International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page:www.iscientific.org/Journal.html

Isolation and Characterization of Probiotics (Lactococcus and Enterococcus) from Processed Straw

Y. CHBAB[1-2], S. EL GHAZA[2], B. OUAKHZAN[2], B. ZAADOUD[2], E. AOUANE[2]

[1] *Higher Institute of Nursing Professions and Health Techniques, Ministry of Health and Social Protection, Beni Mellal, Morocco.*

[2]*Laboratory of Natural Resources and Sustainable Development, Faculty of Science, Ibn Tofail University, Kenitra, Morocco.*

Abstract

Probiotics are microorganisms that can be ingested in food as a fundamental element or as food supplements. They bring an added value exerting a beneficial effect on the host.The main objective of this study is to present probiotic strains isolated and selected from straw. Isolated strains can be recommended for inclusion in food and feed to improve food quality and safety, which can help experts and professionals to recommend and decide on the use of probiotics as elements for improving the quality of food and as agents for the prevention of several diseases and infections.The six strains of lactic acid bacteria selected are of the genera "Lactococcus and Enterococcus", they possess a very significant acidifying power, a high acidity level, and a maximum abundance which qualifies them to be used as a supplement to be added to food formulas. Yeast strains isolated from the same substrate have important characteristics in terms of pH, acidity, and biomass. The use of strains of lactic acid bacteria and yeasts, as a probiotic supplement to food, promotes the enrichment of these foods with beneficial and useful nutrients for health.The use of selected and characterized strains in our study can inhibit the growth of pathogenic bacteria and other undesirable microorganisms present in food. They can also contribute to maintaining the balance of beneficial bacterial flora, which can reduce the risk of bacterial contamination and the incidence of foodborne diseases. By preventing the proliferation of pathogenic bacteria, probiotics can help prevent foodborne illnesses and food poisoning.Additionally, they can improve digestion by producing enzymes that aid in nutrient breakdown. This benefit applies to both humans and animals.However, it is crucial to emphasize that the use of probiotics in human and animal food should be based on robust scientific evidence, considering specific probiotic strains, appropriate dosages, and proper storageconditions to maintain their viability and effectiveness.

Keywords: Food, Lactic acid, Bacteria, Probiotic, straw, yeast, quality, safety

Full-length article **Corresponding author's* e-mail:younesschbab@gmail.com

1. Introduction

Lactic acid bacteria and yeasts which have biotechnological interest have been used for years as elements promoting the fermentation process in the food industry [1]. The spontaneous fermentation of milk has allowed the development of several strains of yeast belonging to six species. The evaluation of certain prerequisites for the evaluation of the probiotic potential of the microorganisms indicated a strain of K. marxianus and a strain of Lc. lactis as the most efficient microorganisms. Their combination allowed the production of fermented milk characterized by technological characteristics in the range of those reported for this category of products until the end of storage [2].

The use of these probiotics in human and animal nutritionis undergoing a great evolution in the field of research. This observation is made and demonstrated by several studies and results. Researchers had an interest and a positive contribution to the quality and safety of food and human health [3, 4].

The controlled use of lactic acid bacteria and yeasts in biotransformation processes or the medical field requires results and conclusions confirmed and validated by competent authorities, in particular the FAO and the WHO [5].This validation aims to highlight their metabolic processes, their interactions with other microorganisms as well as their physicochemical and biochemical, biotechnological properties.

In addition, this study highlights strains of lactic acid bacteria and yeasts isolated and selected from straw alone and treated straw.The isolation and selection of microorganisms from their natural environments make it possible to obtain pure strains intended for biotechnological purposes.Six strains of lactic acid bacteria and six strains of yeast which are isolated and selected can be used in particular in the food industry because they have a very important role in several processes, such as the treatment of raw materials, the manufacture of food additives and in other processes [2].The characterization results of the selected strains demonstrated better performance will improve food quality and safety.

