



Ensiling with Lactic Acid Bacteria: A Review and Bibliometric Analysis

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HIGHLIGHTS:

- Lactic acid bacteria are recognized as safe inoculants due to their preservation properties, safety, and positive impact on silage quality.
- These inoculants remain underutilized in Moroccan silage production, particularly on small farms.
- The keyword analysis clearly identifies lactic acid bacteria, fermentation, bacterial polysaccharides, and probiotics as core, interrelated concepts central to silage quality and animal health.

Article type

Review article

Keywords

Bibliometric Analysis
Microbial Inoculants
Lactic Acid Bacteria
Silage

Article history

Received: 16 Apr 2025
Revised: 29 Jan 2026
Accepted: 25 Feb 2026

Abbreviations

CFU=Colony Forming Units
DM=Dry Matter
FM=Fresh Matter
LAB=Lactic Acid Bacteria
NH₃-N=Ammonia Nitrogen
WSC=Water-Soluble
Carbohydrates
WoS=Web of Science

ABSTRACT

Ensiling is a natural preservation process in which plant matter or agricultural residues are stored under anaerobic conditions, allowing Lactic acid bacteria (LAB) to ferment soluble carbohydrates and generate an acidic environment that ensures preservation. Inoculated silage with Lactic acid bacteria (LAB) inoculants is a promising fermentation method to enhance fermentation efficiency, preserve fodder quality, and produce lactic acid that lowers pH and inhibits undesirable microorganisms; although effectiveness depends on forage type, dry matter content, sugar concentration, strain properties, application rate, and management practices. This review addresses two main aspects: (i) an overview of the ensiling process, including chemical and microbiological changes, commonly used LAB strains, criteria for strains selection, and applications over the last 10 years, and (ii) a bibliometric analysis of research on LAB-inoculated silage published in the last 5 years. The analysis, performed using Web of Science (WoS) and Scopus databases with Bibliometrix and VOSviewer software, identified key publications, authors, and trends. Collaboration networks and key terms, such as fermentation and probiotics, highlight the role of LAB in improving forage preservation and animal health.

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To cite: Issaoui K.E. and Khay E.-O. (2026) 'Ensiling with lactic acid bacteria: A review and bibliometric analysis', *Journal of Food Quality and Hazards Control*, 13(1) pp. 25-39.

Introduction

Feeding livestock during the dry season is a major concern for farmers. During the rainy season, for example, animals exhibit weight gain due to the availability of sufficient, high-quality forage. However, during the dry season (specifically the period from February to June), the animals lose weight as a result of reduced product quality (Dos Santos *et al.*, 2020; Silva *et al.*, 2020). In order to overcome this effect and meet the forage requirements of animals, great interest has been shown in the use of silage for the conservation of forage crops. This method has been used for many decades as a fundamental biological process based on spontaneous fermentation under anaerobic conditions. Silage is a method of preserving food products intended for animal consumption (forage and cereal grains) by fermentation in the absence of oxygen.

This preservation can be achieved either naturally or by the application of a microbiological additive preparation known as starter cultures, which consist of strains belonging to one or more genera of Lactic Acid Bacteria (LAB) (Muck, 2010; Oliveira *et al.*, 2017b; Carvalho *et al.*, 2021).

The use of LAB as an inoculant during silage fermentation is currently becoming an effective application for improving the fermentative quality of silage. Inoculated LAB can provide a reliable and predictable fermentation process, through acidification of the fermentation medium and the production of lactic acid and other metabolites of interest. Under these acidic pH conditions, LAB promotes the development of beneficial bacteria and inhibits harmful and undesirable microorganisms, resulting in improved microbiological and nutritional qualities (Wilkinson and Muck, 2019).

In fact, LAB are a major component of the epiphytic flora of forage and have been identified as *Lactobacillus*, *Enterococcus*, *Weissella*, *Lactococcus*, *Pediococcus*, and *Leuconostoc* (Ni *et al.*, 2015; Puntillo *et al.*, 2020). Thanks to 16S rRNA sequencing, several researchers have been able to identify *Pediococcus*, *Lactobacillus*, *Weissella*, and *Enterococcus* as the dominant epiphytic LAB of various plants such as oats, black tea alfalfa, and sorghum (Chen *et al.*, 2023). In another study by S. Wang *et al.* (2017), a *Pediococcus* strain and three *Lactobacillus* strains were isolated from three types of forage. Crop residues such as sugarcane (Artiles-Ortega *et al.*, 2023), corn stalks (He *et al.*, 2020), and sugar beet tops (El Tawab *et al.*, 2020; Abo-Donia *et al.*, 2023) have been tested as raw materials for silage.

A bibliometric review is defined as a statistical approach that involves analyzing various types of scientific publications (articles, book chapters, and conference papers) in order to gain an overall view of a research field. It also allows for the creation of a map of scientific contributions and the study of collaboration networks between institutes

and individuals (Donthu *et al.*, 2021). This review was aimed to achieve three main objectives: (i) quantify scientific production related to silage based on the use of lactic bacteria from 01-01-2019 to 01-01-2025, to assess the collaboration network among different researchers in this field, and thus to provide information on the most prominent scientific journals, (ii) highlight the effect of LAB in improving silage quality, and (iii) summarize new application results of LAB additives in silage fermentation over the last 10 years and also highlight future prospects for the use of LAB in silage production in Morocco.

