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Fluorescent detection of nisin by genetically modified *Lactococcus lactis* strains in milk and a colonic model: Application of whole-cell nisin biosensors

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Detection of bioactive peptides in complex ecosystems like intestinal environment is a difficult task. In this study, we developed two new bioreporters for nisin based on *Lactococcus lactis* NZ9000 transformed with the vector pNZ:Nis-aFP or pNZ:Nis-mCherry, that encoded for the anaerobic fluorescent protein evoglow-Pp1 (aFP) or the fluorescent protein mCherry, respectively. The biosensors were used to study nisin A production by *L. lactis* INIA 650 in milk and in a colonic model. The use of *L. lactis* NZ9000 pNZ:Nis-aFP as a biosensor allowed the detection of nisin produced by *L. lactis* INIA 650 in milk, but not in the *in vitro* colonic model. In milk, this reporter was induced by direct addition of 10 ng/ml nisin while, in the colonic model, nisin concentrations of 50 ng/ml were necessary. However, the reporter system based on pNZ:Nis-mCherry showed a higher sensibility, detecting nisin concentrations of 1 ng/ml produced by *L. lactis* INIA 650 in colonic media using agar diffusion or cross streak bioassays.

[**Keywords:** Nisin; *Lactococcus lactis*; Biosensor; Fluorescent proteins; Food; Colonic model]

The use of bioprotective cultures in fermented foods have been considered a good alternative to chemical preservatives in the last decades. These bioprotective cultures produce antimicrobial substances as organic acids, hydrogen peroxide, specific enzymes, diacetyl, reuterin or bacteriocins (1–4) that are able to inhibit the growth of spoilage or pathogen bacteria in these foods (5). Bacteriocins are ribosomally produced proteinaceous antimicrobial molecules from bacteria that are exported to the extracellular medium (6,7). Nisin, a bacteriocin produced by several *Lactococcus lactis* strains (8), is the most characterized and commercialized bacteriocin (9). It was approved as a food additive by the Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) in 1969 and was authorized for food preservation (named as E 234) in the European Union by Directive 95/2/EC (10). The European Food Safety Authority (EFSA) authorized initially its use in ripened or processed cheese, certain puddings, clotted cream and mascarpone (11), although this was later extended to unripened cheese and heat-treated meat products (12).

The ability of nisin or nisin producing strains to restrict the growth of gram-positive pathogenic bacteria such as *Listeria monocytogenes* or *Staphylococcus aureus*, alone or in combination with other antimicrobials, have been widely studied in several foods (13–16). However, it is not known whether *L. lactis* strains are able to produce nisin *in situ* in the colon, or whether nisin is completely degraded or inactivated in this environment.

Different immunochemical methods with high sensibility have been published for nisin detection, but they are not totally reliable, due to cross-reactions with compounds structurally related to nisin, which can be present in the testing material (17). In addition, nisin bioassays based on nisin-inducible bioluminescence have been reported. These systems involve bacterial luciferase *luxAB* reporter genes placed under control of a nisin inducible promoter. However, the growth stage of the indicator strain could affect luciferase activity (18,19). In a similar approach, nisin-induced bioassays have been developed using fluorescent reporter genes as the green fluorescent protein gene (GFP) (20) or GFPuv (21). Although the last method has shown a high sensibility, food samples must be diluted before testing as some food components could interfere with the fluorescence. Moreover, the application of these fluorescent proteins in anoxic environments could have limitations since they need the presence of oxygen to express fluorescence (22).

Thus, the objective of the present work is to obtain fluorescent biosensors to assess nisin A production by *L. lactis* INIA 650 strain (23) in a food matrix, and in an *in vitro* colonic fermentation model. Considering this, we developed two new constructs using the no nisin producer *L. lactis* NZ9000, with *NisRK* genes, essentials for a nisin-controlled expression (NICE) system (24), and the plasmid pNZ8048 (with a nisin A inducible promoter P_{nisA}) (25), that controls the expression of anaerobic fluorescent protein gene *evoglow-Pp1* (*L. lactis* NZ9000 pNZ:Nis-aFP) or of *mrfp* gene encoding the fluorescent mCherry protein (*L. lactis* NZ9000 pNZ:Nis-mCherry), in the presence of nisin in the media.

