

Biodiversity, phylogenetic relationship and antibacterial potential of *Bifidobacterium* species isolated from raw milk production chain in Abidjan, Côte d'Ivoire

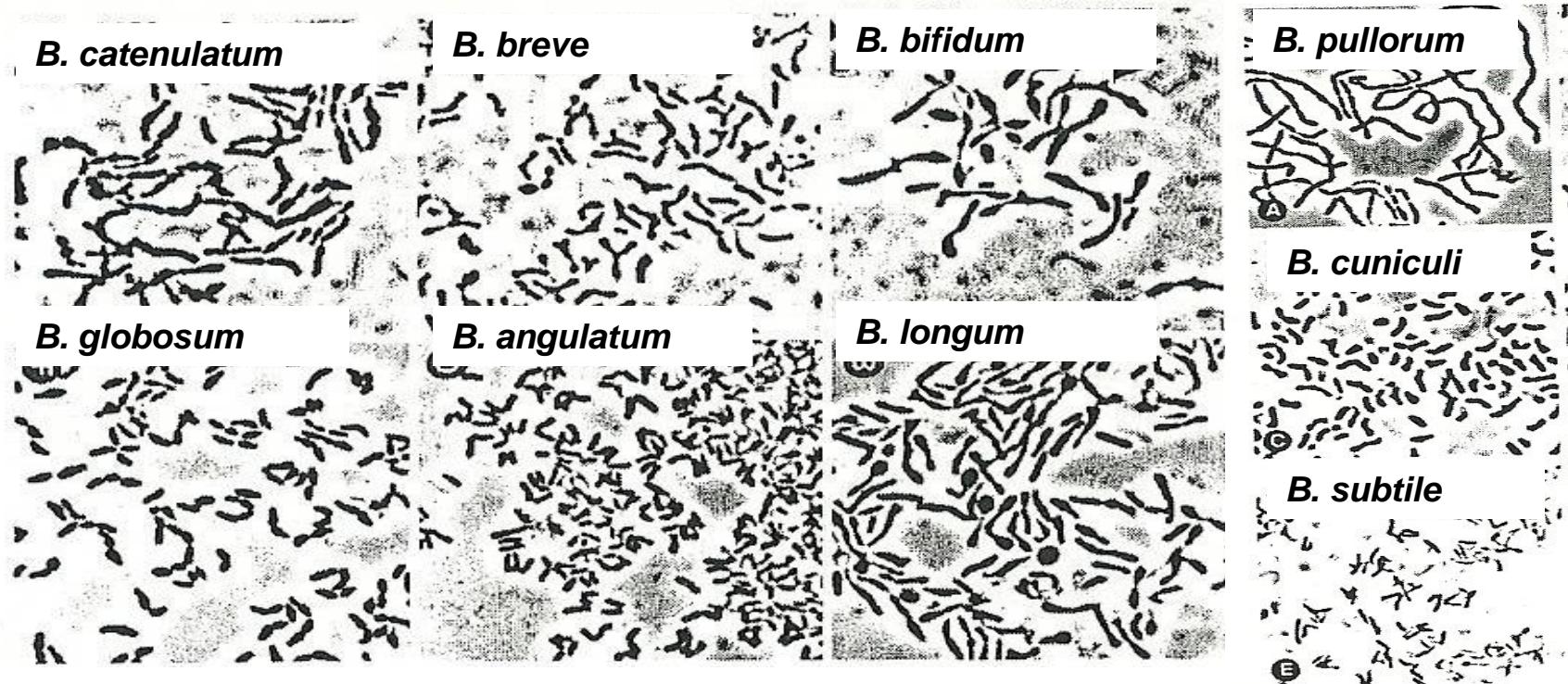
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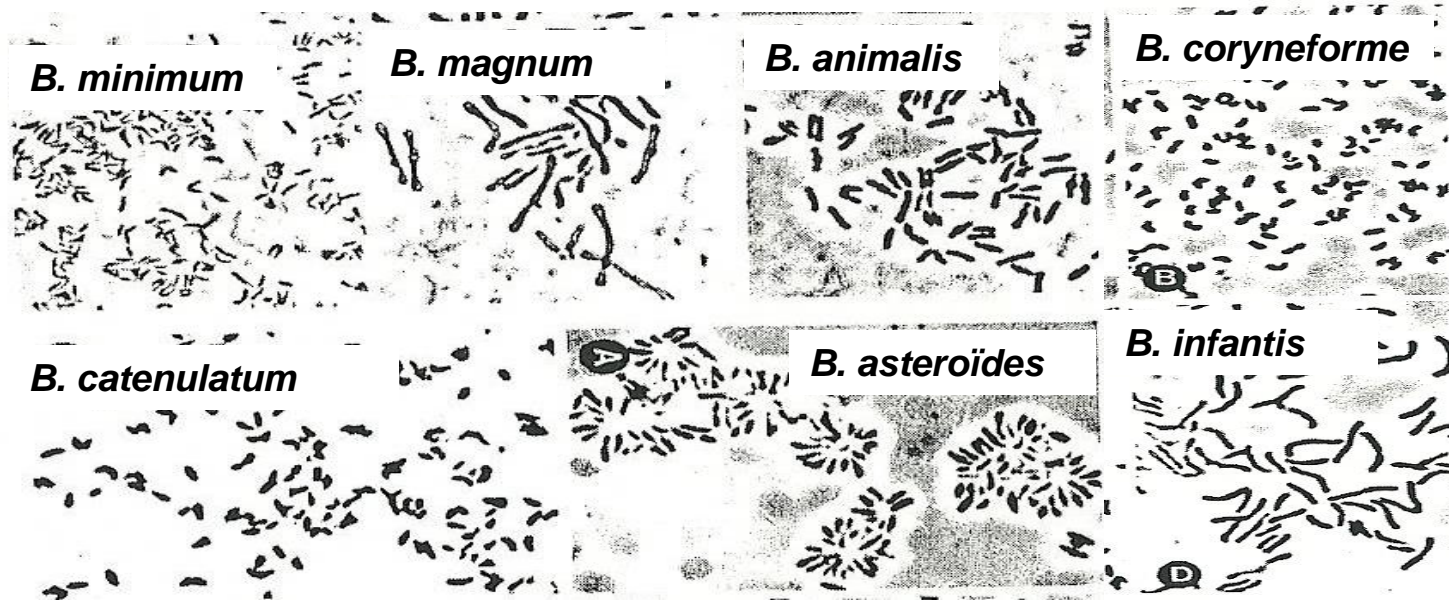
Introduction