Bibliometric analysis of LAB inoculation in silage publications

Bibliometric data collection

Data were retrieved from the Web of Science (WoS) and Scopus databases. Boolean operators were applied to refine the search and ensure comprehensive coverage (Pranckutė, 2021). Publications were screened for relevance to the study's objectives, with inclusion criteria based on publication date (2019–2024), language (English), and document type (articles). Two reviewers independently conducted the screening, and any discrepancies were resolved by discussion to reach consensus. Inclusion and exclusion criteria were predefined based on language, publication type, and topical relevance. This meticulous process guaranteed a strong bibliometric analysis, including a thorough summary of the most pertinent papers, authors, primary keywords, and the development of inoculated silage-related scientific output throughout the previous five years. The research was conducted using the terms "fermentation" AND "lactic acid bacteria" OR "LAB*" OR "probiotic*" to include various expressions related to LAB and probiotic cultures, combined with "silage" OR "ensiling" OR "forage*" OR "crops." This research strategy has allowed for the capture of a wide range of relevant articles in the field. The data analysis was done using R-package software, and conducted using the Bibliometrix and VosViewer software version 1.6.20, which allowed for the identification of trends, author collaborations, as well as co-citation and co-occurrence networks of terms. "VosViewer" and the "Bibliometrix" package software were used to export and analyze the data from the 130 documents for the bibliometric analysis. Duplicates were identified and removed using R-package functions, and irrelevant articles concerning LAB or probiotics were manually filtered based on title, abstract, and relevance to inoculated silage (Arruda *et al.*, 2022; Bukar *et al.*, 2023).

Bibliometric results

There has been an increase in publications related to

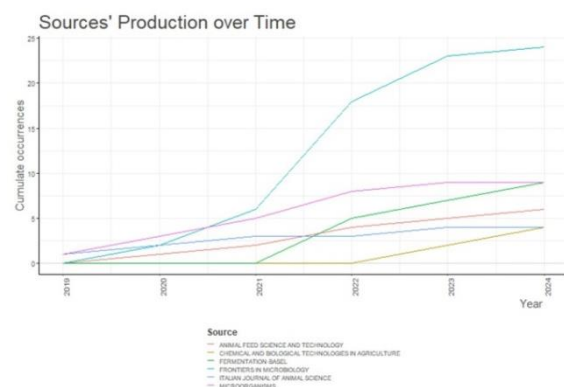
inoculated silage in various scientific journals between 2019 and 2024 (Figure 1a). Frontiers in Microbiology has been the most prolific source, with a marked increase starting in 2021 (24 publications to date), indicating a growing interest in these topics within the fields of microbiology and animal sciences. Other sources, such as Microorganisms and Fermentation-Basel, follow a more moderate progression, while the Italian Journal of Animal Science and the Journal of Chemical and Biological Technologies in Agriculture show a more stable and less voluminous production (4 publications).

The most relevant authors, with at least 10 publications, are recorded in Figure 1b. Yang F. stands out with 18 published articles, followed by Cai Y. and Yang Y., each with 13 publications. Other researchers have made significant contributions with 11 to 12 publications. The number of published documents shows a concentration of scientific output among a few leading authors, suggesting their central role in the studied field.

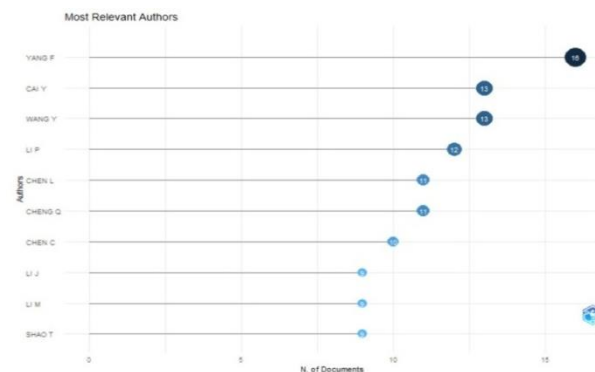
The figure 1c demonstrates the networks of collaborations between different researchers working on studies on silage inoculated by LAB in order to identify research leaders, comprehend cooperation dynamics, and discover possible

high-impact work networks. The graph shows a network of collaborations, generated using VOSviewer. The central author, Cai Y., with a well-connected node, is at the heart of an intense network of collaborations, especially with Ni K. and other close authors, suggesting his major role in the field. Other clusters, less interconnected, indicate regional collaborations or those specific to certain topics.

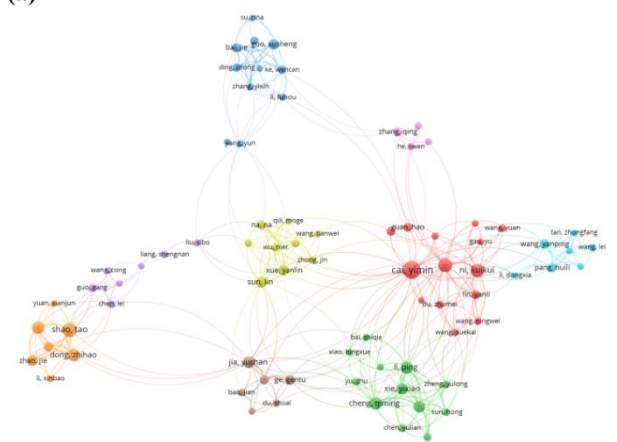
The network of co-occurring keywords was generated using the bibliometric software VOSviewer, with data exported from Scopus and WoS. (Figure 1d). Out of 5075 keywords, 470 have exceeded the threshold set at 5 as the minimum number of occurrences. The figure shows the relationships between the concepts of lactic bacteria and fermentation, which are essential in the silage process. The presence of terms such as metabolism and bacterial polysaccharides suggests that these bacteria also contribute to the structuring of the silage material. Furthermore, the link with probiotics suggests that these bacteria have beneficial effects on animal health by improving their microbiota. The map also highlights the activity of scientific research on improving silage fermentation methods, aiming to maximize the quality and preservation of forage while benefiting animal health.



