

## **Aerobic stability and instability of silages caused by bacteria**

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### **Introduction**

The ensiling process is often divided into 3 phases: aerobic, fermentation, and aerobic deterioration phases. The fermentation phase is sometimes divided into 2 phases: fermentation and storage. Each phase has a considerable impact on the quality of silage fed to animals, and diverse microorganisms can be successively activated and deactivated. Occurrence of and changes in the microbial population depend mainly on crop composition and silo management, i.e., crop species, dry matter (DM) content, water-soluble carbohydrate (WSC) content, buffering capacity, degree of anaerobiosis, and contamination of soil and manure. The microorganisms identified predominantly in silages are facultative anaerobes; hence, many bacteria and yeasts have the potential to participate in all phases of the ensiling process.

If good anaerobiosis is achieved by prompt sealing, air trapped in the forage is rapidly consumed by plant respiration and aerobic microorganisms. This may terminate the aerobic phase within a few hours and initiate the fermentation phase, during which facultative and obligate anaerobes compete for substrate (Driehuis and Oude Elferink, 2000). The fermentation phase may continue for several weeks to a year. Lactic acid bacteria (LAB) are the most important microorganisms in this phase, because if they can overwhelm other bacteria, acidification would rapidly and loss of nutrients would be minimized. In the initial stage of the fermentation phase, however, large numbers of enterobacteria occasionally proliferate and produce large amounts of ethanol and 2,3-butanediol (McDonald et al., 1991). Anaerobic yeast growth could also occur vigorously in some cases, resulting in fermentation of sugars to ethanol and CO<sub>2</sub>. These alcohols do not contribute to acidification and preservation, and thus indirectly impair silage stability during the

subsequent aerobic deterioration phase. Growth of *Clostridium* spp. during the late stage of fermentation is a concern, but the growth will not be intensified if acidification is sufficient and anaerobiosis is secured. With respect to aerobic stability, silages with high butyric acid content resist deterioration.

In the aerobic deterioration phase, air penetrates into a hitherto anaerobic environment; aerobic and facultative anaerobic microorganisms gain another opportunity to proliferate. Yeasts, molds, and acetic acid bacteria are mainly associated with aerobic deterioration, whereas *Bacillus* spp., LAB, and *Enterobacteria* may also abound in the aerobic deterioration phase (Merry and Davies, 1999). Several studies have shown that *Clostridium* spp. (obligate anaerobes) grow in air-exposed silages (Jonsson, 1991; Vissers et al., 2007).

Under aerobic conditions, yeasts can oxidize lactic acid and raise silage pH, which in turn facilitates the growth of other microorganisms. Therefore, yeasts are considered to be primarily responsible for the initiation of aerobic spoilage, and a population of  $10^5$  cfu/g at silo opening is regarded as a critical level, above which silages may deteriorate in the presence of air (McDonald et al., 1991). The roles of bacteria in initiating aerobic deterioration would not be greater than the roles of yeasts. Among acetic acid bacteria, *Bacillus* spp., *Enterobacteria*, and *Clostridium* spp., only acetic acid bacteria have been shown to initiate spoilage in the absence of simultaneous or previous yeast growth (Woolford and Wilkie, 1984). Therefore, acetic acid bacteria have been considered the most important targets for spoilage control, with the others being follow-up or opportunistic bacteria that may grow after increase in pH and acid oxidation. However, acetic acid bacteria are detectable almost exclusively in corn silages (Oude Elferink et al., 2001). This is in contrast to the fact that production of ethanol, the major substrate for acetic acid bacteria, is often minimal in corn silages compared to low-moisture grass silages. Furthermore, Kan and Nishino (2011) have shown that inoculation of *Acetobacter pasteurianus*, isolated from bunker-made untreated corn silage, does not enhance aerobic spoilage of corn silage, irrespective of the time of inoculation, i.e., either before ensiling or after silo opening. It is reasonable to state that obligate aerobic bacteria may initiate

aerobic spoilage by metabolizing fermentation products. However, the role of acetic acid bacteria is not conclusive and awaits further investigation.