The acidifying power, the acidity rate, and the high abundance rate are quality criteria for integrating these strains into the food industry. Several strains of lactic acid bacteria and yeasts are used as probiotics, which confer beneficial effects on human health.This research will allow microbiologists and industrialists to choose the best strains and improve the productivity, quality, and safety of the final products, including the improvement of industrial lactic acid bacteria strains without the use of recombinant DNA technology[6]. The term probiotic is defined by the FAO and WHO as "live microorganisms administered in adequate amounts that are beneficial to the health of the host". These micro-organisms, when they exist in sufficient quantity in food, they have positive effects on human and animal health.Probiotics are made up of lactic acid bacteria and yeasts. The genera Lactobacillus and Bifidobacterium are exceptional for the group of lactic acid bacteria, while for yeasts, the species Saccharomyces boulardii and cerevisiae. These microorganisms meet quality and safety criteria. Lactococcus, Enterococcus, and Bacillus species are also used.

Many probiotic strains have been isolated from the human intestine, recommended by the FAO/WHO for human use. Others are isolated only from human faeces and less frequently from the human stomach, **[**7].The main food sources of probiotics are yogurts and fermented milk. These foods contain high levels oflactic acid bacteria and yeasts. They provide a very favorable pH, favoring the survival of probiotic bacteria. Numerous studies have shown that probiotic microorganisms are also found in non-dairy fermented substrates such as cereals, legumes, cabbage, and vegetables and processed and fermented straw feed [8].In livestock farming, probiotic microorganisms are used as biological agents for the preservation of fodder by acidifying fermentation. Their use in silage limits or inhibits certain undesirable metabolic pathways and improves the nutritional quality of forage [9]. Thus, a 5 to 11% increase in zootechnical performance such as digestibility has been observed in livestock[10]. Weight gain can reach a percentage of 42%.

The 100,000 billion probiotic bacteria, which mainly colonize the small intestine and the colon, participate in digestive, but also metabolic, neurological, and immune functions [11]. New-generation probiotics have better-defined properties and clinical indications[12].The specific acids and quantities produced by lactic acid bacteria in fermented products have major consequences on the properties of the final product. Acid production by LABs is therefore a desirable property to modulate and control [6].These micro-organisms consist of bacteria and/or yeasts which must meet safety criteria such as the history of non-pathogenicity of these micro-organisms and the absence of risk of transmission of resistance genes to antibiotics, probiotics, on the functional level, must demonstrate immunostimulatory capacities and produce antimicrobial substances against pathogens.

First, they have a role in strengthening the intestinal barrier, for example by increasing the production of mucus or by competing on adhesion sites with certain pathogens[13]. They have an antimicrobial effect through the production of molecules, bacteriocinscapable of inducing the death of certain Gram-positive bacteria and through their ability to lower the intestinal pH, thus inhibiting the development of certain Gram-negative bacteria. Finally, they have an immunomodulatory capacity by stimulating innate immunity through the production of TH1-type cytokines, and adaptive immunity by the production of secretory IgA during an infectious episode[14]. Since probiotics are generally recognized as safe and can be eliminated with antimicrobial agents, their use should be considered in patients of all ages. and the reduction of side effects of antibiotics in the eradication of Helicobacter pylori. Since probiotics are generally recognized as safe and can be eliminated with antimicrobial agents, their use should be considered in patients of all ages [15].

2. Materials and Methods

2.1. Chopping of the straw

A straw bale is the elementary cubic unit of stock in the barn. Each unit is put into a crusher at 1500 revolutions per min. The ground material obtained is composed of pieces of fibers of about 5 to 10 mm. It is stored in plastic drums for further processing.

2.2. Lactic acid bacteria

2.2.1. Chopped and processed straw

The shredded straw obtained is made up of pieces of fibers of about 5 to 10 mm. The ground material is treated with different concentrations of urea. The one whose proportion of urea is 5% is added in another trial of molasses. The incorporations were carried out on samples with a humidity rate of 60% to 70% and subjected to room temperature. The duration of treatment was set at 30 days.