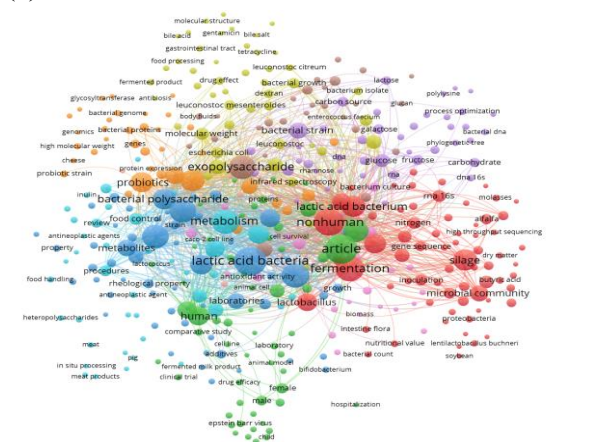
(a)



(b)



(c)



(d)

Figure 1: Bibliometric analysis of silage inoculated with Lactic Acid Bacteria (LAB); (a) sources' production over time; (b) most relevant authors; (c) co-authorship network; (d) co-occurrence of keywords

Silage procedure

The success of silage depends on the biochemical characteristics of the biomass, including its LAB load, soluble carbohydrate content, moisture, buffering capacity, Dry Matter (DM) content, and packing density (Franco *et al.*, 2016). Consequently, effective silage-making begins with the selection of appropriate raw materials. Cereals, such as some sorghum and maize cultivars, are known to be drought-tolerant and have very high DM yields (Williams and Shinnors, 2012; Pholsen *et al.*, 2016). The inclusion of legumes, natural grasses, cultivated forages, as well as a combination of grasses and legumes are likely to improve the nutritional quality of silage. Van Nevel and Demeyer (2008) have specified 3 main criteria to be considered for each silage: DM content, sugar content, and resistance to acidification.

Ensiling is an essential technique for protecting silage. The process is based on a set of four key stages that must be taken into consideration to ensure a high-quality end product (Figure 2). The first stage involves harvesting the forage crop at the ideal stage of maturity. Several studies have shown that the harvesting period has a direct influence on the quality of the ensiled forage.

A study by Kalač (2011) reported that harvesting grasses at the last stages of growth, with low soluble sugar content affects the speed of forage fermentation by delaying the pH decrease necessary for effective preservation. Atis *et al.* (2013) tested the effect of harvesting plants at different stages of maturity on the silage quality of four sorghum cultivars; the results showed that the stage of maturity is the optimum time for harvesting forage sorghum for all four cultivars. In another study by Zamir *et al.* (2020) harvesting at the milking stage improved the biochemical quality of the silage by causing a significant reduction in pH through the production of lactic acid and an increase in protein content, which was low when harvesting at an advanced stage of maturity. Similarly, Liu *et al.* (2023)

reported that harvesting corn at the milking stage produced silage of high nutritional value. In the same study, ensiling a mixture of alfalfa and corn improved the sensory and nutritional quality of the fermented product, characterized by high soluble carbohydrate and lactic acid content, were significantly high, and low pH and ammoniacal nitrogen. In addition, the effect of harvest time combined with other additives, such as urea and molasses, was also tested for its impact on silage quality (Zamir *et al.*, 2020; Tarekegn, Nurfeta and Bayssa, 2024). In fact, silage additives have been divided into two broad categories: inhibitors (acids, salts, and solvents) and stimulators (LAB, sugars, and enzymes) of fermentations (Pitt, 1990; McDonald, Henderson and Heron, 1991). The second silage stage consists of chopping the harvested forage in the field into small pieces, generally between 6 and 60 mm. This step is critical, as it facilitates the expulsion of the air contained in the grass (moisture content >80%), and together with adequate sealing and compaction, promotes the anaerobic environment required for lactic fermentation (Soundharrajan *et al.*, 2021; Okoye *et al.*, 2023). The chopped forage is then packaged in a previously prepared silo. Various silo models can be used: open, vertical, horizontal, and bag silo. During filling, the floor of the silo must have a slope of 2-5% to allow drainage of free effluent. The cover may be made of plastic, concrete, or cement, and the dimensions must be adapted to the quantity of forage to be ensiled. The type of silo has been shown to significantly affect the chemical and physical characteristics of silage, principally pH and the amount of acetic acid produced (Kızılsimsek, Erol and Calıslar, 2005). At the end of the filling process, molasses provides fermentable sugars, and salt can be used as silage preservatives at a concentration of 30-50 kg and 5-10 kg per ton of forage respectively. Silage can be stored for more than a year, the storage time depends on silo type, sealing quality, and forage characteristics.

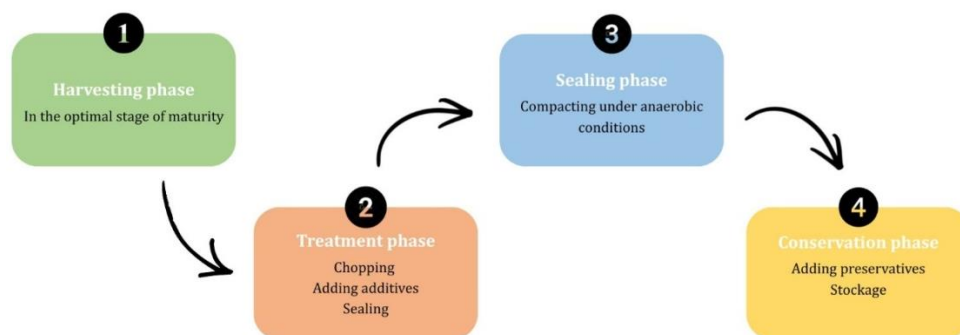


Figure 2: Main steps in the ensiling process

Biochemical and microbiological changes during ensiling

Silage is stored under anaerobic conditions, where the

epiphytic LAB present in the raw material acidifies the mass. Depending on the biochemical and microbiological transformations, silage can be subdivided into three main

phases (Franco, Buffière and Bayard; Kalač, 2011; 2016) (Figure 3):

- Initial phase characterized by the respiration and metabolic activity of plant cells and the bacterial enzymatic activity, favored by the presence of water and oxygen. Glucose and fructose are the main fuel products used in respiration reactions. By releasing heat, CO₂, and water, proteolysis reactions are triggered by the hydrolases contained in the burst lysosomes, leading to a flow of cell contents and the resulting juice.
- Early fermentation phase: During the first 48 h, in the presence of oxygen, facultative anaerobes develop, mainly enterobacteria. The fermentative activity of these microorganisms generates a negligible yield of acetic acid, alcohol, and gas. The fermentation reactions carried out by these microorganisms slow down the acidification of the forage, resulting in a loss of nutrients.
- Late fermentative phase (lactic fermentation): when favorable conditions such as anaerobiosis, availability of fermentable sugars, pH >4 and 10 < T°C <40 are maintained, LAB proliferate and promote acidification.