For the suppression of microorganisms, undissociated acid is of primary importance, and dissociated molecules have minor effects (Courtin and Spoelstra, 1990; Muck and Pitt, 1991). Undissociated acid can passively diffuse into microbial cells through the cell membrane and split into anions and protons inside the cell, depending on the internal pH, thereby acidifying the cytoplasm and disrupting proton-motive force, as well as inhibiting substrate transport, energy-yielding processes, and macromolecular synthesis (Ostling and Lindgren, 1993). The pH has a marked influence on the proportion of acids present in undissociated or dissociated forms (Table 1); hence, the inhibitory activities of lactic, acetic, and propionic acids largely depend on pH (Matsuda et al., 1994). Lactic acid exhibits a strong antibacterial activity under acidic conditions, such as the antibacterial activities in well-preserved silages (pH 4.0); under insufficiently acidic conditions (pH 5.0 and 6.0), the activity of lactic acid against non-LAB is lower than the activity of acetic and propionic acids. Inhibition of yeasts and molds by lactic acid, however, hardly occurs at any pH; concentration of lactic acid at 50 g/L is equivalent to 115 g/kg DM in silages with a DM content of 300 g/kg. The minimum inhibitory concentrations of acetic acid and propionic acid against yeasts and molds at pH 4.0 are 5 g/L and 2.5 g/L, respectively, indicating that the antifungal activity of acetic and propionic acids is more than 10 and 20 times greater, respectively, than that of lactic acid. The antifungal activity is markedly reduced if the surrounding pH increases to 5.0 and 6.0, but acetic and propionic acids are yet better for fungal suppression than lactic acid is. During the actual ensiling process, lactic, acetic, and propionic acids exhibit synergistic action against yeasts (Moon et al., 1983). However, lactic acid may contribute minimally to the antifungal effects of fermentation products. Many yeast species such as *Pichia* and *Candida* spp. can assimilate lactic acid under aerobic conditions. This is yet another reason that yeasts are primarily responsible for the initiation of aerobic spoilage.

Silages considerably contribute to ruminant feeding in temperate and cold regions

(Wilkinson, 1999). In Japan, silages account for more than 70% of conserved forages (Ohmomo et al., 2002). Owing to the expansion of big bale machinery, preparation of low-moisture silages with long cut lengths, restricted fermentation, and insufficient acidity (pH >5.0) has increased. This may have raised the risk of contamination with undesirable *Enterobacteria*, *Bacillus* spp., yeasts, and molds, leading to lowered stability in both the fermentation and aerobic deterioration phases. In large-scale farming, by contrast, silage is more often made in bunker silos, in which a large area of the silo face is inevitably exposed to air. Management of aerobic deterioration is now considered critical and must be researched further.

Prediction of the onset of silage deterioration and the types of microorganisms that may initiate and carry out the spoilage on the basis of silo opening analysis is yet impossible. In this regard, a high concentration of acetic acid is useful against bacteria and fungi, and *Lactobacillus buchneri* has been considered a promising agent. However, alternatives to the heterofermentative LAB are necessary, because excessive acetic acid production remains a concern. Improvements, especially in the area of novel bacterial inoculant preparation, are necessary. A goal is to discover homofermentative or facultatively heterofermentative LAB with antifungal activity, and sources from which this target can be achieved are available.

Table 1. Minimum inhibitory concentrations (g/L) at different pH of lactic, acetic, and propionic acids against bacteria and fungi (Matsuda et al., 1994)

Microorganisms	Lactic acid			Acetic acid			Propionic acid		
	pH	pH	pH	pH	pH	pH	pH	pH	pH
	4.0	5.0	6.0	4.0	5.0	6.0	4.0	5.0	6.0
<b>Bacteria</b>									
<i>Escherichia coli</i>	1.25	15	25	5	15	35	<0.5	0.5	1.25
<i>Bacillus cereus</i>	5	25	40	nd	2.5	15	nd	0.5	0.5
<i>Bacillus licheniformis</i>	2.5	25	40	nd	0.5	5	nd	2.5	5
<i>Staphylococcus aureus</i>	nd	20	35	nd	5	30	nd	0.5	1.25
<i>Lactobacillus casei</i>	2.5	15	30	5	20	>50	5	15	>50