2.3. Treatments adopted

Test n°1: The proportions of ureas added to the straw are: 5%, 10%, 15%, and 20%.

Test n°2: On straw with 5% urea are added different proportions of molasses: 5%, 10%, 15%, and 20%.

Test n°2: control (straw alone).

2.4. Search for strains of lactic acid bacteria

2.4.1. Enumeration of lactic acid bacteria

The lactic acid bacteria are enumerated on solid MRS medium (Man, Rogosa, and Sharpe), inoculated deeply l ml of the 10^{-1} to 10^{-7} dilutions, and the dishes were incubated at a temperature of 30°C for 48 hours.

2.4.2. Isolation and purification

The isolation is carried out on the same medium, several colonies have been isolated, purified five times, and stored on a solid MRS medium at 4°C.

2.5. Characterization of the selected strains: pH and titratable acidity

The bacterial strains were cultured for three days on liquid MRS medium in flasks at 30 ± 2°C and in the dark. The initial and final pH is measured using an Orion Research-type pH meter with the combined electrode, previously calibrated. The titration of the acidity is carried out on 10 ml of culture with a 0,1 N sodium hydroxide solution using a Mohr burette with stopcock, in the presence of a drop of a methanolic solution of 1% phenolphthalein used as a color indicator. The acidity is expressed in mg of lactic acid (MW = 90,08 g) per 100 ml of culture.

2.6. Characterization of selected strains: Catalase test and Gram staining

The catalase test is used by emulsifying a bacterial culture in a drop of them oxygenated at 30 g/l placed on a slide. The positive reaction is reflected by the presence of a release of gas bubbles.The cultures are examined by the classic gram staining technique, the bacteria stained in purple are gram-positive while the others stained in pink are gram-negative.

2.7. Yeast strains

2.7.1. Isolation of yeast strains

A quantity of soft wheat straw crushed using a hammer mill was added with 5 kg of urea purchased from a dealer and 55 kg of well water. Only 10 kg of molasses was then added to the previous mixture. The medium is contained in plastic drums in ambient air at a temperature of 25°C. Every five days, samples were taken for the isolation of yeast strains on a PDA medium sterilized for 15 min at 121°C. The cultures are incubated for 24 hours at 30°C. in Petri dishes 90 mm in diameter containing 20 ml of medium. After five successive cycles of subculturing, the isolated yeasts are stored in test tubes on a PDA medium inclined at +4°C in the dark.

2.7.2. Characterization of strains

The selected yeast strains were monitored on a semi-synthetic medium in Petri dishes 90 mm in diameter containing 15 ml of medium. Sterilization takes place at 105°C for 15 min. The incubation is carried out for 24 hours at 30°C. It is sought to obtain at least one correct growth strain. Three parameters were monitored every 5 days for 30 days: pH, biomass, and acidity.The pH of the cultures is measured using an Orion Research-type pH meter previously calibrated at pH 7 and 4.The growth of the yeast strains is monitored by measuring the absorbance of the medium using a UV-2004 type spectrophotometer at 600 nm against the control (semi-synthetic medium).Since organic acids can retain the proton H+ at certain pHs, it is necessary to combine the monitoring of the pH with that of the acidity. Ten ml of the supernatant is transferred to a 100 ml beaker. A few drops of 1% phenolphthalein are added to it. The titration is carried out with a solution of NaOH N/9 until the colored indicator changes to pink. Acidity is expressed as a percentage of lactic acid (MW = 90,08 g) per 100 ml of culture.

