Regulation [38]. A high contamination level (4.57 ± 0.87 Log CFU/g) of fresh beef sold in butcher shops of Addis Ababa, Ethiopia, was also noticed by Zerabruk et al. [1].

However, despite the poor sanitary quality of fresh beef observed in this study, sulphite-reducing *Clostridium* was not found in any sample. This result was very surprising considering the fact that slaughtering, evisceration, dressing, and cleaning of the carcasses are carried out on the ground. Regarding *Pseudomonas* spp., they were present in all samples at loads which significantly ($p < 0.05$) vary from 4.75 ± 0.06 (CC) to 5.05 ± 0.03 Log CFU/g (MD). Their presence in the fresh beef samples is indicative of a high risk of spoilage. In fact, bacteria belonging to *Pseudomonas* genus are generally recognized to actively contribute to fresh meat spoilage owing to their ability to degrade sugars, lipids, and proteins even at refrigeration temperature [3, 41]. As observed in Table 1, *Bacillus* spp. were found in all samples with a contamination level which significantly varies from one site to another. The high contamination level was observed in the site MBT (5.43 ± 0.07 Log CFU/g). Yeasts and moulds were enumerated in the different samples and results

TABLE 1: Mean microbial loads in Log CFU/g of fresh beef samples collected from different markets of the city of Ngaoundéré.

Microbial types	PM (<i>n</i> = 10)	GM (<i>n</i> = 10)	MBT (<i>n</i> = 10)	MBG (<i>n</i> = 10)	MD (<i>n</i> = 10)	CC (<i>n</i> = 10)	Norms
Total aerobic count	5.93 ± 0.02 ^d	5.38 ± 0.01 ^a	5.82 ± 0.06 ^c	5.84 ± 0.01 ^c	5.51 ± 0.12 ^b	5.80 ± 0.13 ^{cd}	7.00
Total coliforms	5.81 ± 0.01 ^d	5.26 ± 0.08 ^b	5.02 ± 0.02 ^a	5.63 ± 0.05 ^c	5.29 ± 0.01 ^b	5.93 ± 0.04 ^e	3.00
Faecal coliforms	5.20 ± 0.01 ^d	5.08 ± 0.02 ^b	4.95 ± 0.03 ^a	5.08 ± 0.11 ^{abc}	5.19 ± 0.01 ^c	5.38 ± 0.01 ^e	2.70
Faecal <i>Streptococci</i>	4.33 ± 0.02 ^a	4.65 ± 0.07 ^b	5.11 ± 0.14 ^c	4.70 ± 0.12 ^b	4.39 ± 0.08 ^a	4.76 ± 0.14 ^b	—
<i>Staphylococcus</i> spp.	4.65 ± 0.02 ^b	4.35 ± 0.06 ^a	4.30 ± 0.13 ^a	4.82 ± 0.11 ^{cd}	4.97 ± 0.03 ^d	4.87 ± 0.03 ^c	3.00
<i>Salmonella</i> spp.	+	+	+	+	+	+	0
SR- <i>Clostridium</i>	—	—	—	—	—	—	0
<i>Bacillus</i> spp.	5.36 ± 0.09 ^d	5.26 ± 0.05 ^c	5.43 ± 0.07 ^e	5.09 ± 0.01 ^b	5.29 ± 0.02 ^c	4.48 ± 0.02 ^a	—
<i>Pseudomonas</i> spp.	4.81 ± 0.02 ^a	4.93 ± 0.06 ^c	4.82 ± 0.10 ^{abc}	4.87 ± 0.03 ^b	5.05 ± 0.03 ^d	4.75 ± 0.06 ^a	2.00
Yeasts	4.31 ± 0.01 ^c	4.05 ± 0.02 ^a	4.20 ± 0.10 ^{bc}	4.26 ± 0.04 ^b	4.44 ± 0.02 ^d	4.67 ± 0.11 ^e	—
Moulds	1.76 ± 0.01 ^d	1.46 ± 0.05 ^a	1.57 ± 0.03 ^b	1.63 ± 0.01 ^c	2.29 ± 0.08 ^f	1.83 ± 0.02 ^e	—

n = number of samples per site; — = absence; + = presence; PM = “Petit Marché”; GM = “Grand Marché”; MD = “Dang market”; CC = “Centre Commercial”; MBG = “Bamnyanga market”; MBT = “Bantail market”; SR-*Clostridium* = sulphite-reducing *Clostridium*; values with different letters within a line are significantly different (*p* < 0.05) according to Duncan’s multiple range test; norms = microbiological criteria of fresh beef established by the European Commission Regulation (EC, 2005).