The genus *Bifidobacterium* consists:

- **Gram+ anaerobes** with a variety of rod morphologies
- most prevalent microflora in the GIT of humans and animals (Scardovi, 1984).
- includes 34 species.



Introduction



Probiotic abilities → human and animals good health:

- Prevent constipation
- Improve lactose tolerance
- Reduce risk of colon cancer
- Reduce blood cholesterol
- Improve immune response
- Involved in resorption of intestinal infections (by organic acids and bacteriocin production: **bifidin; bifidocine B**)
- Restore intestinal microflora



Introduction

- Bifidobacteria are normal intestinal flora in humans and animals. Their properties => bifidobacteria are used as **fecal contamination indicators**.

- By their intestinal ecology, milk is not the natural environment of bifidobacteria.

Their presence in milk is a sign of fecal contamination <==> a **marker of level of hygiene** in dairy production chain (Beerens et al., 2000).

Introduction

The local dairy industry, from farm to retail point is informal and often escapes to monitoring of quality.

Most stakeholders lack knowledge on sanitary aspects of their production, which can result in

- > poor production standards
- > contamination of local raw milk by pathogens
- > causing diseases and food poisoning in consumers..

Introduction

Data relating on safety management of dairy production chain are scarce in Côte d'Ivoire.

Furthermore, no study has been conducted on species of *Bifidobacterium* contaminating milk and their antibacterial abilities using sequence analysis of hsp60.

The objective of this study was to determine the diversity and phylogenetic relationship of species *Bifidobacterium* present in the milk production chain in Abidjan and assess their potential to inhibit pathogenic bacteria.