2.7.3. Determination of pH and biomass of selected strains

The strains isolated from straw treated with urea and molasses were grown on a semi-syntactic medium with different concentrations of urea as nitrogen sources and incubated at a temperature of 30°C for 24 hours.The pH is measured using an Orien Research-type pH meter. The measurement values are taken after the calibration of the device. The standards used are pH 7 and 4.The growth of the yeast strains is monitored by measuring the optical density of the medium using a standard spectrophotometer (UV-2004 POWER: 110/220V-50/60Hz). The density reading is made at 600 nm against an uninoculated control (semi-synthetic medium).

3. Results and Discussions

3.1. Phenotypic characterization of lactic acid bacteria strains

3.1.1. Colony morphology

Lactic acid bacteria are living cells, prokaryotes, heterotrophs, and chemo-organotrophs. With few exceptions, lactic acid bacteria are generally Gram-positive, immobile, asporulated, anaerobic but aerotolerant, and lacking catalase (some strains possess pseudocatalase), nitrate reductase, andcytochrome oxidase. They have numerous nutritional requirements (amino acids, peptides, salts, fatty acids, and carbohydrates) [16].

All lactic acid bacteria have a strictly saccharolytic fermentative metabolism which, by using carbohydrates, they can produce either:

- lactic acid exclusively (strict homolactic bacteria),
- lactic acid and acetic acid (optional heterolactic bacteria),
- lactic acid, acetic acid, or ethanol and CO2: strict heterolactic bacteria [17].

Microscopic observation is necessary to define the shape of bacterial cells (cocci or bacilli).The Gram staining of the isolates after 72 h of culture on modified MRS makes it possible to ensure that we are indeed in the presence of the Gram-positive bacteria (violet staining).Lactic acid bacteria are cocci or rods [18], and they are generally aerotolerant. However, certain species inhabiting the digestive tract of animals, for example, are strict anaerobes; even in the presence of O2, they are incapable of carrying out oxidative phosphorylation. They are Gram-positive; usually immobile and asporulated [19].They do not possess catalase, nitrate reductase, or cytochrome oxidase.In addition, they do not liquefy gelatin or produce indole or hydrogen sulfide [19].These bacteria are spherical or ovoid cocci grouped in pairs or chains, generally immobile, from carbohydrates their metabolism is homofermentative, and they produce a certain number of antimicrobial agents [18, 20].

3.1.2. Characterization of isolated and selected strains

The taxonomy of lactic acid bacteria was based on Gram staining and it is possible to classify them according to the nature of the products of bacterial metabolism obtained from carbohydrates. In a similar study by Drouault[21], lactic acid bacteria are Gram-positive bacteria, and the catalase test was negative. These results are consistent with those found by Lairini [22].

3.1.3. Catalase test

Catalase is an iron-containing enzyme, it catalyzes the decomposition of hydrogen peroxide into water and oxygen. The detection test for this enzyme in a bacterial strain consists of adding hydrogen peroxide to the bacterial cells, the presence of catalase is marked by the formation of gas bubbles (oxygen).

3.1.4. Temperature values

The recorded temperature values vary between 20 and 30°C. This interval is perfectly suited to the temperature required by the majority of microorganisms, favoring maximum multiplication.