MATERIALS AND METHODS

Bacterial strains and culture conditions All bacteria used in this study, *L. lactis* subsp. *lactis* INIA 650 and *L. lactis* subsp. *lactis* NZ9000 and their transformed strains are listed in Table 1 (23–26). *Lactococcus* strains were grown at 30 °C in M17

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broth (Scharlau Chemie SA, Barcelona, Spain) supplemented with 5 g/L glucose (Sigma–Aldrich, St Louis, MO, USA) (GM17) under aerobic conditions and subcultured twice from frozen stocks before the experiments.

Erythromycin or chloramphenicol (both from Sigma–Aldrich) were added, if necessary, as a selective agent at 5 μg/ml. For milk assays, the strains were grown previously in skimmed milk reconstituted to 10 % and sterilized. Bacterial counts were determined by plating decimal dilutions on duplicate plates of GM17 alone or with its corresponding antibiotic and incubated at 30 °C for 24 h.

Construction of plasmid pNZ:Nis-aFP The *evoglow-Pp1* anaerobic fluorescence protein gene was first amplified by PCR using pGlow-C-Pp1 as the template and the primers For-aFP TTTTCCATGGTCAACGCAAAACTCCTGCAACTG/Rev-aFP TTTTTCTAGATCAGTGCTTGGCCTGGCCCTGCTG. The PCR product was digested with *Nco*I and *Xba*I and ligated into the corresponding restriction sites of the vector pNZ8048, which contains the nisin A inducer promoter (NICE system) and translational initiation region. Restriction endonucleases and T4 DNA ligase came from New England Biolabs (Hitchin, UK). *L. lactis* NZ9000 strain was transformed with the ligation mixture containing pNZ:Nis.aFP according to Landete et al. (27). Transformants were checked by restriction mapping and sequencing of the inserted fragment.

Construction of plasmid pNZ:Nis-mCherry The synthetic *mrfp* gene, whose codon usage was optimized for LAB and encoding the monomeric red fluorescence protein mCherry (28), was amplified from pRCR12 plasmid (26) with the primers F-mCherry (5′-TTTCCATGG ATTCAGATATGGTTTCAAAAGGGAGG-3′) and R-mCherry (5′-TTTCTAGATCATTTATATAATTCGTCCATGCCACCTG-3′). After, this PCR fragment and pNZ8048 were digested with *Nco*I/*Xba* I and ligated. The mixture of ligation was transformed in *L. lactis* NZ9000 according to Landete et al. (27). Transformants were checked by restriction mapping and sequencing of the inserted fragment.

Nisin production in milk Nisin production by *L. lactis* INIA 650 inoculated at 1% (v/v) in sterile reconstituted milk and incubated for 24 h was studied at two different conditions: 30 °C under aerobic conditions and 37 °C under anaerobic atmosphere (Whitley DG250 anaerobic workstation, Don Whitley Scientific Ltd., Shipley, UK), as well as in the presence of the *L. lactis* NZ9000 based biosensors. Supernatants for nisin detection were obtained after centrifugation at 12,000 ×*g* for 5 min, followed by pH adjustment to 6.0 with 2 mol l^{-1} NaOH and filtration through 0.22 μm pore size low protein binding filters (Millex GV, Millipore, Molsheim, France). Negative control supernatants were obtained from milk, in which pH was decreased up to 4.2 to precipitate its protein content before centrifugation. Filtered supernatants were stored at − 80 °C until use.

***In vitro* colonic fermentation model** The colonic model consisted of an *in vitro* fermentation system as previously described (29). Batch fermentors with 15 ml of a sterilized faecal suspension and 135 ml growth media (10% v/v) were magnetically stirred and maintained at 37 °C with a continuous sparring with oxygen-free nitrogen (Whitley DG250 Anaerobic workstation). In each experiment, a 3 ml sample was taken from each vessel at 0.5, 6 and 24 h. Supernatants were obtained from these samples as explained before. Each trial was performed in duplicate.