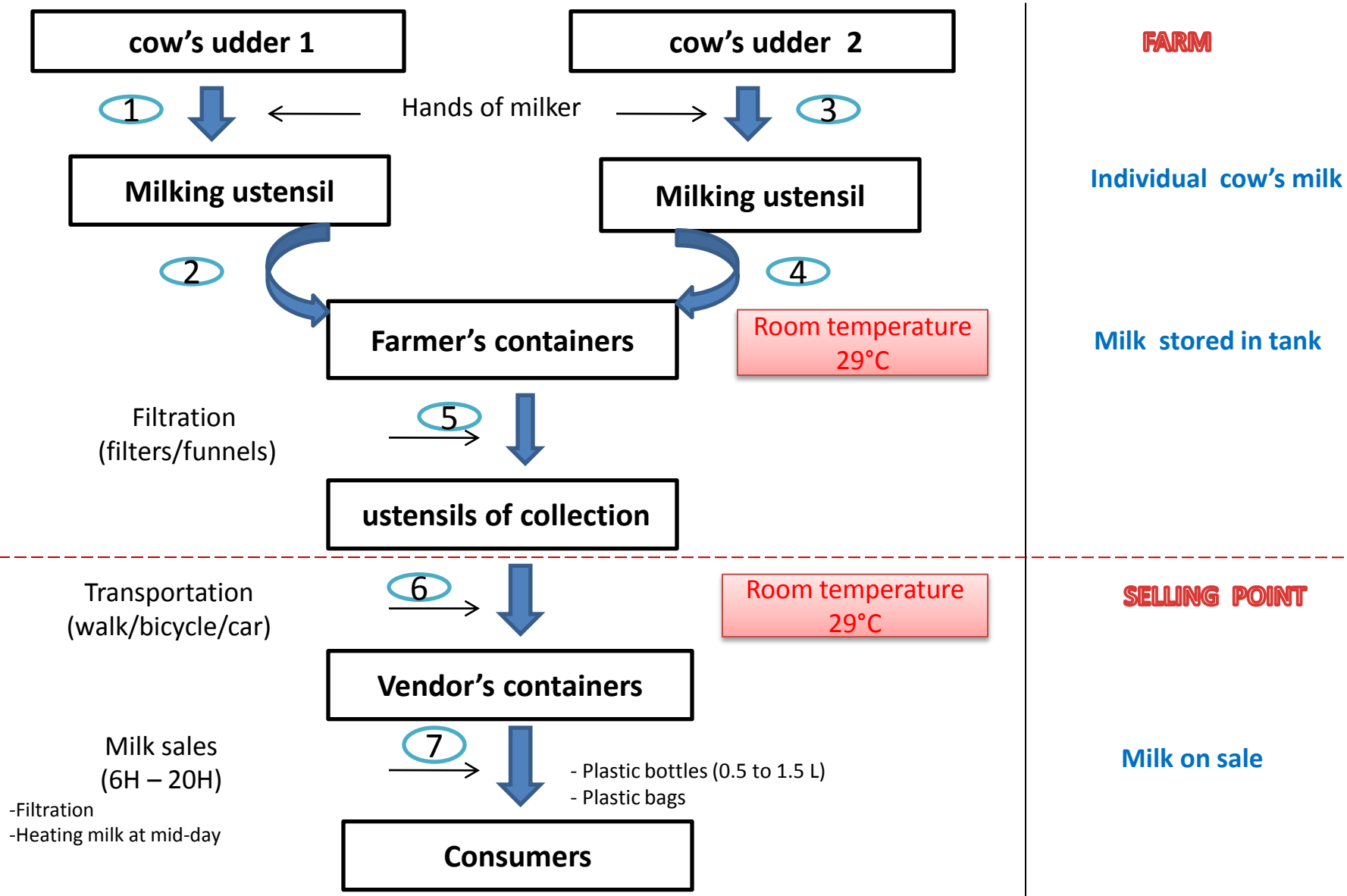


Figure 1: Local dairy production chain diagramm

Material and methods

Data collection was conducted from October to December 2008 in three sites of Abidjan: Port-Bouet, Lièvre rouge and Abobo.

A total of 189 samples were collected during milking in small dairy farms and retail point:

Samples	
Swabs of cow's udder	65
swabs of milkers's hands	14
milk taken from cow's udder (individual cow's milk)	74
raw milk stored in tank (milk from the farmer's containers)	6
raw milk on sale (vendor's containers)	30

Material and methods

❖ Bacterial isolation

Samples were analysed by Beerens (1998) culture based methods modified by Delcenserie et *al.* (2005).

❖ Molecular analysis

-to identify the genus *Bifidobacterium*:

-> the chromosomal DNA extraction,

-> DNA amplification (hsp60 gene, 16sDNA gene)

-> sequencing of specific amplified fragments (217 bp, 1050 bp)

-The resulting sequences were analyzed using BLAST (National Center for Biotechnology Information) to identified Bifidobacteria species

-**Tree** was also drawn using the ClustalX program and was visualized with the TreeView program.

Material and methods

❖ Evaluation of antibacterial activity of bifidobacteria

Screening ability to inhibit pathogens :

- spot test (Bernet et *al.*,1993),
- agar diffusion method (Tagg et *al.*,1976).