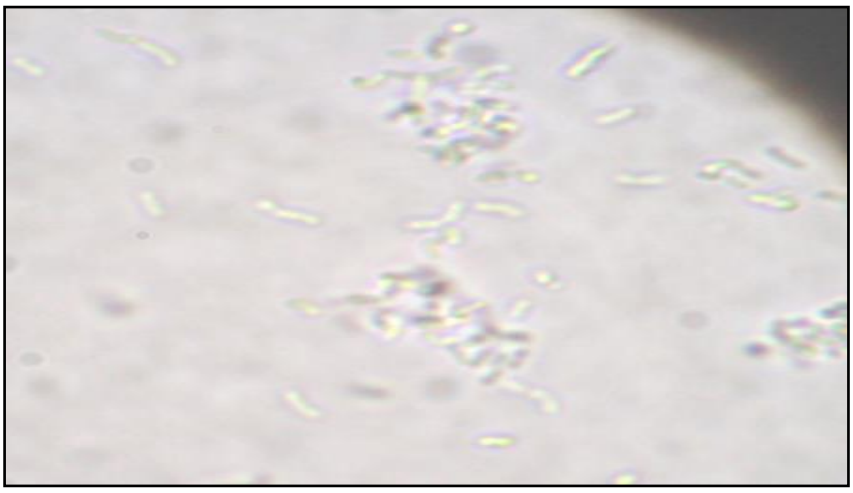

Figure 1: Microscopic observation of lactic acid bacteria isolated from straw

Table 1: Characterization of isolated lactic acid bacteria strains

Isolatedstrains	Gram Coloration	Catalase Test	T (°C)	Origin	pH_i	pH_f	Acidity mg of lacticacid/ 100 ml	DOi	DOf	Gender
BL_{ys8}	+	-	20 to 30	Witness	6,8	3,76	0,37	0,816	0,915	*Streptococcus*
BL_{ys9}	+	-	20 to 30	Trial 1	6,8	3,89	0,35	0,283	0,863	*Enterococcus*
BL_{ys10}	+	-	20 to 30	Trial 2	6,8	3,82	0,14	0,540	0,905	*Lactococcus*
BL_{ys12}	+	-	20 to 30	Trial 3	6,8	3,94	0,25	0,628	0,817	*Lactococcus*
BL_{ys13}	+	-	20 to30	Trial 4	6,8	3,97	0,21	0,700	0,850	*Lactococcus*
BL_{ys15}	+	-	20to 30	Trial 5	6,8	3,78	0,38	0,672	0,944	*Streptococcus*

- : Negative+: Positive

Table 2: Identification of isolated lactic acid bacteria

Isolatedstrains	Origins	Genrders
BL_{ys8}	Witness	*Streptococcus*
BL_{ys9}	Trial 1	*Enterococcus*
BL_{ys10}	Trial 2	*Lactococcus*
BL_{ys12}	Trial 3	*Lactococcus*
BL_{ys13}	Trial 4	*Lactococcus*
BL_{ys15}	Trial 5	*Streptococcus*

Table 3: Variation in pH and absorbance A of the culture media of six yeasts at 30°C and initial pH of 5,50

Strains	pH_{final}	$A_{initial}$	A_{final}	Acidity (mg/100 ml)
LVcy1	6,65	0,65	0,92	0,13
LVcy2	6,64	0,60	0,93	0,22
LVcy3	6,61	0,53	0,92	0,18
LVcy4	6,48	0,62	0,89	0,16
LVcy5	6,54	0,51	0,72	0,24
LVcy6	6,47	0,61	0,75	0,18

Table 4: Evolution of the pH and biomass of three yeast strains in the presence of five concentrations of urea on a semi-synthetic medium at 30° C. and initial pH of 5,50

Urea	Strain	Final PH	Initial Acidity	Final Acidity
1g/l	LVcy1	6,66	0,51	0,75
	LVcy2	6,66	0,39	0,73
	LVcy3	6,42	0,50	0,74
2g/l	LVcy1	6,84	0,58	0,99
	LVcy2	6,81	0,53	0,83
	LVcy3	6,77	0,52	0,78
3g/l	LVcy1	6,80	0,57	0,79
	LVcy2	6,71	0,36	0,75
	LVcy3	6,77	0,58	0,69
4g/l	LVcy1	7,09	0,57	0,98
	LVcy2	7,02	0,63	0,72
	LVcy3	6,93	0,78	0,72
5g/l	LVcy1	7,16	0,29	0,79
	LVcy2	7,31	0,49	0,72
	LVcy3	6,84	0,51	0,81

Table 5: Origin of yeast strains tested on Urea Tryptophan medium at 30°C and pH 5,50