Batch fermentors were inoculated at 1% v/v with the nisin-producing strain *L. lactis* INIA 650. Supernatants were obtained as previously described. For assays including the biosensor, *L. lactis* NZ9000 pNZ:Nis-aFP strain was inoculated at 1% with *L. lactis* INIA 650 or with 10 ng/ml nisin A (from *L. lactis* 2.5%, Sigma–Aldrich). Batches without nisin or nisin-producing strain were kept as controls. Milk samples with *L. lactis* INIA 650 or 10 ng/ml nisin incubated with the bioreporter strain were used as positive control. Samples were diluted, plated and checked for fluorescent *L. lactis* NZ9000 pNZ:Nis-aFP colonies.

Nisin detection and quantification by agar diffusion bioassays Plate diffusion bioassays were performed as described by Tramer and Fowler (30) and modified by Dodd et al. (31). Assays were carried out in triplicate to detect nisin A antimicrobial activity in culture supernatants.

GM17 plates were overlaid with 0.7 % soft agar and inoculated at 0.1 % with *L. lactis* NZ9000 (control), *L. lactis* NZ9000 pNZ:Nis-aFP or *L. lactis* NZ9000 pNZ:Nis-mCherry. A volume of 25 μl of each supernatants were placed in 5 mm diameter wells. Nisin (Sigma–Aldrich) or supernatants from *L. lactis* INIA 650 grown in GM17 were included as positive controls. Control media was used as negative control. Plates were maintained approximately for 1 h at 4 °C and incubated overnight at 30 °C, at aerobic conditions (32). Then, plates were examined for inhibition zones and fluorescence. Nisin concentration in supernatants was calculated according to the equation described by Tramer and Fowler (30) by comparison of the inhibition zone areas obtained in our samples and nisin standards (Sigma–Aldrich) ranged from 0 to 150 ng/ml. Agar diffusion assays in plates inoculated with the reporter strains *L. lactis* NZ 9000 pNZ:Nis-aFP or *L. lactis* NZ 9000 pNZ:Nis-mCherry and known nisin concentrations (0–1000 ng/ml) diluted in PBS or colonic media were also performed.

Nisin detection and quantification by the cross streak technique *L. lactis* INIA 650 and *L. lactis* NZ9000 pNZ:Nis-mCherry were previously grown in GM 17 broth for 18 h. Then, 1 ml culture was centrifuged, washed three times and finally resuspended in 500 μl peptone water. A single streak of *L. lactis* INIA 650 inoculum was placed in the centre of a colonic agar plate with colonic fermentation media with 1:10 filtered sterilized faecal suspension (as previously) and 1.5 % agar. After 30 min, the plates were seeded with the reporter bacteria, or the parental strain *L. lactis* NZ9000 as negative control, by a single streak at a 90° angle to the other streak and plates were incubated for 24 h under anaerobic (anaerobic chamber) and microaerophilic conditions (Oxoid CampyGen sachets, Thermo Fisher Scientific, Basingstoke, UK). Plates were then incubated for 1 h at room temperature and analyzed for fluorescence.

Detection of fluorescence in colonies and agar diffusion bioassays *L. lactis* NZ9000 pNZ:Nis-aFP colonies from milk and batch fermentors as well agar diffusion bioassays were checked for fluorescence by a ChemiDoc MP imaging system (Bio-Rad Laboratories S.A., Madrid, Spain) with blue epi-illumination (Alexa 488) and emission filter of 530/28 nm as described previously by Landete et al. (22). White epi illumination standard filter was also used as control. Agar diffusion plates with parental *L. lactis* NZ9000 strain were used as negative controls.

In agar diffusion bioassays and cross streak plates using *L. lactis* NZ9000 pNZ:Nis-mCherry as indicator strain, red fluorescence was checked using a Chem-iDoc MP imaging system with green epi-illumination (Alexa 546) and emission filter of 605/50 nm. Agar diffusion and cross streak plates with parental *L. lactis* NZ9000 strain were used as negative controls.

Statistical analysis Results from the experiments performed in duplicate were submitted to one-way analysis of variance using program Statview, version 5.0 (SAS Institute, Cary, NC, USA). This statistical analysis was used for the comparison of means, with significance assigned at $P < 0.05$.