obtained show that yeasts were present in these samples with loads which range from 4.05 ± 0.02 (GM) to 4.67 ± 0.11 Log CFU/g (CC). Moulds’ count varies from 1.46 ± 0.05 (GM) to 2.29 ± 0.08 Log CFU/g (MD). The presence of these microorganisms in fresh beef samples could result from the contamination during slaughtering, dressing, and evisceration processes. Moreover, air exposition of meat during selling could lead to meat contamination by mould spores.

3.2. Identification of Microorganisms Isolated from Fresh Beef Samples. The strains isolated from the different fresh beef samples were purified and identified by their macroscopic, microscopic, cultural, and biochemical properties. The results obtained show that all the 151 strains isolated belonged to five genera: *Pseudomonas*, *Staphylococcus*, *Salmonella*, *Escherichia*, and *Candida*. These isolates were identified at species level using API galleries. Table 2 presents the identification percentages gathered from the fermentative profile of the different isolates on API 20 E, API 20 NE, API 20 Staph, and API 20 AUX, respectively. Twelve species (12) were identified with at least 97% of identity as follows: *Pseudomonas putida* (12 strains), *Pseudomonas aeruginosa* (13 strains), *Pseudomonas* sp. (26 strains), *Escherichia coli* 1 (5 strains), *Escherichia coli* (14 strains), *Salmonella enteritidis* (9 strains), *Salmonella* sp. (15 strains), *Staphylococcus epidermidis* (8 strains), *Staphylococcus xylosus* (12 strains), *Staphylococcus aureus* (18 strains), *Candida albicans* (5 strains), and *Candida* sp. (14 strains).

The different isolates identified in this study were grouped by genus, species, and sampling sites (Table 2). The highest microbial diversity (10 species) was observed in the sampling sites PM and MBG while the lowest diversity was noticed in the site GM (6 species). Accordingly, the high proportion of strains (20.52% and 25.82%) was isolated from fresh beef sold in the sites MBG and PM, respectively. 15.23, 12.58, 17.88, and 7.94% of strains were, respectively, obtained from fresh beef sold in the sites CC, MD, MBT, and GM.

For the genus *Pseudomonas*, 51 strains belonging to three species: *P. putida* (12), *P. aeruginosa* (13), and *Pseudomonas*

sp. (26) were isolated (Table 2). The proportion of strains isolated varies with the species identified and the sampling sites. The high proportions of *P. putida* (33.33%) and *Pseudomonas* sp. (23.07%) were noticed in the site CC, and that of *P. aeruginosa* (38.46%) was observed in the site MBG. The high proportions of strains belonging to the genus *Pseudomonas* observed in this study (33.77%) could be justified by the fact that they are versatile bacterium ubiquitous in nature and are mostly found in water and soils. During slaughtering, evisceration, and dressing processing which are generally carried out on the ground, they could easily contaminate the meat. Strains of *Pseudomonas* sp. and *P. aeruginosa* were also identified in fresh beef sold in Calabar metropolis, Nigeria [14], and in Kenyan markets [42].

The second most important proportion of strains isolated in this study was those belonging to the genus *Staphylococcus* (25.16%). A total of 38 strains regrouped in 3 species: *S. xylosus* (12), *S. epidermidis* (8), and *S. aureus* (18) were isolated (Table 2). The high proportion of *S. aureus* (27.77%) and *S. epidermidis* (50%) was isolated from the site MBT while *S. xylosus* was isolated only in the sites MBG (66.66%) and PM (33.33%). It is important to highlight that the strain *S. xylosus* is useful in meat processing products as sausage due to its ability to degrade biogenic amines. *S. aureus* considered to be a well-known pathogen to humans was the predominant strain of the genus *Staphylococcus* with 47.36%. Its predominance could result from contamination through skin, mouth, hand, and noses of butchers as this strain colonized at 40 to 60% the nasal mucosa and skin of healthy human being. *S. xylosus*, *S. epidermidis*, and *S. aureus* were isolated by Goja et al. [15] in fresh beef sold in markets of Khartoum, Omdurman, and Bahri cities, Sudan.