Table 1: Pathogenic microorganisms tested

Espèces	LMDA n°*
<i>Listeria monocytogenes</i>	S0154
<i>Listeria monocytogenes</i>	S0580
<i>Staphylococcus aureus</i>	S0155
<i>Staphylococcus aureus</i>	S0156
<i>Salmonella typhimurim</i>	S0157
<i>Salmonella Hadar</i>	S0066
<i>Escherichia coli</i> O157 :H7	S0231
<i>Escherichia coli</i> O26	S0347



Results

❖ Distribution of *Bifidobacterium* species

17(8,9%) samples were positive for *Bifidobacterium* spp.;

(5) five different species have been identified:

Strains	Effective	Cow's udders	Hand of milker	Milk taken from cow's udder	Milk on sale
<i>B. minimum</i>	9	3	2	3	1
<i>B. pseudolongum</i>	5	5			
<i>B. thermophilum</i>	1				1
<i>B. thermacidophilum</i>	1	1			
<i>B. magnum</i>	1			1	
Total	17	9	2	4	2

❖ Origin of milk contamination

Type of samples	Total of samples (n)	<i>Bifidobacterium</i> positives	Prevalence of <i>Bifidobacterium</i> % [95% CI]*
Hand of milker	14	2	14.3% [2.5% - 43.8%]
Cow's udder	65	9	13.8% [6.9% - 25.2%]
Raw milk taken from cow's udder	74	4	5.4% [1.7% - 13.9%]
Raw milk stored in tank	6	0	0.0% [0.0% - 48.3%]
Milk on sale	30	2	6.7% [1.2% - 23.5%]
Total	189	17	8.9% [5.5% - 14.2%]

The Generalized Linear Model (GLM) with binomial errors indicated no significant difference in the prevalence of *Bifidobacterium* ($P = 0.3$) by type of samples.

❖ Sequence similarity in partial hsp60 genes

For all 17 different bifidobacteria species, the partial hsp60 DNA sequences were conserved, with a similarity ranging from **89% to 100%** (mean 97.24%).

Interspecies and intraspecies	Hsp60 sequence similarity rate	Means
Species of <i>B. minimum</i>	95 - 99%	97.56%
Species of <i>B. pseudolongum</i>	95 - 100%	98.2%
<i>B. thermophilum</i>	98%	
<i>B. thermacidophilum</i>	97%	
<i>B. magnum</i>	89%	