RESULTS

***L. lactis* NZ9000 pNZ:Nis-aFP as nisin bioreporter in milk or colonic environment** *L. lactis* INIA 650 viability in milk at different conditions and in a colonic model was studied. Bacterial counts after 24 h incubation showed no significant differences between the incubation conditions of the milk, reaching up to 10 log cfu/ml after 24 h. On the other hand, *L. lactis* INIA 650 counts in an *in vitro* colonic model reached levels up to 8.2 ± 0.2 log cfu/ml after 6 h and 5.7 ± 0.8 log cfu/ml after 24 h.

When L. *lactis* INIA 650 was incubated for 24 h in both milk at 30 °C under aerobic conditions and at 37 °C under anaerobic

TABLE 1. Bacterial strains and plasmids used in this work.

Strain/plasmid	Relevant phenotype or genotype	Reference
Strain		
L. lactis subsp. *lactis* INIA 650	Nisin A-producing parental strain (previously TAB50)	23
L. lactis subsp. *lactis* NZ9000	None nisin-producer; harboring the NICE system	24
L. lactis NZ9000 pNZ:Nis-aFP	Harbors pNZ:Nis-aFP plasmid; Cm^r	This work
L. lactis NZ900 pNZ:Nis-mCherry	Harbors pNZ:Nis-mCherry plasmid; Cm^r	This work
Plasmid		
pNZ8048	Expression vector for gram-positive bacteria harboring the inducible nisin promoter (P_{nisA}); Cm^r	25
pGlow-C-Pp1	Template for the anaerobic fluorescence protein gene *evoglow-Pp1* (aFP); Amp^r	Evocatal[a]
pRCR12	Template for the synthetic *mrfp* gene encoding the monomeric red fluorescence protein mCherry; Cm^r	26
pNZ:Nis-aFP	pNZ8048 with P_{nisA} and anaerobic *evoglow-Pp1*; Cm^r	This work
pNZ:Nis-mCherry	pNZ8048 with P_{nisA} and *mrfp*; Cm^r	This work

[a] Evocatal GmbH (Düsseldorf, Germany).

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conditions, concentrations higher than 500 ng nisin/ml were quantified by using *L. lactis* NZ9000 or *L. lactis* NZ9000 pNZ:Nis-aFP as indicator strain. The presence of fluorescence around inhibition halos confirmed that the inhibition was due to nisin (Fig. 1A–D). Nisin production decreased slightly (to approximately 450 ng/ml) in milk when *L. lactis* INIA 650 was incubated together with *L. lactis* NZ9000 pNZ:Nis-aFP. No nisin activity was detected from the supernatants obtained from the colonic batch fermentors inoculated with *L. lactis* INIA 650 (Fig. 1A–D).

Fluorescent colonies from the bioreporter *L. lactis* NZ9000 pNZ:Nis-aFP were observed when this was cocultured with *L. lactis* INIA 650 or by the addition of 10 ng/ml nisin to milk, and incubated at 30 °C during 24 h (Fig. 1F–H). No fluorescent colonies were detected when this assays was carried out in the colonic model (Fig. 1E–G). Inhibition zones and fluorescence were obtained from supernatants from milk incubated with 10 ng/ml nisin, but no inhibition zones or fluorescence were obtained from supernatants from colonic model with the same nisin concentration (data not shown). Assays performed in colonic media with different nisin concentrations (0–1000 ng/ml) indicate a detection limit of 50 ng/ml of nisin using *L. lactis* NZ9000 pNZ:Nis-aFP as bioreporter (Fig. 2A).

***L. lactis* NZ9000 pNZ:Nis-mCherry as nisin bioreporter under colonic environment** Agar diffusion assays performed in colonic media with different nisin concentrations (0–1000 ng/ml) indicate a detection limit of 1 ng/ml nisin when *L. lactis* NZ9000 pNZ:Nis-mCherry was used as a bioreporter strain (Fig. 2B). Supernatants obtained from colonic batch fermentors with *L. lactis* INIA 650 showed nisin activity when *L. lactis* NZ9000 pNZ:Nis-mCherry was used as indicator microorganism in the agar diffusion assays. Nisin production levels was estimated at around 10 ng/ml after 0.5 and 6 h under colonic conditions, and it decreased after 24 and 48 h to concentrations of approximately 1 ng/ml (Fig. 3A and B). Experiments using this biosensor in the colonic model with different added nisin concentrations showed a time dependent nisin degradation (Fig. S1) at low nisin concentrations.