<i>Lactobacillus brevis</i>	2.5	15	40	5	25	>50	5	15	>50
<i>Lactobacillus helveticus</i>	nd	15	20	1.25	10	30	2.5	10	45
<i>Pediococcus pentosaceus</i>	5	15	25	2.5	15	40	<0.5	10	45
Yeasts and molds									
<i>Saccharomyces cerevisiae</i>	>50	>50	>50	5	15	>50	2.5	5	25
<i>Pichia anomala</i>	>50	>50	>50	2.5	10	35	1.25	5	25
<i>Candida krusei</i>	>50	>50	>50	5	20	30	2.5	10	>50
<i>Aspergillus oryzae</i>	>50	>50	>50	0.5	1.25	35	0.5	1.25	2.5

Medium composition: glucose 10 g/L, peptone 5.0 g/L, yeast extract 3.0 g/L, KH<sub>2</sub>PO<sub>4</sub> 0.3 g/L, K<sub>2</sub>HPO<sub>4</sub> 0.3 g/L, and MgSO<sub>4</sub> 0.1 g/L.

### **Bacteria detectable in deteriorated silage**

#### *Enterobacteria*

Enterobacteria are facultative anaerobes and can survive in the silage environment if acidification is insufficient. They can also be detected in deteriorated silage after increase in pH, which is a result of lactic and acetic acid oxidation. Lindgren et al. (1985) counted a high number of enterobacteria ( $>10^8$  cfu/g) in severely deteriorated corn silage, even though the pH was low and lactic acid concentration was high at silo opening. Because most enterobacteria do not proliferate at pH lower than 4.5, they are regarded as follow-up microflora; however, spoilage-associated species have not been well studied. Li and Nishino (2011a, b) identified, by culture-independent community analysis, *Erwinia*, *Rahnella*, *Pantoea*, *Morganella*, and *Klebsiella* spp. in deteriorated grass and corn silage. *Erwinia* and *Rahnella* spp. were also found as the dominant enterobacteria in fresh grass (Heron et al., 1993), but their presence in the aerobic deterioration phase was not examined.

The number of enterobacteria in standing crops sometimes exceeds that of LAB, and an increase in the numbers of both enterobacteria and LAB has been observed during wilting. Manure application may increase the number of enterobacteria in standing crops; however, the number will decrease with time (Ostling and Lindgren, 1991). Manure application, therefore, does not affect the

proportion of enterobacteria in silage crop, but slow acidification may occur if the growth of enterobacteria is vigorous during initial stages of the fermentation phase. Contamination with *Escherichia coli* is an important risk factor in terms of animal health and food hygiene (Wilkinson, 1999), but *E. coli* constitutes a small proportion (approximately  $10^{-3}$  times) of the total number of enterobacteria during ensiling (Ostling and Lindgren, 1991).

The minimum inhibitory concentration of undissociated lactic acid against enterobacteria is 0.5–0.8 g/L, which is much lower than the normal content (approximately 10 g/L) observed in silages with desirable lactic acid fermentation (Wilkinson, 1999). Therefore, even when large numbers of enterobacteria are present in silage crops, they may not be prevalent, and spoilage will be initiated by microorganisms other than enterobacteria. However, if the growth of LAB is retarded by extensive wilting or delayed sealing, enterobacteria may predominate and produce ethanol and 2,3-butanediol at levels higher than the levels of lactic and acetic acids. This alcoholic fermentation may retain the silage pH at above 5.0, and low concentrations of undissociated acids may facilitate the growth of aerobic yeasts and molds. Proliferation of enterobacteria during the initial stages of ensiling could therefore be regarded as a causal factor of aerobic spoilage.

### *Bacillus*

*Bacillus* spp. are spore-forming obligate or facultative anaerobes. Their density varies ( $10^1$ – $10^5$  cfu/g) during silo opening, with larger populations observed in grass silage than in corn silage (Te Giffel et al., 2002). Soil is the main habitat of *Bacillus* spp.; therefore, their numbers can increase if the silage is contaminated with soil or manure at harvest. Silage in the surface layer of a bunker silo may also have a high number of *Bacillus* spp., because air would infiltrate more into the silage mass located close to the surface compared with silage mass in deeper positions (Lindgren et al., 1985).

Inoculation of *Bacillus* spp. during silo opening did not enhance aerobic deterioration of  $\gamma$ -irradiated corn silage (Woolford and Wilkie, 1984). Thus, although a marked increase in the

number of *Bacillus* spp. (Lindgren et al., 1985) and change in bacterial composition from LAB to *Bacillus* spp. (Inglis et al., 1999) were observed after aerobic exposure, *Bacillus* spp. are not regarded as initiators of aerobic deterioration. Many *Bacillus* species, including *B. cereus*, *B. coagulans*, *B. circulans*, *B. pumilus*, and *B. licheniformis*, are found in deteriorated silage (Woolford, 1990; Inglis et al., 1999; Li and Nishino, 2011a, b); however, whether the various species prefer specific crops and impair aerobic stability remains unknown.