Strain	Origin
LVyc1	Straw + urea (5%) + molasses (10%)
LVyc2	Straw + urea (5%) + molasses (15%)
LVyc3	Straw + urea (5%) + molasses (20%)
LVyc4	Strawalone
LVyc5	Strawalone
LVyc6	Strawalone

3.1.5.pH values and acidity

Monitoring of pH and acidity shows a gradual decrease in pH for all the strains isolated. The BLy8, BLy9, BLy10, and BLy15 strains prove to be the most efficient with final pH values of 3,76; 3,89; 3,82, and 3.78 and acidity levels of 0,35; 0,14; 0,25; 0,21; 0,38 of lactic acid per 100 ml of culture medium which indicates that strains of Lactococcuslactisssplactis show the highest acidifying activity compared to other species.

3.1.6. Growth biomass

The results relating to the growth biomass show an exponential evolution in the multiplication of isolated lactic acid bacteria with a final optical density that varied between 0,817 and 0,944. All the selected bacteria have a great capacity to multiply to have very good growth.

3.1.7. Identification of isolated lactic acid bacteria

After identification of the isolated strains, we selected three genera of Lactococcus, these are BLys10, BLys12, and BLys13. Two strains of the Streptococcus genera are the bacteria BLys8 and BLys15. A single bacterium genus Enterococcus: BLys9.The genera isolated and selected from treated straw, in particular, Lactococcus and Enterococcus, can be used as very useful probiotic strains in animal feed, providing added value in terms of food quality and safety.

3.2. Characterization of yeast strains

3.2.1. PH and Acidity values

During the physicochemical analyzes carried out on the isolated yeast strains, a slight increase in the final pH is observed for the cultures of the six yeasts (Table 3). The pH fluctuates between 6,47 and 6,65. The organic acid content varies between 0,13 and 0,24 mg/100.

3.2.2. Abundance of biomass

For the biomass, approximated by measuring the absorbance, two different evolutionary trends are observed (Table I): the LVyc1, LVyc2, and LVyc3 strains have an almost identical final absorbance (0,92 to 0,93) followed by LVyc4 (0,89). The absorbances are lower (0,72 to 0,89) for the LVyc5, LVyc6, and LVyc4 yeasts.The LVyc1, LVyc2, and LVyc3 strains were selected for the urea confrontation test (Table 4). They showed correct growth and interesting values for the pH and acidity of the previous trials.The final pH varies between 6,42 and 7,16. LVyc1 and LVyc2 could not lower the pH to low values when grown in the presence of high urea concentrations (4 to 5 g/l).

The neutralization of ammonia molecules still seems difficult. LVyc1 gives the best growth. It achieves a high absorbance (0,98).The strains tested are yeasts isolated from the previously prepared fermentation mash. The table below gives the origin of each strain.The yeast strains shown in the table were selected from different biotopes, treated straw, and straw alone. The isolated yeasts constitute strains of high-performance biotechnological interest, to be used in animal feed. The integration of these strains in feed intended for breeding will, in particular, improve the quality of feed.Only the LVyc1 strain presents a marked urease activity, this activityismainlydue to the presence of the urease's enzyme. This shows the important interest of the incorporation of this ureolytic strain in the process of straw treatment with urea. These results are consistent with those of The use of urease of microbial origin (LVyc1) is a beneficial alternative for farmers who use soyflour, the most known

product for its urease property for the treatment of straw for cattle feed [23, 24].

The nitrogen molecule which result from the hydrolysis of urea bythe urease's enzyme [25], is amajor source for the synthesis of proteins of the animal which is directly involved in the compensation of the need of production and maintenance [26]. The total and rapid degradation of urea into ammonia ispossible by using strains of the LVyc1genus.Work for optimizing the processing conditions are in progress to determine the humidity rates, the amount of the enzyme, the concentration of the urea and the molasses for the successful treatment of straw. In the end, can say that the strawis a vegetable carrier which contains microorganisms with urease properties capable of multiplying in a biotope rich in urea as a nitrogen source, and therefore the complete hydrolysis of urea.