Cross streak experiments performed in faecal semisolid agar showed red fluorescence in the intersection of *L. lactis* INIA 650 and *L. lactis* NZ9000:Nis-mCherry under microaerobic conditions, but not in the intersection of *L. lactis* INIA 650 and the parental strain *L. lactis* NZ9000 (Fig. 3C–F). The detected red fluorescence in the bioreporter strain indicates presence of nisin produced by *L. lactis* INIA 650.

DISCUSSION

L. lactis is a species normally used as starter or bioprotective culture in cheese production (33) that can be metabolically active in the gut (34). Some *L. lactis* strains are able to produce nisin, an antimicrobial agent active against food-borne microorganisms, of which inhibitory activity has been studied in different foods (5). However, little is known about its antimicrobial activity once it goes through the human gastrointestinal tract (GIT). Previous studies have shown degradation of nisin when it goes through the GIT (35), however, it could be produced by *L. lactis* in the colon, extend its protective antimicrobial activity, and even affect the host microbiota. *L. lactis* INIA 650 has been previously shown to be able to grow and survive in milk and dairy products, producing large yields of nisin (23,36). Two new biosensors with a nisin inducible promoter were developed in this work in order to track and detect

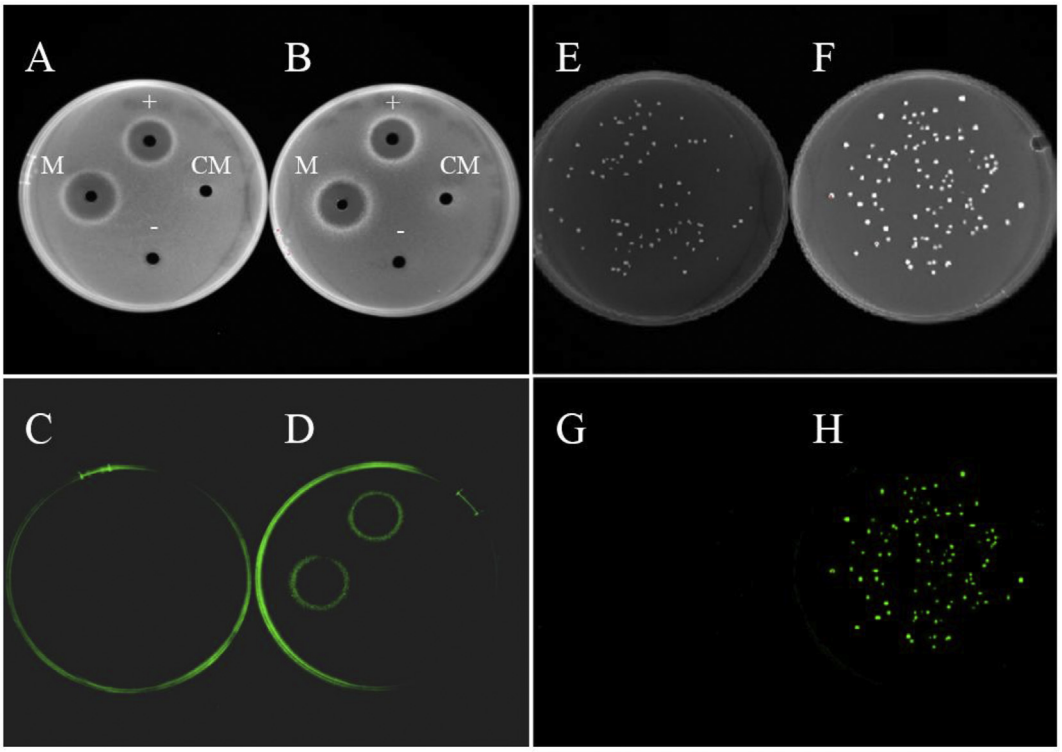

FIG. 1. Determination of fluorescence and antimicrobial activity of culture supernatants in agar diffusion assays in GM17 0.7 % agar incubated at 30 °C with 0.1 % *L. lactis* NZ9000 parental (A, C) or pNZ:Nis-aFP reporter strain (B, D). Supernatants were obtained from *L. lactis* INIA 650 grown in GM17 (+), control milk (−), *L. lactis* INIA 650 grown in a colonic model for 24 h (CM) or grown in milk at 37 °C under anaerobic conditions (M). Detection of fluorescent colonies in plates inoculated with *L. lactis* NZ9000 pNZ:Nis-aFP incubated for 24 h with 10 ng/ml nisin in a colonic model at 37 °C under anaerobic conditions (E, G) and in milk at 30 °C for 24 h (F, H). Filters used were white epi-illumination, standard filter (A, B, E, F) and blue epi-illumination, 530:28 filter (C, D, G, H).