Silage is identified as the main source of aerobic and anaerobic spores in milk (Te Giffel et al., 2002). *Bacillus* spp. are ubiquitous in nature; therefore, silage and milk will inevitably be contaminated by a few *Bacillus* spores. The species detected in silage have been shown to cause food spoilage; hence, *Bacillus* growth and spore formation must be discouraged both during ensiling and after exposure to air.

### *Clostridium*

*Clostridium* spp. are also spore formers but are unable to grow under aerobic conditions. The number of proteolytic and lactic acid-assimilating species may increase during the later stage of fermentation phase and produce ammonia and butyric acid, thereby lowering the nutritive value of silages (McDonald et al., 1991). Among fermentation products, butyric acid is a strong inhibitor of fungal growth; therefore, from an aerobic spoilage inhibition perspective, silages with high butyric acid content are stable.

Because *Clostridium* spp. are obligate anaerobes, their presence in silages has been related to anaerobic instability in the late fermentation phase and not to aerobic instability in the aerobic deterioration phase (Driehuis and Oude Elferink, 2000). However, Jonsson (1991) observed an increase in *Clostridium* spores from  $10^5$  cfu/g at silo opening to  $10^6$  or  $10^7$  cfu/g after aerobic spoilage in *C. tyrobutyricum*-inoculated grass silage. Furthermore, Vissers et al. (2007) reported that *Clostridium* spores at density higher than  $10^5$  cfu/g were observed in grass and corn silages, especially in the surface layers and in the molded spots of bunker and clamp silos. Growth of strictly

anaerobic *Clostridium* spp. in air-exposed silages is difficult to envision but may be explained as follows: Oxygen penetrating the silage mass might have initiated the growth of aerobic and acid-tolerant microorganisms (both bacteria and yeasts), which might have oxidized residual sugars and fermentation acids and increased the silage pH. When oxygen was completely consumed, anaerobic niches with an elevated pH might have developed, enabling the multiplication of *Clostridium* spp. in the deteriorated silage. While this hypothesis needs to be validated by further experiments, survival of *Clostridium* spp. in the fermentation and aerobic deterioration phases should be minimized.

#### *Acetic acid bacteria*

Besides yeasts, acetic acid bacteria, which are Gram-negative and catalase-positive rods, are known to play a role in initiating the aerobic deterioration of corn silages. The species isolated from silages belong to the *Acetobacter* genus, although 10 genera are presently recognized as members of the acetic acid bacteria family (Yamada and Yukphan, 2008). Oxygen is considered necessary for the growth of acetic acid bacteria; however, a strain capable of surviving under anaerobic conditions has been identified (Du Toit et al., 2005). Oxidation of ethanol to acetic acid is the best-known characteristic of acetic acid bacteria; however, oxidation of lactic and acetic acids to CO<sub>2</sub> and H<sub>2</sub>O is also an important function of these bacteria.

Inoculating corn silage with acetic acid bacteria accelerates the onset of heating in the presence of air (Spoelstra et al., 1988). In this study, ethanol was first oxidized to acetic acid without an increase in the silage pH. Thereafter, when ethanol was depleted, lactic and acetic acids were oxidized to set off distinctive spoilage. These reactions were observed with or without simultaneous proliferation of yeasts, indicating that acetic acid bacteria can be solely responsible for initiating aerobic deterioration. An interesting property of acetic acid bacteria in ensiling is crop difference; acetic acid bacteria are found exclusively in corn and other cereal silages (Oude Elferink et al., 2001). Although extensive ethanol fermentation may occur at a higher rate in low-moisture grass silages



than in corn silages, acetic acid bacteria are seldom detected in aerobically stable or unstable grass silages. The reason for the absence of acetic acid bacteria in grass silages is unknown and needs to be examined.