In this study, 19 strains of *E. coli* belonging to the species *E. coli* (14) and *E. coli* 1 (5) were isolated (Table 2). The highest proportion of *E. coli* strains (28.57%) was observed in the sites CC, MBG, and PM. Strains of *E. coli* 1 were noticed only in the sites MD (40%) and MBT (60%). The results obtained in the present study corroborate with those mentioned by Balcha et al. [43] who found, on fresh beef sold in Mekelle, Quiha, and Wukro markets of Ethiopia, strains

TABLE 2: Genus, species, identification percentages, and proportions of strains isolated from fresh beef samples collected from different markets of the city of Ngaoundéré.

Genus	Species	ID percentages (%)	Number of strains	Numbers and proportions of strains per sampling sites					
				CC	MD	MBG	MBT	GM	PM
<i>Pseudomonas</i>	<i>Pseudomonas putida</i>	98.50	12	4 (33.33%)	2 (33.33%)	4 (16.66%)	0 (0%)	0 (0%)	2 (16.66%)
	<i>Pseudomonas aeruginosa</i>	99.80	13	2 (15.38%)	3 (23.07%)	5 (38.46%)	0 (0%)	1 (7.69%)	2 (15.38%)
	<i>Pseudomonas</i> sp.	97.40	26	6 (23.07%)	3 (11.53%)	3 (11.53%)	8 (30.76%)	2 (7.69%)	4 (15.38%)
<i>Escherichia</i>	<i>Escherichia coli</i>	99.60	14	4 (28.57%)	0 (0%)	4 (28.57%)	0 (0%)	2 (14.28%)	4 (28.57%)
	<i>Escherichia coli</i> 1	99.40	5	0 (0%)	2 (40.00%)	0 (0%)	3 (60.00%)	0 (0%)	0 (0%)
<i>Salmonella</i>	<i>S. enterica</i> ser. Enteritidis	98.60	9	0 (0%)	3 (33.33%)	3 (33.33%)	0 (0%)	0 (0%)	3 (33.33%)
	<i>Salmonella</i> sp.	99.10	15	2 (13.33%)	0 (0%)	4 (26.66%)	3 (20.00%)	2 (13.33%)	4 (26.66%)
<i>Staphylococcus</i>	<i>Staphylococcus aureus</i>	98.60	18	2 (11.11%)	4 (22.22%)	3 (16.66%)	5 (27.77%)	1 (5.55%)	3 (16.66%)
	<i>Staphylococcus xylosus</i>	100.00	12	0 (0%)	0 (0%)	8 (66.66%)	0 (0%)	0 (0%)	4 (33.33%)
	<i>Staphylococcus epidermis</i>	98.30	8	0 (0%)	0 (0%)	2 (25.00%)	4 (50.00%)	0 (0%)	2 (25.00%)
	<i>Candida albicans</i>	98.60	5	0 (0%)	0 (0%)	2 (40.00%)	1 (20.00%)	0 (0%)	2 (40.00%)
<i>Candida</i>	<i>Candida</i> sp.	98.30	14	3 (21.42%)	2 (14.28%)	1 (7.14%)	3 (21.42%)	4 (28.57%)	1 (7.14%)
Total			151	23 (15.23%)	19 (12.58%)	39 (25.82%)	27 (17.88%)	12 (7.94%)	31 (20.52%)

ID = identification percentages of the strains from API data; ser. = serovar; PM = "Petit Marché"; GM = "Grand Marché"; MD = "Dang market"; CC = "Centre Commercial"; MBG = "Bamnyanga market"; MBT = "Bantail market."

of *E. coli* at proportions ranging from a market to another. They also highlighted that some strains of *E. coli* isolated from fresh beef were identified as Enterohemorrhagic *E. coli* (*E. coli* O157: H7). Giving that some strains of *E. coli* such as Enterohemorrhagic *E. coli* (EHEC) are recognized as food-borne pathogens associated with several outbreaks of diseases [44], its presence in fresh beef as observed in this study is worrisome. Molecular identification of the strains isolated is required in order to bring more information about their pathogenicity.