In fact, the quantity of lactic acid produced during ensiling depends on the type of dominant flora.

- In an environment dominated by homofermentative bacteria (e.g., *Lactobacillus plantarum* and *Lactobacillus casei*), lactic acid is produced exclusively from glucose and fructose. In contrast, dominant heterofermentative bacteria (e.g., *Leuconostoc* spp. and *Lactobacillus brevis*) yield less than 45% lactic acid while also producing ethanol, acetic acid, and CO₂. Under unfavorable conditions, the process can shift to butyric fermentation by clostridial spores in the silage. This undesirable pathway results in nutritional loss from protein catabolism, reduced medium acidity, promoting other spoilage fermentations, deteriorated organoleptic and sanitary quality. During ensiling, LAB are the primary microbial group responsible for fermentation quality. They metabolize Water-Soluble Carbohydrates (WSC; e.g., glucose, fructose, sucrose, and fructans) into lactic acid via homo- or heterofermentative pathways depending on the fermentation mode (Tanizawa *et al.*, 2015).

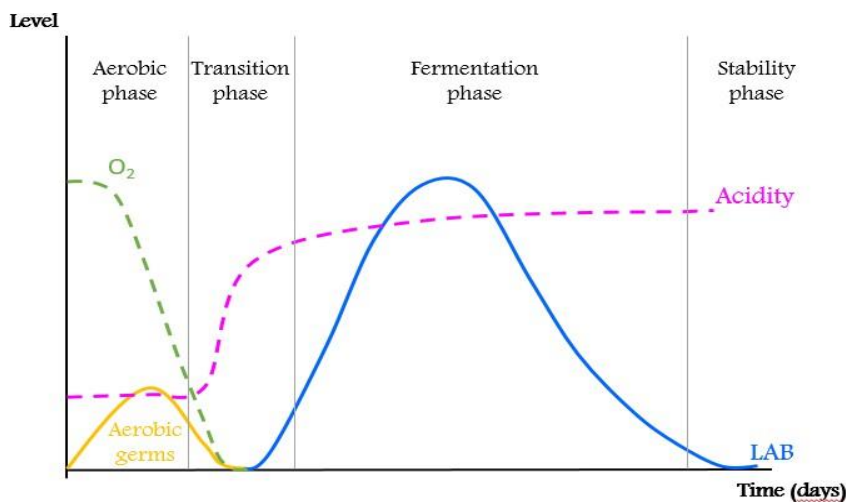


Figure 3: Chemical and microbiological evolution during a silage
LAB=Lactic Acid Bacteria

LAB uses the glycolysis pathway to break down one glucose molecule into two pyruvate molecules, two ATP and two NADH. The NADH molecules are then oxidized to reduce pyruvate to lactic acid. Homofermentative LAB such as *Lactococcus* and some *Lactobacillus* produce lactic acid as the main by-product of fermentation, rapidly lowering pH and preventing undesirable microbial growth. However, heterofermentative LAB such as *Leuconostoc* produce lactic acid, ethanol, and CO₂, among others, of which lactic acid is the major product (Silva *et al.*, 2020; Soundharajan *et al.*, 2021).

Factors affecting silage quality

Several factors, including microbial populations, environmental conditions, and management practices, can influence fermentation and ultimately silage quality.

Microbiological factors

The use of high-quality silage, free of toxins and pathogenic microorganisms, is necessary to preserve animal health and maximize performance (Ogunade *et al.*, 2016). Enterobacteriaceae, mainly some *Escherichia coli* and *Hafnia alvei*, are among the pathogenic microorganisms most commonly found in ensiled forages (Ni *et al.*, 2017). They can contaminate forage via irrigation water, and compete with LAB for nutrients

during early fermentation, when acidification is still low (McGarvey *et al.*, 2013). Wang *et al.* (2019) reported a relative abundance of enterobacteria in alfalfa and stylo silage at the start of ensiling; subsequently, during the fermentation process this dominance was replaced by lactic flora especially *Lactobacillus*, *Leuconostoc*, *Pediococcus*, and *Weissella*. In contrast, Cai *et al.* (2021) detected a low load of LAB (<3.73 log₁₀ Colony Forming Units [CFU]/g Fresh Matter [FM]) alongside a high aerobic bacteria count (>5.39 log₁₀ CFU/g FM), in sorghum silage, resulting in poor quality. Previous studies indicate that at least 5 log₁₀ CFU/g FM of epiphytic LAB is required to inhibit undesirable microorganisms and preserve silage quality (Cai *et al.*, 1999; Kaiser, Weiss and Zimmer, 1997).

Listeria monocytogenes, *Clostridium* spp. and *Bacillus* spp. are also considered the main Gram-positive contaminants of silage. Being less acid-tolerant, their presence is frequently linked to inadequate fermentation. *L. monocytogenes* has been detected in samples of untreated corn or corn treated with a concentration of 4 g/t of bacterial additives (Sharif *et al.*, 2023). Several studies have shown a high correlation between the presence of *L. monocytogenes* and silage pH (Queiroz *et al.*, 2018). A study by Nucera *et al.* (2016) showed that the presence of *Listeria* in silage is not always due to its presence in the soil and on vegetation, but can also be due to contamination during ensiling. In the same study, *Listeria* was predominantly detected in high pH silage bales and in molded areas. In fact, LAB are known for their anti-listerial activity (Ellis *et al.*, 2016) via a range of bioactive metabolites, such as organic acids, reuterin, hydrogen peroxide, and bacteriocins, which have similarly shown the ability to control *Clostridium* and *Bacillus* spores (Liao and Nyachoti, 2017; Vimont *et al.*, 2019).