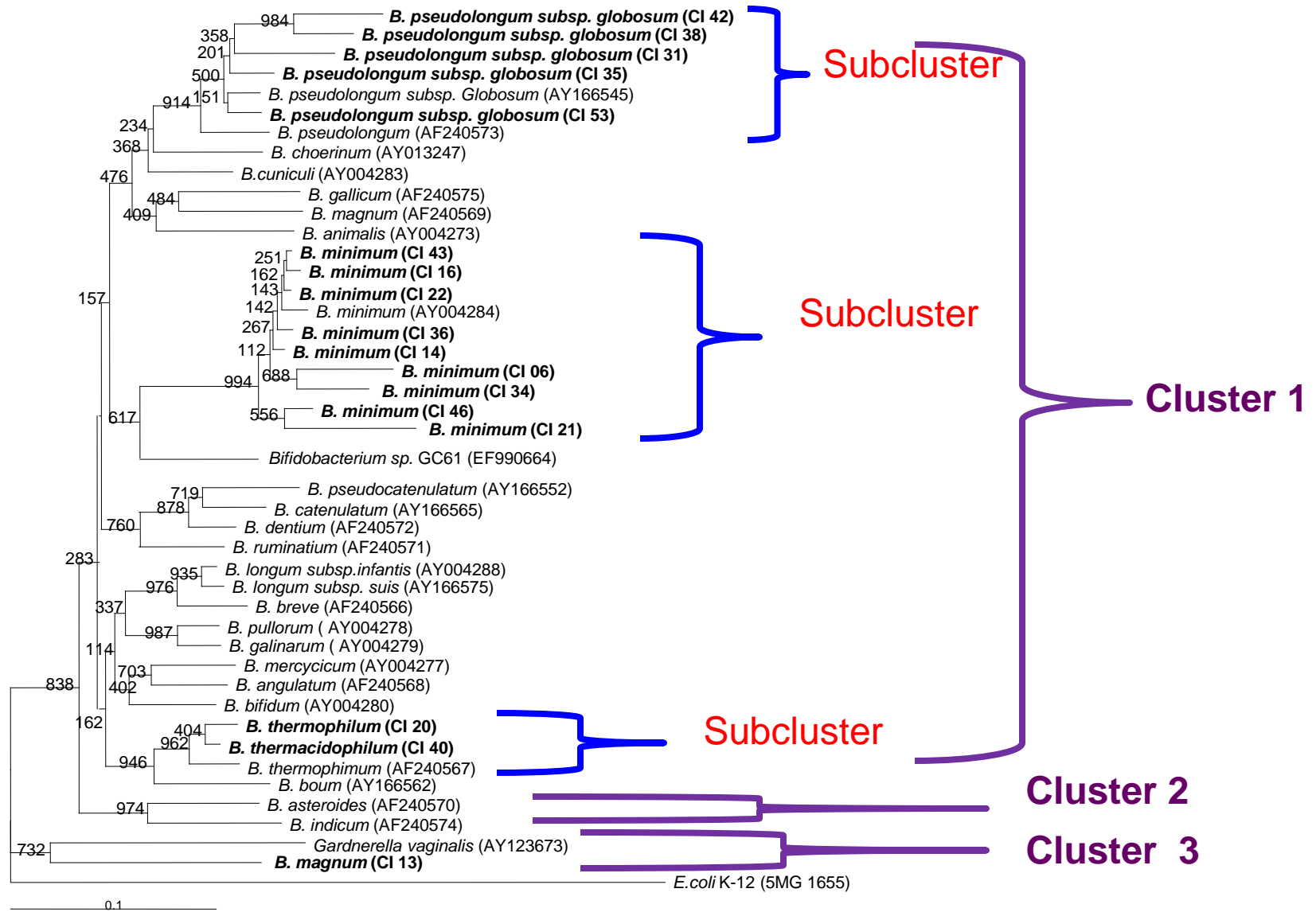


Fig. 1. Phylogenetic tree based on partial hsp 60 gene sequences. The tree was rooted with *Escherichia coli* and constructed using a neighbor-joining algorithm. Bootstrap values of 1000 data sets, are given at each node. Numbers in parentheses correspond to the GenBank accession numbers. Strains of Côte d'Ivoire are written in bold. Bar. 0.1 sequence divergence.

❖ Pathogens inhibition of bifidobacteria isolated

Bifidobacteria of Côte d'Ivoire	pH of the culture supernatant in MRS	Diameter of inhibition (mm)							
		<i>Salmonella</i>		<i>Staphylococcus</i>		<i>Escherichia</i>		<i>Listeria</i>	
		<i>hadar</i> (S066)	<i>typhimurim</i> (S0157)	<i>aureus</i> (S0156)	<i>aureus</i> (S0155)	<i>coli</i> O26 (S0347)	<i>coli</i> O157:H7 (S0231)	<i>monocytogenes</i> (S0580)	<i>monocytogenes</i> (S0154)
CI* 6	3.75	35	27	28	30	22	12	20	23
CI 16	3.76	23	28	23	28	20	30	20	23
CI 22	3.76	30	30	24	24	22	24	33	25
CI 21	3.77	21	26	22	25	22	30	20	29
CI 40	3.77	17	22	18	32	20	26	21	23
CI 42	3.77	22	27	16	32	20	22	28	23
CI 14	3.81	20	20	22	30	22	28	28	23
CI 34	3.86	21	26	19	28	21	23	25	20
CI 13	4.09	25	30	22	31	26	25	20	22
CI 31	4.11	22	20	14	30	29	12	20	26
CI 36	4.33	16	8	8	19	16	9	20	18
CI 46	4.42	0	0	0	0	0	0	0	0
CI 43	4.43	0	0	0	0	0	0	0	0
CI 35	4.55	0	0	0	0	0	0	0	0
CI 20	4.64	0	0	0	0	0	0	0	0
CI 38	4.78	0	0	0	0	0	0	0	0
CI 53	4.9	0	0	0	0	0	0	0	0

Evaluation of antibacterial activity of bifidobacteria was shown that 11 out of 17 bifidobacteria strains (64.7%) showed an inhibition zone ranging from 8 to 35 mm



Conclusion

This is the first time a study is conducted on bifidobacteria in Côte d'Ivoire.

The hygienic quality of local raw milk can be assessed from the detection of the bifidobacteria in raw milk.

Bifidobacterium species isolated in this study did not produce bacteriocin to inhibit growth of pathogenic microorganisms.

The raw milk fermentation with Bifidobacteria should inhibit growth of contamination germs to improve microbiological quality of local milk.

Only lactic acid bacteria cannot solve problem of hygienic quality of local milk.

To make effective any action at this level, we need a quality policy with good hygiene practices related to cleanliness of animals and their environment and sanitation of milking (milking, milking utensils).

- ❖ Centre Suisse de Recherches Scientifiques en Côte d'Ivoire, CSRS
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- ❖ IFS
- ❖ AUF
- ❖ Thank you to livestock producers and vendors of milk