The role of acetic acid bacteria in ensiling and aerobic spoilage is still inconclusive. Kan and Nishino (2011) examined the effects of *A. pasteurianus* inoculant, which was isolated from bunker-made corn silage. Their study revealed that addition of *A. pasteurianus* ( $10^5$  cfu/g) during ensiling elicited a slight improvement in aerobic stability of corn silage but not grass silage. Spoilage was not accelerated in either corn or grass silage when *A. pasteurianus* ( $10^5$  cfu/g) was added after silo opening, although survival of *A. pasteurianus* during the spoilage process was confirmed by the detection of 16S rRNA and PQQ-dependent alcohol dehydrogenase genes. Acetic acid bacteria are acid tolerant, aerobic, and capable of oxidizing ethanol, lactic acid, and acetic acid; hence, the hypothesis that acetic acid bacteria and yeasts initiate aerobic deterioration is undoubtedly reasonable. However, unaccountable differences exist between grass and cereal silages, and acetic acid bacteria are not usually counted despite the wide recognition of their importance in aerobic deterioration. Microbial ecology of yeasts and other bacteria is well understood, but there is room for improvement in our understanding of acetic acid bacteria in forage conservation.

### *Lactic acid bacteria*

Lactic acid bacteria are facultative anaerobes, and a large population is occasionally detected in aerobically deteriorated silages. The species identified are mainly *Lactobacilli*, i.e., *L. plantarum*, *L. brevis*, *L. buchneri*, and *L. bulgaricus*, but *Enterococcus* and *Pediococcus* spp. have also been detected (Woolford, 1990). Lactic acid bacteria are acid tolerant and well adapted to silage environment, and thus, major populations found at silo opening can be LAB with desirable lactic acid fermentation. These surviving LAB may have a greater opportunity to prevail over the other bacteria after silo opening. Furthermore, some LAB have been shown to metabolize lactic acid into acetic acid and CO<sub>2</sub> under aerobic conditions in the absence of sugars (Quatravaux et al., 2006).

Inoculation of LAB, however, did not enhance aerobic deterioration of  $\gamma$ -irradiated corn silage (Woolford and Wilkie, 1984). Further, changes in silage pH may be minimal even if lactic acid is oxidized to acetic acid and CO<sub>2</sub>. Because LAB have limited proteolytic and deaminating activity, the growth of LAB during the aerobic deterioration phase may not increase the concentrations of amines and ammonia. Therefore, although some LAB species exhibit distinctive persistence during the spoilage process (Li and Nishino, 2011b), LAB are considered to have little or marginal impact on the aerobic instability of silages.

### **Bacteria capable of spoilage inhibition**

#### *Propionibacteria*

Because the antifungal activity of propionic acid is greater than that of lactic and acetic acids, propionibacteria became the popular choice for inhibiting aerobic deterioration in the 1980–1990s. Propionibacteria can ferment glucose or lactic acid to produce acetic and propionic acids, with the latter being produced at higher levels than the former (Merry and Davies, 1999). Thus, if the bacteria were successfully grown in rapidly fermenting silages where lactic acid bacteria play a major role, the concentration of antifungal propionic acid could increase substantially. However, silages may not be suitable for the growth or survival of propionibacteria, because only a few groups have successfully isolated propionibacteria from silages. Published results indicate inconsistent or unreliable effects of propionibacteria on propionic acid production and stability improvement (Merry and Davies, 1999). Therefore, after the appearance of *L. buchneri* as an alternative inoculant, attention shifted to the heterofermentative LAB species.

#### *Lactic acid bacteria*

The majority of aerobic spoilage is initiated by yeasts, but attention should always be paid to suppressing the growth of bacteria. In this regard, synergistic action of antibacterial lactic acid and antifungal acetic acid is better than the singular action of propionic acid-producing bacteria. Because