Fungi and/or mycotoxins may also be present in the silage product. Their presence may be the result of pre- or

post-harvest contamination, mainly by *Fusarium*, *Penicillium*, and *Aspergillus* (Alonso *et al.*, 2013; Gallo *et al.*, 2015). Aflatoxins produced by *Aspergillus* have been considered the most toxic and carcinogenic (Mobashar *et al.*, 2010). Some LAB have been shown to inhibit fungal growth and consequently mycotoxin production. They form aggregates with fungi and inhibit the expression of genes encoding toxin production (Mieszkin *et al.*, 2017; Sadhasivam *et al.*, 2019).

Environmental factors

Exposure to air during ensiling or storage promotes the growth of lactate-assimilating yeasts. This raises the silage pH and temperature, which can exceed 40 °C (Chen *et al.*, 2013), thus favoring the development of undesirable microorganisms (Kung Jr *et al.*, 2018).

Management and treatment factors

Forage ensiled alone or containing less than 5–8% WSC in DM may not reach a low enough pH to produce quality silage (Adesogan and Newman, 2010). It has been shown that treatment with WSC (molasses, fructose, and glucose) and many other chemical additives (Table 1) increases DM digestibility and leads to a rapid increase in lactic acid content at the start of ensiling, contributing to higher-quality silage (Gao *et al.*, 2021; Dong *et al.*, 2020; Ni *et al.*, 2017). On the other hand, limitations in the use of chemical additives have been reported by several researchers; for example, the addition of molasses alone can induce the proliferation of undesirable bacteria and consequent loss of DM (Cao *et al.*, 2010). Additionally, organic acids preserve forage, while their corrosive effects mainly concern equipment and concrete (Oladosu *et al.*, 2016). The use of formic acid during ensiling resulted in the accumulation of ammoniacal nitrogen with lower levels of WSC (Grøseth *et al.*, 2024).

Table 1: Chemical additives used in silage

Chemical additives	Concentration	Silage matrix	Effect	References
Molasses	5% FM	Sudangrass	- Lactic Acid Bacteria (LAB)-treated silage exhibited reduced bacterial diversity and a higher relative abundance of <i>Lactobacillus</i> , <i>Pediococcus</i> , and <i>Sporolactobacillus</i> , which improved fermentation outcomes.	(Wang <i>et al.</i> , 2020)
Chestnut tannins, Oak tannins, Zeolite erythritol wood molasses	8 g/kg DM 10 g/kg DM 100 g/kg DM 60 g/kg DM 20 g/kg DM	Rygras and red clover	- Tannins protected proteins from plant and microbial enzymes by forming complexes that resist silage fermentation and in vitro protease activity.	(Herremans <i>et al.</i> , 2019)
Propionic acid Tea polyphenols	4 g/kg FM 4 g/kg FM	Alfalfa	- Propionic acid kept silage aerobic stability and best conserved fatty acid of silage. - Tea polyphenol relieved synthesis of C6:0 and had a slight lipolysis of unsaturated fatty acids.	(Liu, Dong and Shao, 2018)
Formic acid Molasses Fibrolytic enzyme	0.2% FM 0.4% FM 0.3% FM	Napier grass	The three additives improved Napier grass silage by increasing sugars, reducing lignocellulose, and enhancing enzymatic digestibility.	(Desta <i>et al.</i> , 2016)

Chemical additives	Concentration	Silage matrix	Effect	References
sodium benzoate potassium sorbate sodium nitrite	200 g/kg FM 100 g/kg FM 50 g/kg FM	Shelled corn	- All additive treatments markedly enhanced aerobic stability and improved dry matter recovery compared with untreated High Moisture Corn (HMC).	(Da Silva <i>et al.</i> , 2015)
Mixture of: formic acid (42.5%), propionic acid (10.0%), ammonium formate (30.3%), benzoic acid (2.2%).	(4 l/t) DM	Maize	- Silages were well fermented, reaching a pH below 4.0 after 10 days. - The chemical additive did not affect lactic acid concentration but increased acetic acid levels.	(Tyrolová, Bartoň and Loučka, 2017)
Sugarcane molasses	3% FM	Sugarcane trash Sugarcane stalks	- Decrease in pH from 5.7-5.9 to 3.8-4.2 through formation of organic acids (mainly acetate and lactate) - Improving the digestibility of cellulosic biomass for methane production.	(Janke <i>et al.</i> , 2019)
Propionic acid	2.2 g/kg DM	Alfalfa	- Propionic acid suppressed yeast and mold growth during ensiling and subsequent aerobic exposure, while maintaining pH below 5.0.	(Ogunade <i>et al.</i> , 2016)

DM=Dry Matter; FM=Fresh Matter

Lab additives in silage fermentation

To better control fermentation during ensiling, LAB have been used as bacterial additives to increase the ratio between beneficial and undesirable microbes in the ensiling (Bai *et al.*, 2021). Microbiological additives generally belong to the genera *Lactobacillus*, *Enterococcus*, *Pediococcus*, *Lactococcus* (Ellis *et al.*, 2016; Muck *et al.*, 2018). *L. plantarum* is the species most commonly used as a starter culture, alone or in co-culture with other LAB species (Table 2).