The main reservoir of zoonotic *Salmonella* is food from animal origin, and the main sources of infections incriminated *Salmonella* in industrialized and developing countries are animal-derived products, notably fresh meat products [45]. In this study, 24 strains of *Salmonella* were isolated among which there were 37.50% of *S. enterica* serovar Enteritidis and 62.50% of *Salmonella* sp. (Table 2). Regarding *S. enterica* serovar Enteritidis, it was found only in sites MD, MBG, and PM at the proportion of 33.33%. *Salmonella* sp. was absent in the site MD and present in the other sites. Sites MBG and PM rank first with 26.66% each. Ukut et al. [14] also identified *Salmonella* spp. in fresh beef sold in Calabar market, Nigeria. In another study conducted by Obeng et al. [39], *Salmonella* spp. were isolated and identified in fresh meat sold in Tolon and Kumbungu districts of the Northern Region of Ghana.

For yeasts belonging to the genus *Candida*, Table 2 shows that 19 strains were isolated and identified as the species *C. albicans* (5 strains) and *Candida* sp. (14 strains). Strains of *C. albicans* were found only in sites MBG, MBT, and PM at proportions of 40, 20, and 40%, respectively. Strains of *Candida* sp. were identified in fresh beef samples collected from the different sites and the site GM ranks first with 28.57% of strains. The results of this study are in accordance with those reported by Comi and Cantoni [46] who showed that strains of *Candida* spp. are dominant yeasts of fresh beef.

In order to visualize the association between the microbial species identified in this study and the sampling sites, a principal component analysis was carried out. Figure 1 presents the distribution of microbial species isolated from fresh beef and sampling sites on the axis system F1 × F2. As seen in Figure 1, three main groups were formed. The first group shows that the strains belonging to the species *C. albicans*, *Salmonella* sp., *P. aeruginosa*, *P. putida*, *S. xylosus*, and *S. enteritidis* are associated with the sites PM and MBG. The second group shows that the strains belonging to the species *Pseudomonas* sp., *E. coli* 1, *S. epidermis*, and *S. aureus* are associated with site MBT. Finally, the third group shows that the strains belonging to the species *Candida* sp. are mainly associated with the sites MD, CC, and GM. This principal component analysis pointed out the specific relationship

FIGURE 1: Distribution of microbial species isolated from fresh beef and sampling sites on F1 $\times$ F2 axis. PM = "Petit Marché"; GM = "Grand Marché"; MD = "Dang market"; CC = "Centre Commercial"; MBG = "Bamnyanga market"; MBT = "Bantail market."

between the microbial diversity of fresh beef and the sampling sites. It enables to observe associations between the sampling sites and the microbial profile of fresh beef sold in Ngaoundéré. The use of 16S rRNA gene sequencing and a high number of meat samples will improve this PCA representation through an increase of the microbial diversity.

3.3. Antiadhesive Activity of Biosurfactant. Food spoilage and disease transmission due to microorganisms attached into surfaces are one of the main problems faced by food industries, particularly in the meat industry. In this study, the antiadhesive property of biosurfactant was tested against some selected bacterial and fungal strains isolated from fresh beef and against some reference cultures. The biosurfactants derived from *L. paracasei* subsp. *tolerans* N2 inhibited the adhesion of all the tested strains in a dose-dependent manner (Table 3). Biosurfactant was active even at 0.01 against all the tested microorganisms. At that concentration, 33.96 ± 2.36 and $27.70 \pm 0.75\%$ of inhibition were recorded against *S. aureus* MTCC 1430 and *S. aureus* STP1. Complete inhibition (100%) of microbial adhesion was observed against *Bacillus* sp. BC1, *S. aureus* STP1, and *S. xyloso* STP2 at biosurfactant concentration of 10 mg/mL. The least antiadhesive activity ($56.12 \pm 0.16\%$) of biosurfactant was noticed against the yeast strain *C. albicans* LV1. This antiadhesive activity of biosurfactants observed in this study could be due to their ability to change the hydrophobicity of the interface between the strain and the surface of the polystyrene plate. Hence, microbial adhesion to the surface will decrease as biosurfactant concentration increases. Sharma and Saharan [21] have explained the antiadhesive activity of biosurfactants of *L. helveticus* by their ability to form a thin film which modifies the wettability of the surface and thus decreases the adhesive properties of microorganisms. The inhibition percentages observed in Table 3 are not different from the values reported by Merghni et al. [22] with