homofermentative LAB exclusively produce lactic acid, inoculation of LAB species often fails to improve or sometimes worsens aerobic stability. Inoculation of heterofermentative LAB can increase antifungal acetic acid content, but typical species such as *L. brevis* and *L. fermentum* may not yield sufficient levels (>50 g/kg DM) of acetic acid to prevent spoilage (Danner et al., 2003; Wang and Nishino, 2009). Inoculation of *L. buchneri*, in contrast, can yield sufficient acetic acid levels if added at  $10^{5-6}$  cfu/g during ensiling, because *L. buchneri* can metabolize lactic acid to acetic acid and 1,2-propanediol under anaerobic conditions (Oude Elferink et al., 2002). This metabolism reduces the level of lactic acid during fermentation; hence, oxidation of lactic acid by yeasts can also be reduced after silo opening. Anaerobic degradation of lactic acid into acetic acid is also observed with *L. plantarum* (Lindgren et al., 1990); however, the activity is limited to conditions in which sugars are unavailable, and thus, manipulation of acetic acid production using this metabolism is rather insecure and unreliable. In silages inoculated with *L. buchneri*, 1,2-propanediol can be degraded anaerobically by *L. diolivorans* into propionic acid and 1-propanol (Krooneman et al., 2002), both of which can further promote stability after exposure to air (Danner et al., 2003). Because excessive acetic acid production may suppress silage intake, commercial preparations are often mixed with homofermentative LAB species to attenuate the activity of *L. buchneri*.

Increasing attention has been directed toward the use of antifungal substances produced by LAB. Although most studies on antimicrobial activity have focused on antibacterial effects, a few studies have described inhibitory effects of LAB on spoilage yeasts and molds. If homofermentative or facultatively heterofermentative LAB with strong antifungal activity are identified, concerns about DM recovery and feed intake can be minimized. A leading strain for this concept is *L. plantarum* MiLAB 393, which was isolated from grass silage (Strom et al., 2002). This strain produces 3 antifungal substances, i.e., phenyllactic acid, cyclo(L-Phe-L-Phe), and cyclo(L-Phe-*trans*-4-OH-L-Phe). The same research group that isolated these antifungal substances screened more than 1,200 LAB isolates by using *Aspergillus fumigatus* as an indicator and found strong inhibitory activity in 42 isolates (Magnusson et al., 2003). *Lactobacillus coryniformis*, *L.*

*plantarum*, and *Pediococcus pentosaceus*, which were frequently identified, produced unknown antifungal substances other than phenyllactic acid, cyclo(L-Phe-L-Phe), and cyclo(L-Phe-*trans*-4-OH-L-Phe). None of the antifungal isolates could inhibit the growth of *Pichia anomala* and *Penicillium roqueforti*, both of which are important spoilage fungi in silages; however, these works should be expanded to develop sound LAB inoculants besides *L. buchneri*.

Silage and fermented foods of long shelf life may be a good source of novel LAB inoculants. High survival and well adaptation to the fermentation process are key factors, and propionibacteria have failed to fulfill these criteria. In this regard, total mixed ration (TMR) silage is an interesting material. In Japan, some companies and contractors manufacture commercial TMR silages by mixing wet byproducts with dry feed. Wet brewers grains and soybean curd residue are often used as the main ingredients, and feedstuffs such as grass hay, legume hay, cracked corn grain, soybean meal, and wheat bran are added in lower proportions. A beneficial property of TMR silages is their high stability in the presence of air; no heating would occur even in summer, although non-ensiled TMR mixtures will easily deteriorate within a day or 2. Furthermore, the TMR silage was stable even with large yeast populations ( $10^6$  cfu/g) at silo opening, despite the fact that silage with  $>10^5$  cfu/g of yeasts is prone to deterioration in the presence of air (Nishino et al., 2004; Wang and Nishino, 2008). We isolated *L. buchneri* as the predominant LAB from a TMR silage containing wet brewers grains (Wang and Nishino, 2008), and detected sourdough LAB, i.e., *L. panis*, *L. hammesii*, *L. mindensis*, *L. frumenti*, *L. farciminis*, and *L. pontis* (Meroth et al., 2003; De Vuyst et al., 2009), in commercial TMR silages collected from several regions and manufacturers (Wang and Nishino, 2010). Both TMR silage and sourdough have a long shelf life; hence, the ability of sourdough LAB to inhibit aerobic spoilage is worth investigating.

## Conclusions

Because yeasts are well known to play a major role in initiating spoilage, less attention has been paid lately to the involvement of bacteria in the aerobic deterioration process. The role of

bacteria had been investigated intensively in the 1970–1980s, and those studies have greatly contributed to our present understanding. However, there is room for further improvement with respect to how and when *Acetobacter* spp. and *Clostridium* spp. can be activated, and whether previous culture-based knowledge can be modified by incorporating culture-independent analysis. The search for novel homofermentative LAB inoculants capable of producing antifungal substances seems attractive and challenging. To achieve these goals, research is indeed worth continuing.

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