4. Conclusion

The strains of yeasts and lactic acid bacteria are known and have been used for decades in different processes of fermentation and traditional food preservation. The purpose of study is to highlight a collection of yeast and lactic acid bacteria strains isolated from a natural food; straw and which can be used as probiotics. At the end of research study, six strains of lactic acid bacteria and six strains of yeast were isolated and selected. The results of morphological and physicochemical characterization of the strains have shown that the straw contains different kinds of lactic acid bacteria and yeasts with indicators and performance criteria in terms of food preservation.According to the findings and the results observed, deduce that the selected strains have acidifying powers and good technological functionalities and therefore they can contribute to the inhibition of the main pathogens in situ in foods intended for human consumption and/or animal.

Acknowledgement

We thank all the professors and authors for their participation in carrying out this study and this work, as well as the reviewers and reviewers of the journal for their effort and support.

References

[1] K. Katina, and K. Poutanen. (2013). Nutritional aspects of cereal fermentation with lactic acid bacteria and yeast. In Handbook on Sourdough biotechnology. Springer, Boston, MA. 229-244.https://doi.org/10.1007/978-1-4614-5425-0_9

[2] V. Galli, M. Venturi, E. Mari, S. Guerrini, L. Granchi. (2022). Selection of Yeast and Lactic Acid Bacteria Strains, Isolated from Spontaneous Raw Milk Fermentation, for the Production of a Potential Probiotic Fermented Milk. Fermentation.8(8): 407.https://doi. org/10.3390/fermentation8080407.

[3] B.D. Chaves, M.M. Brashears, K.K. Nightingale. (2017). Applications and safetyconsiderations of Lactobacillus salivarius as a probiotic in animal and humanhealth. Journal of Applied Microbiology. 123(1): 18-28.

[4] F. De Filippis, E. Pasolli, D. Ercolini. (2020). The food-gut axis: lacticacidbacteria and theirlink to food, the gut microbiome and humanhealth. FEMS microbiology reviews. 44(4): 454-489.https://doi.org/10.1093/ femsre/fuaa015

[5] C. Hill, F. Guarner, G. Reid, G.R. Gibson, D.J. Merenstein, B. Pot B, et al., (2014). Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nature reviews. Gastroenterology &hepatology.11(8): 506-614.https://doi.org/10.1038/nrgastro.2014.66

[6] M.F.P. Derkxet, Thomas Janzen , Kim I Sørensen , Jeffrey E Christensen , Birgitte Stuer-Lauridsen , Éric Johansen (2014). The art of strain improvement of industrial lactic acid bacteria without the use of recombinant DNA technology.Microbial CellFactories. 13(1): 475-2859-13-S1-S5.DOI:10.1186/1475-2859-13-S1-S5.

[7] Zielinska et Kolozyn-Krajewska, Food-Origin Lactic Acid Bacteria May Exhibit Probiotic Properties: Review. 5063185. doi : 10.1155/2018/5063185. eCollection 2018.

[8] Y.Chbab et al. (2011). Monitoring of the evolution of the microbiological floraì Treated Strawî. Biotechnology: An Indian Journal. 5(1): 66-72.

[9] Y.Chbab et al, (2012). Selection of an ureolytic yeast from straw treated by urea and molasses. BioTechnology An Indian Journal. 6(10): 299-303.

[10] Mustapha Bello Salawu, Hélène Warren, Adegbola T Adesogan, (2001). Characteristics of fermentation, aerobic stability and ruminal degradation of bi-croppea/wheat forages ensiled. 81(13):1263-1268. https://doi.org/10.1002/jsfa.937

[11] G. Huys et al, (2013). Caractérisation microbienne des probiotiques – Rapport consultatif du groupe de travail « 8651 Probiotiques » du Conseil Supérieur de la Santé (CSS) belge. Molecular Nutrition and Food Research.57(8):1479–1504. Doi: 10.1002/mnfr.201300065.