biosurfactants from *L. casei* ATCC 393 LZ9 and *L. casei* ATCC 393 LBL. They noticed inhibition percentages ranging from 68.84 to 84.86% against *S. aureus* 6538, *S. aureus* 9P, and *S. aureus* 29P at biosurfactant concentration of 12.5 mg/mL. As shown in Table 3, Gram-negative bacteria were more resistant to the antiadhesive activity of biosurfactant. A similar observation was noticed by Sambanthamoorthy et al. [47] with biosurfactants from *L. jensenii* and *L. rhamnosus*. This resistance of Gram-negative bacteria could result in their ability to produce, once adhered to surfaces, homoserine lactone acyl (HLA) molecules which increase their resistance to antiadhesive compounds [48].

Besides, the adhesive properties of microorganisms isolated from fresh beef samples as shown in this study suggest that cutting meat surfaces represent an ecological kennel of continuous contamination of meat. Hence, the antiadhesive activity of biosurfactant from *L. paracasei* subsp. *tolerans* N2 against these strains shows that these molecules could be useful in the reduction of meat contamination through cutting surfaces.

4. Conclusion

This study showed that fresh beef sold in the markets of Ngaoundéré contained both pathogenic and spoilage microorganisms at levels higher than the threshold values recommended by the European Commission. Twelve microbial species colonized these fresh beef: *P. putida*, *P. aeruginosa*, *Pseudomonas* sp., *E. coli* 1, *E. coli*, *S. enteritidis*, *Salmonella* sp., *S. epidermidis*, *S. xyloso*, *S. aureus*, *C. albicans*, and *Candida* sp. with *Pseudomonas* sp. as the most abundant strains. The presence of *Salmonella* in all samples makes no doubt on the safety status of the meat. In the same way, the presence of microorganisms like *P. putida* and *P. aeruginosa* which can lead to a quick spoilage of fresh beef suggests a reduced shelf life of the product. However, identification of these strains through 16S rRNA gene analyses needs to be done to confirm their identity. The results

TABLE 3: Microbial inhibition percentages (%) obtained from the antiadhesive assays with the biosurfactant from *L. paracasei* subsp. tolerans N2.