In a recent study by You *et al.* 2022, the application of *L. plantarum* PS-8 during alfalfa silage production demonstrated a positive correlation between the lactic microbiome including *L. plantarum*, *Weissella paramesenteroides*, *L. brevis*, *Lactobacillus curvatus*, and *Lactobacillus farciminis*, and four organic acids including lactic acid, which increased the stability and shelf life of the silage product following the acceleration medium acidification during fermentation. In the same study, the microbial load of molds and coliforms was considerably reduced to levels below the detection limit. Similar results were found by Bai *et al.*, 2021; *Lactobacillus*, *Weissella*, and *Pediococcus* were positively correlated with concentrations of lactic acid, propionic acid, and the ratio of lactic acid to acetic acid; on the other hand, *Hafnia* sp. was negatively correlated with concentrations of the acids discussed.

It has been concluded that the chemical and nutritional composition of silage is species-dependent (Campbell *et al.*, 2020). *Enterococcus* is generally recognized as capable of accelerating lactic acid fermentation (Wang *et al.*, 2020). However, *Enterococcus faecalis* used in alfalfa silage was less acidifying than *L. plantarum* and *Pediococcus pentosaceus*, which were able to lower pH levels (Bai *et al.*, 2021). This finding aligns with the report by Auerbach *et al.* (2020), who attributed the lack of a significant LAB effect on lactic acid production to the high

population of competitive epiphytic LAB already present on the crop at ensiling. Under such conditions, the addition of LAB inoculants was insufficient to dominate the fermentation process. In addition to pH and organic acids, Ammonia Nitrogen (NH₃-N) and DM content are among the performance indicators guaranteeing silage quality (Ni *et al.*, 2020). NH₃-N is generally considered to be the result of deamination of amino acids, which reduce the nutritional value of silage. The accumulation of NH₃-N is a typical feature of *Clostridia* fermentation. In silage controlled by the addition of LAB additives, reduced *Clostridium* fermentation was detected as a result of the bacteriostatic and bactericidal actions of organic acids disrupting the absorption of amino acids by bacteria (Ni *et al.*, 2020). Recent studies have highlighted the synergistic effect of a combination of lactic inoculants on improving silage quality (Kim, Lee and Choi, 2021). *L. plantarum* and *Enterococcus faecium* have shown a very high acidification potential, inhibiting the proteolytic activity of other microorganisms and consequently reducing the NH₃-N concentration in the silage medium (Oliveira *et al.*, 2017a).

Under effective silo management, DM losses should not exceed 5% (Borreani *et al.*, 2018). Studies have shown that DM content is lower in untreated silage at the end of storage compared to silage inoculated with LAB (Bai *et al.*, 2021; Liu *et al.*, 2019b; Zhao *et al.*, 2021). This loss is attributed to undesirable fermentation resulting from the conversion of lactic acid to butyric acid (Muck, 2010). Consistent with these findings, Zhao *et al.* (2020) reported that silage treated with *L. plantarum* retained significantly higher DM content, which corresponded with a reduced abundance of undesirable microorganisms.

Contrary to these observations, several studies have reported significant DM losses when specific forage-inoculant combinations were used. For instance, Auerbach and Nadeau (2020) observed losses in a mixture of timothy

(*Phleum pratense* L.), meadow fescue (*Festuca pratensis* L.), and red clover (*Trifolium pratense* L.) ensiled with *Lactobacillus buchneri*. Similarly, Auerbach *et al.* (2020) reported losses in whole-crop rye (*Secale cereale* L.) treated with *L. plantarum* and *Lactobacillus paracasei*, and Restelatto *et al.* (2019) found losses in corn silage and ryegrass inoculated with *L. buchneri* and *L. plantarum*. Dunière *et al.* (2017) attributed these variations in DM retention to several factors, including ensiling duration and conditions (aerobic vs. anaerobic), the density and diversity of epiphytic flora on the forage, and the type of additive used (chemical, biological, or a combination).

It has been generally concluded that chemical additives are safer in terms of silage product quality than organic additives. Abebaye *et al.* (2020) demonstrated that ensiling

green maize stalks with 3% molasses improved organic matter digestibility, resulting in higher metabolizable energy and crude protein content compared to fermentation with fermented juice. Similarly, Fang *et al.* (2022) reported that adding molasses alone or with LAB inoculants promoted lactic acid fermentation, favoring the growth of *Lactobacillus* and increasing lactic acid content. In another study by Kumari *et al.* 2023, the positive effect of a combination of stimulators (*L. plantarum*, *Lactobacillus fermentum*, xylanase and cellulase), and inhibitors (propionic acid) was endorsed. Oladosu *et al.* 2016 demonstrated the effectiveness of a combination of fibrolytic enzymes and LAB on the degradability of forage material.

Table 2: Lactic Acid Bacteria (LAB) inoculant applications on silage fermentation