[12] P. Marcella et al, (2022).Next-Generation Probiotics for Inflammatory Bowel Disease, International Journal of Molecular Sciences.23(10): 5466.doi:10.3390/ijms23105466.

[13] C. Vanderpool, F. Yan, D.B. Polk. (2008). Mechanisms of probiotic action: implications for therapeutic applications in inflammatory bowel diseases. Inflammatory Bowel Diseases. 14(11): 1585-96.

[14] M.C. Moreau, P. Raibaud, M.C. Muller. (1982). Relationship between the development of the intestinal IgA immune system and the establishment of microbialflora in the digestive tract of youngholoxenicmice. Annals of Immunology. 133D (1): 29-39.

[15] A. Taibi and E.M. Comelli. (2015). Practical approaches to probiotics use. Applied Physiology, Nutrition, and Metabolism. 39(8): 980-986. DOI : 10.1139/apnm-2013-0490.

[16] W.H. Holzapfel et WH Holzapfel 1, P Haberer , R Geisen , J Björkroth , U Schillinger. (2011). Taxonomy and Important Features of ProbioticMicroorganisms in Food and Nutrition. American Journal of Clinical Nutrition. 73(2): 3655-373S.

[17] P. Vandamme, B. Pot, M. Gillis, P. DeVos, K. kersters, E.J. Swings. (1996). Polyphasictaxonomy, a consensus approach to bacterial systematics. Microbiological Review.60(2): 407-38.

[18] J.P. Larpent. (1990). Food Microbiology, Technique and Documentation. Lavoisier. 10(72): 2-3-17.

[19] F. Dellaglio, H.D. Roissart, S.Torriani, M.C.Curk and D.Janssens. (1994). Bactéries lactiques. volume 1 Uriage : Lorica : 25-30-48.

[20] N. Baliyan, M. Kumari, P. Kumari, K. Dindhoria, S. Mukhia, S. Kumar et al., (2022). Probiotics in fermented products and supplements. InCurrent Developments in Biotechnology and Bioengineering. Elsevier 8(4):73-107.

[21] S. Drouault, G. Corthia. (2001). Effects of lacticacidbacteriaingestedwithfermentedmilks on health. VeterinaryResearch. BioMed Central. 32 (2): 101-117.

[22] S. LAIRINI, N. BEQQALI, R. BOUSLAMTI , R. BELKHOU et F. ZERROUQ., (2014). Isolation of lactic acid bacteria from traditional dairy products, Moroccans and formulation of a fermented milk similar to Kefir. Afrique Science.10(4): 267 - 277.

[23] P.E.V. Williams, A. Macdearmid. (1984). Innes, general manager ; Gauld, SA,. Barley straw treated with ammonia and rolled barley offered either together, in a mixed ration, or successively to beef cattle. Animated. Feed Sci. Technol. 10: 247-255.

[24] P. Hassoun, F. Geoffroy, G. Saminadin, P. Avant, M. Beramis. (1990). Studies on the ammoniation of sugar cane bagasse with urea. Effects of humidity, urea levels, urease source and treatment periods on composition, in vitro dry matter digestibility and evolution of ureolytic bacteria. Animated. Feed Sci. Technol. 29 (1) :113-1292.

[25] S. Sahnoune, J.M. Besle, M. Chenost, J.P. Jouany, D. Combes. (1991) Ammoniation of straw via the hydrolysis of urea. Ureolysis in low water medium. Anim Feed Science and Technology. (34): 75-93.

[26] V.R. Kumarasuntharam, M.C.N. Jayasuriya, H.B. Perdok. The effect of the method of urea-ammonia treatment on the subsequent utilization of rice straw by cross bred bull calves. Agric. Wastes. Volume 10(3): 195-203.