Test organisms	Biosurfactant concentration (mg/mL)									
	0.01	0.1	0.5	1	2.5	5	7.5	10		
<i>S. aureus</i> MTCC 1430	33.96 ± 2.36 ^{aG}	49.15 ± 2.48 ^{bl}	69.03 ± 0.06 ^{cH}	76.26 ± 0.38 ^{dl}	82.51 ± 1.43 ^{eG}	92.09 ± 0.23 ^{fG}	97.34 ± 1.09 ^{gH}	99.18 ± 0.10 ^{hl}		
<i>M. luteus</i> MTCC 106	22.59 ± 2.59 ^{aD}	42.38 ± 2.18 ^{bH}	65.73 ± 1.47 ^{cG}	78.90 ± 3.33 ^{dl}	87.17 ± 3.08 ^{eH}	92.63 ± 0.41 ^{fG}	94.11 ± 0.51 ^{gF}	95.59 ± 0.71 ^{hG}		
<i>L. monocytogenes</i> MTCC 839	20.51 ± 1.19 ^{aD}	32.41 ± 0.09 ^{bE}	41.50 ± 0.43 ^{cE}	50.94 ± 0.19 ^{dF}	58.29 ± 1.48 ^{eF}	62.22 ± 0.24 ^{fE}	68.40 ± 1.55 ^{gE}	75.15 ± 0.53 ^{hF}		
<i>E. coli</i> MTCC 118	19.95 ± 0.32 ^{aD}	30.24 ± 0.69 ^{bD}	38.77 ± 2.40 ^{cD}	48.62 ± 1.94 ^{dE}	54.65 ± 1.46 ^{eD}	60.08 ± 0.16 ^{dD}	65.78 ± 0.48 ^{dD}	72.14 ± 0.33 ^{hE}		
<i>P. aeruginosa</i> PSB2	15.46 ± 0.75 ^{aC}	21.64 ± 2.25 ^{bb}	31.30 ± 1.00 ^{cB}	40.72 ± 0.10 ^{dB}	47.90 ± 0.95 ^{eB}	51.70 ± 0.55 ^{fB}	56.80 ± 0.15 ^{gB}	61.70 ± 0.55 ^{hB}		
<i>P. putida</i> PSJ1	13.10 ± 0.10 ^{aB}	23.15 ± 1.81 ^{bb}	30.71 ± 1.52 ^{cB}	40.30 ± 0.40 ^{dB}	46.95 ± 1.50 ^{eB}	52.71 ± 0.52 ^{fB}	57.22 ± 0.82 ^{gB}	60.71 ± 0.50 ^{hB}		
<i>S. enteritidis</i> SL2	14.45 ± 0.65 ^{aC}	26.80 ± 1.10 ^{bC}	34.73 ± 0.14 ^{cC}	42.10 ± 0.20 ^{dC}	50.40 ± 0.90 ^{eC}	58.21 ± 0.05 ^{fC}	61.20 ± 0.10 ^{gC}	66.10 ± 0.50 ^{hC}		
<i>E. coli</i> E2B	19.32 ± 0.90 ^{aD}	27.18 ± 0.45 ^{bC}	36.10 ± 0.40 ^{cD}	45.90 ± 0.15 ^{dD}	53.50 ± 0.35 ^{eE}	59.10 ± 0.75 ^{fD}	64.15 ± 1.50 ^{gD}	70.10 ± 0.45 ^{hD}		
<i>C. albicans</i> LV1	5.78 ± 0.35 ^{aA}	12.19 ± 2.30 ^{bA}	24.80 ± 2.11 ^{cA}	36.78 ± 1.91 ^{dA}	43.10 ± 0.66 ^{eA}	47.90 ± 0.25 ^{fA}	53.62 ± 0.31 ^{gA}	56.12 ± 0.16 ^{hA}		
<i>Bacillus</i> sp. BC1	24.25 ± 0.90 ^{aE}	41.82 ± 1.14 ^{bH}	67.70 ± 0.67 ^{cG}	70.30 ± 0.50 ^{dG}	81.90 ± 0.22 ^{eG}	90.62 ± 0.20 ^{fH}	98.78 ± 0.50 ^{gH}	100.00 ± 0.00 ^{hJ}		
<i>S. aureus</i> STP1	27.70 ± 0.75 ^{aF}	40.01 ± 0.23 ^{bH}	66.17 ± 1.80 ^{cG}	73.38 ± 0.70 ^{dH}	82.21 ± 0.82 ^{eG}	93.30 ± 0.75 ^{fI}	99.25 ± 0.82 ^{gH}	100.00 ± 0.00 ^{hJ}		
<i>S. xyloso</i> STP2	21.32 ± 2.10 ^{aD}	38.41 ± 0.25 ^{bG}	65.70 ± 1.50 ^{cG}	73.13 ± 0.70 ^{dH}	81.92 ± 0.31 ^{eG}	92.40 ± 0.21 ^{fI}	98.16 ± 1.10 ^{gH}	100.00 ± 0.00 ^{hJ}		
<i>S. epidermis</i> STP3	21.80 ± 2.29 ^{aD}	37.11 ± 0.31 ^{bF}	60.20 ± 1.66 ^{cF}	71.76 ± 0.50 ^{dG}	80.68 ± 1.79 ^{eG}	88.18 ± 0.13 ^{fF}	95.94 ± 0.05 ^{gG}	98.70 ± 0.050 ^{hH}		

Negative controls were set at 0% to indicate the absence of biosurfactant. Positive percentages indicate the reductions in microbial adhesion when compared to the control. Results are expressed as means ± standard deviation of results from triplicate experiments.

of this study demonstrated the antiadhesive activity of biosurfactant against microbial strains isolated from fresh beef. Efforts made by the Government to improve the microbiological quality of fresh beef should deal not only with the respect of good slaughtering, evisceration, and hygienic practices but also with the cleaning process of surfaces where meat is cut and sold.

Data Availability

The data used in this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare no conflicts of interest.

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