Silage type	LAB inoculant	Inoculation rate (CFU/g)	Dry matter (%)	Ensiling duration (days)	Effect on silage quality	References
Alfalfa	<i>Lactobacillus plantarum</i> <i>Pediococcus pentosaceus</i> <i>Enterococcus faecalis</i>	10 ⁵	31.19	60	The combination of several LAB inoculants ensures better fermentation quality and modulates the diversity, the interaction, and the metabolic pathways of the bacterial community during ensiling.	(Bai <i>et al.</i> , 2021)
Barley	<i>Lactobacillus plantarum</i> <i>Lactobacillus casei</i> <i>Lactobacillus buchneri</i>	10 ⁵	29.57	60	The use of LAB in silage increased populations of beneficial <i>Lactobacillus</i> while suppressing undesirable microorganisms.	(Liu <i>et al.</i> , 2019a)
Soybean	<i>Lactobacillus plantarum</i> <i>Pediococcus pentosaceus</i>	10 ⁶	24	60	LAB treatment can enhance beneficial <i>Lactobacillus</i> counts and limit the proliferation of undesirable microorganisms such as <i>Clostridia</i> and <i>Enterobacter</i> .	(Ni <i>et al.</i> , 2017)
Paddy Rice	<i>Lactobacillus plantarum</i> <i>Lactobacillus casei</i>	10 ⁶	36.71	60	<i>Lactobacillus casei</i> can tolerate acidic conditions and improve silage quality, resulting in a lower pH and higher lactic acid levels.	(Ni <i>et al.</i> , 2015)
Alfalfa	<i>Pediococcus acidilactici</i> <i>Pediococcus pentosaceus</i>	10 ⁶	31.3	56	During the initial day of ensiling, pH decreased sharply, while acetic acid levels remained low.	(Silva <i>et al.</i> , 2016)
Guinea grass Napier grass	<i>Lactobacillus plantarum</i> <i>Lactobacillus casei</i>	10 ⁵	20.18 17.88	60	Silages treated with cellulase had significantly elevated crude protein and decreased Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) contents compared with LAB-inoculated silage.	(Khota <i>et al.</i> , 2016)
Corn	<i>Lactobacillus plantarum</i>	10 ⁶	38.1	90	Lactic acid levels increased in the treated silage, whereas microbial diversity decreased compared to the untreated silage.	(Keshri <i>et al.</i> , 2018)
Alfalfa	<i>Lactobacillus plantarum</i>	10 ⁵	24	56	Poor preservation of alfalfa silage was linked to the absence of fast-acidifying LAB and the presence of <i>Clostridia</i> .	(Zheng <i>et al.</i> , 2017)
Sugar can	<i>Lactobacillus hilgardii</i> and 13 other LAB strains	10 ⁹	26.48	61	The best silage characteristics were achieved, including reduced Dry Matter (DM) loss, low ethanol, elevated LAB counts, and minimal butyric acid content.	(Ávila <i>et al.</i> , 2014)
Rice straw	<i>Lactobacillus plantarum</i>	10 ⁴	41.79	60	The synergistic action of hemicellulase and <i>Lactobacillus plantarum</i> enhanced cellulose conversion and increased glucose yield during enzymatic hydrolysis.	(Zhao <i>et al.</i> , 2018)

Silage type	LAB inoculant	Inoculation rate (CFU/g)	Dry matter (%)	Ensiling duration (days)	Effect on silage quality	References
ryegrass	<i>Lactobacillus plantarum</i> <i>Lactobacillus buchneri</i>	10 ⁶	18.8	60	Inoculating silage with LAB reduced microbial diversity and promoted the growth of <i>Lactobacillus</i> , <i>Pediococcus</i> , and <i>Sporolactobacillus</i> , enhancing fermentation quality.	(Li <i>et al.</i> , 2019)
Napier grass	<i>Lactobacillus farciminis</i> <i>Lactobacillus plantarum</i> <i>Weissella thailandensis</i> <i>Lactococcus lactis</i>	10 ⁵	21.2	90	All LAB inoculants decreased silage pH and ammonia-N concentration.	(Wang <i>et al.</i> , 2019)
Corn	<i>Lactobacillus buchneri</i> <i>Lactobacillus plantarum</i> <i>Leuconostoc mesenteroides</i>	10 ⁶	35.5	90	The inoculation of silage with <i>Lactobacillus</i> enhanced its aerobic stability.	(Wang <i>et al.</i> , 2019)
Moringa oleifera Leaf	<i>Lactobacillus plantarum</i>	10 ⁶	24.88	120	Inoculated silage showed reduced pH and NH ₃ -N, along with higher lactic acid levels.	(Wang <i>et al.</i> , 2018)
Hedychium gardnerianum	<i>Lactobacillus plantarum</i> , <i>Pediococcus acidilactici</i> , <i>Pediococcus pentosaceus</i> <i>Propionibacterium acidipropionici</i>	10 ⁶	19.54	60	Molasses together with LAB inoculation resulted in optimal silage characteristics, including stable lactic and acetic acid levels, low pH, minimal dry matter loss, absence of butyric acid, and low NH ₃ -N content.	(Moselhy, Borba and Borba, 2015)
Corn stover	<i>Lactobacillus brevis</i> <i>Lactobacillus parafarraginis</i>	10 ⁶	-	45	Corn stover silages treated with the two strains exhibited lower pH and reduced acetic acid accumulation.	(Xu <i>et al.</i> , 2017)
Grass Silage	<i>Lactobacillus plantarum</i>	10 ⁶	-	60	<i>Lactobacillus plantarum</i> especially when combined with molasse, improved fermentability of grass silage (higher lactic acid content, fast pH drop, and undesirable microorganism inhibition).	(Li <i>et al.</i> , 2022)
Oat and common vetch	<i>Lactobacillus plantarum</i> <i>Lactobacillus buchneri</i>	5×10 ⁵	-	60	The combination of oat-common vetch silage with LAB treatment significantly improved fermentation quality, pH and lactic acid content.	(Ma <i>et al.</i> , 2025)
Oat	<i>Lactiplantibacillus plantarum</i> <i>Lactiplantibacillus brucei</i>	225,1×10 ⁶	-	60	Treatment with LAB showed increased lactic acid content, decreased pH value, and high antioxidant activity of oat.	(Wang <i>et al.</i> , 2024)

LAB=Lactic Acid Bacteria

Selection criteria

Generally, LAB is considered the most desirable strains of biological additives for silage due to their safety and preservative properties. They positively affect silage quality and improve animal performance. However, application results of these bio-additives have shown a large inter-species difference (Amaral *et al.*, 2020; Zhao *et al.*, 2020), requiring conditional selection of these microorganisms while following a set of criteria to ensure an efficient ensiling process and preserve the nutritional quality of the forage. The main criteria include the ability to rapidly lower pH, efficient acid production, rapid growth, resistance to stress conditions (different pH and temperatures), inhibition of undesirable microorganisms, and adaptation to the silage environment (Carvalho *et al.*, 2021). In the majority of studies, researchers follow a similar strategy while taking into account the selection objective. Some inoculants are specially formulated to prevent the growth of undesirable molds, yeasts or

bacteria, while others are designed to improve nutritional quality or reduce fermentation losses.

The first selection stage consists of obtaining strains through isolation. The second stage involves morphological, physiological, and biochemical characterization tests. Key traits assessed include tolerance to environmental conditions, including the ability to survive and function efficiently under variations in temperature and pH. Rapid acidification to inhibit the growth of undesirable microorganisms is also evaluated, along with metabolism classification (homofermentative or heterofermentative). Additional criteria include the ability to reduce fermentation losses such as nutrient DM losses and the production of volatile compounds, antimicrobial activity (Nascimento Agarussi *et al.*, 2019; Wang *et al.*, 2017). Finally, safety is confirmed through tests for enterotoxin production, hemolytic activity, gelatinase and Deoxyribonuclease (DNase) activity, and antibiotic resistance.

After screening, the next step is to identify the selected

strains using various molecular techniques, including 16S rRNA Gene Sequencing (Alhaag *et al.*, 2019; Silva *et al.*, 2020) and MALDI-TOF MS (Puntillo *et al.*, 2020), as the most commonly used. The third stage consists of performance testing during silage production, by measuring DM digestibility, chemical and microbiological composition, and evaluation of aerobic stability during storage (Carvalho *et al.*, 2021; Silva *et al.*, 2020). The final stage is based on an assessment of animal performance by measuring feed consumption, growth (Gallo *et al.*, 2015), lactation, metabolism, and digestibility of ruminal nutrients (Monteiro *et al.*, 2021).

Inoculated silage: Moroccan case study

In Morocco, forage production plays a central role in livestock feeding, although it remains insufficiently valued in relation to national needs. According to the report by the Moroccan National Institute of Agronomic Research, the area dedicated to forage crops is estimated at approximately 500,000 hectares, with an annual production of about 1.7 billion Forage Units (FU). These crops represent about 4 to 6% of the useful agricultural area and generate nearly 18.8 million tonnes of green matter per year (INRA Morocco, 2024). The main materials ensiled include oat forage (rather than whole grain), fodder corn, and fodder beet roots—the latter requiring mixing with fibrous material to ensure proper fermentation. During ensiling, urea (1%) and molasses (2%) are added as chemical additives to support the fermentation process. Depending on the moisture content of the plants (dry or wet), wheat straw or water is added to reduce or increase DM levels, respectively. Nutrients are then added to enrich the silage, such as calcium carbonate, sodium carbonate, dried beet pulp, the mineral salt appreciated by the animals, and industrially prepared vitaminized mineral supplements.

Despite the widespread use of LAB in silage production worldwide, and the wealth of data in the literature describing the possible inoculation of forages with specific inoculants, this application is not yet widely practiced in Morocco, especially on small farms or in regions where farming practices are less intensive. Moreover, very little research has been carried out on the effect of bacterial inoculants on silage quality and animal performance in Morocco. Trials have been carried out on the conservation of olive industry residues (pomace, margines, margions) via controlled ensiling using selected endogenous LAB. Others have studied the ensiling of fish waste (*Sardina pilchardus*), comparing two types of process: biological by *L. plantarum* and chemical using 2% phosphoric acid (Loughzal, Tahri and Faid, 2003). In the same vein, three LAB strains (*Lactobacillus plantarum*, *Lactococcus lactis* and *Pediococcus spp.*) were selected and combined for use

as lactic ferments to improve the hygienic and nutritional quality of barley, which can be used as a feed ingredient for broilers (Choukri *et al.*, 2023).

This lack of widespread practice in Morocco could be due to a lack of information and awareness about the benefits of using inoculants for silage and their effects on forage quality. In addition, the acquisition of these inoculants can be expensive and difficult for farmers due to their limited availability on the local market. Traditional farming practices and lack of resources can also make farmers reluctant to adopt new and more advanced farming techniques. On the other hand, climatic variation in some regions may be less favorable to the practice of silage making, such as prolonged periods of drought, and highly irregular precipitation directly impacting the quantity and quality of forages available for ensiling, particularly in rainfed areas receiving less than 350 mm of annual rainfall. These conditions may limit farmers' interest in the use of inoculants (El Housni *et al.*, 2001).

Faced with these constraints, several concrete opportunities can be deployed to promote the use of LAB-based inoculants in Morocco. These include the development of local inoculants from indigenous strains adapted to Moroccan agro-climatic conditions, reducing costs and dependence on imported products, as well as strengthening training and agricultural extension programs for farmers.

Conclusion

In conclusion, silage inoculated with LAB proves to be a promising method for optimizing the fermentation process, preserving forage quality, improving nutrient conservation, and enhancing animal performance. This review highlights the key microbiological and chemical mechanisms governing silage quality, with particular emphasis on the importance of appropriate LAB strain selection. The bibliometric analysis, based on the WoS and Scopus databases, revealed a significant growth in research over the past five years, with emerging trends focusing on multifunctional and indigenous LAB strains, microbial interactions during fermentation, and the links between silage quality, animal health, and feed safety. These trends identify future research priorities centered on locally adapted inoculants and their practical impact under diverse agro-climatic conditions. In Morocco, the ensiling process relies mainly on the use of chemical additives, while inoculation with LAB remains limited, particularly on small-scale farms. This situation highlights the need to strengthen applied research, raise farmer awareness, and develop cost-effective, locally available inoculants to promote the adoption of inoculated silage as a sustainable strategy for improving the productivity of Moroccan livestock systems.

Author contributions

K.E.I. conducted the literature search, drafted and wrote the initial manuscript. E.-O.K. critically reviewed the literature, provided conceptual guidance, and revised the manuscript. Both authors read and approved the final version.

Acknowledgments

There is no appreciation.

Conflicts of interest

The authors declare that there is no conflict of interest.

Funding

This research received no specific grant from any funding agency in the public, commercial, or non-profit sectors.

Ethical consideration

Not applicable.

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