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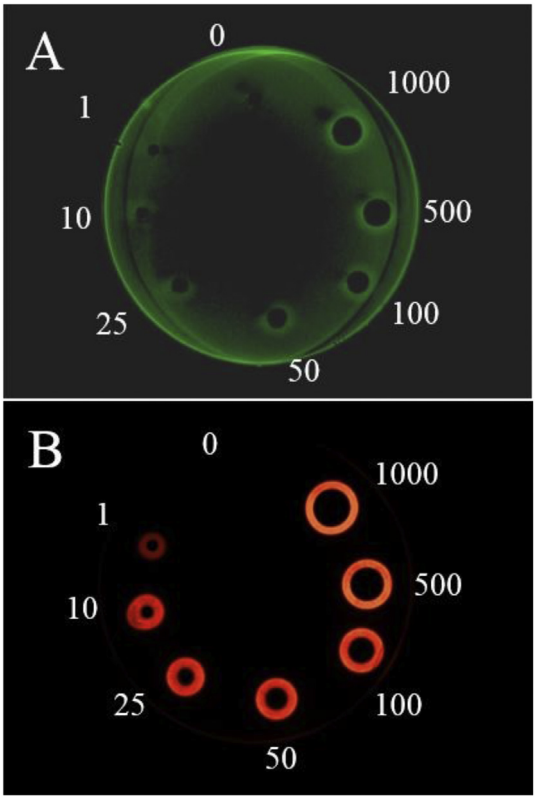

FIG. 2. Fluorescence determination in agar diffusion assays in GM17 0.7% agar incubated at 30 °C with 0.1 % *L. lactis* NZ9000 pNZ:Nis-aFP (A) or *L. lactis* NZ9000 pNZ:Nis-mCherry (B) reporter strains and supernatants of different concentrations of nisin (0–1000 ng/ml) in colonic fermentation media. The filters used were: blue epi-illumination, 530:28 filter (A) and green epi-illumination, 605:50 filter (B).

nisin production in milk, as a food model, and in an *in vitro* human colonic fermentation model (29,37).

Plate diffusion bioassays with a sensitive indicator strain have been frequently used for quantification of nisin. However, several factors could interfere with the accuracy and sensitivity of this method (38,39). Microbiological, immunochemical and nisin-inducible bioluminescence methods have been used to detect nisin in pure solutions with high sensitivity; however, their detection limit is increased in complex food matrices (17–19,32). Bacterial biosensors based on luciferase need the presence of the substrate luciferin and oxygen, being necessary to verify that there is enough oxygen for the reaction to occur, as well as the growth stage of the indicator strain (40). An advantage of whole-cell fluorescent protein-based nisin biosensors is that they do not require any substrate or additional cofactors for fluorescence and showed an easily detectable phenotype. The application of these biosensors can reduce the detection limit in complex food matrices (0.2–45 ng/ml) and/or the time to perform the bioassay (20,21). The strict requirement for molecular oxygen for the synthesis of fluorophores could be also a major drawback of the use of fluorescent proteins. Flavin-mono-nucleotide-based fluorescent proteins, as the Evoglow-Pp1 protein tested here, could overcome oxygen restrictions, although their limited brightness can be an obstacle (41).

Here, we created two different biosensors. In the first one, *L. lactis* NZ9000 was transformed with the plasmid pNZ8048:Nis-aFP, harboring the *evoglow-Pp1* gene (22) and the P_{nisA} promotor. The use of this anaerobic fluorescent protein has been of special interest in the case of anaerobic lactic acid bacteria or environments, like the colonic one. Furthermore, its expression is independent of pH in a range from 3 to 8, being suitable to fermented foods and colon anaerobic conditions, unlike the other approaches in which the presence of oxygen was necessary for the fluorescence emission. In addition, since the induction and the fluorescence are

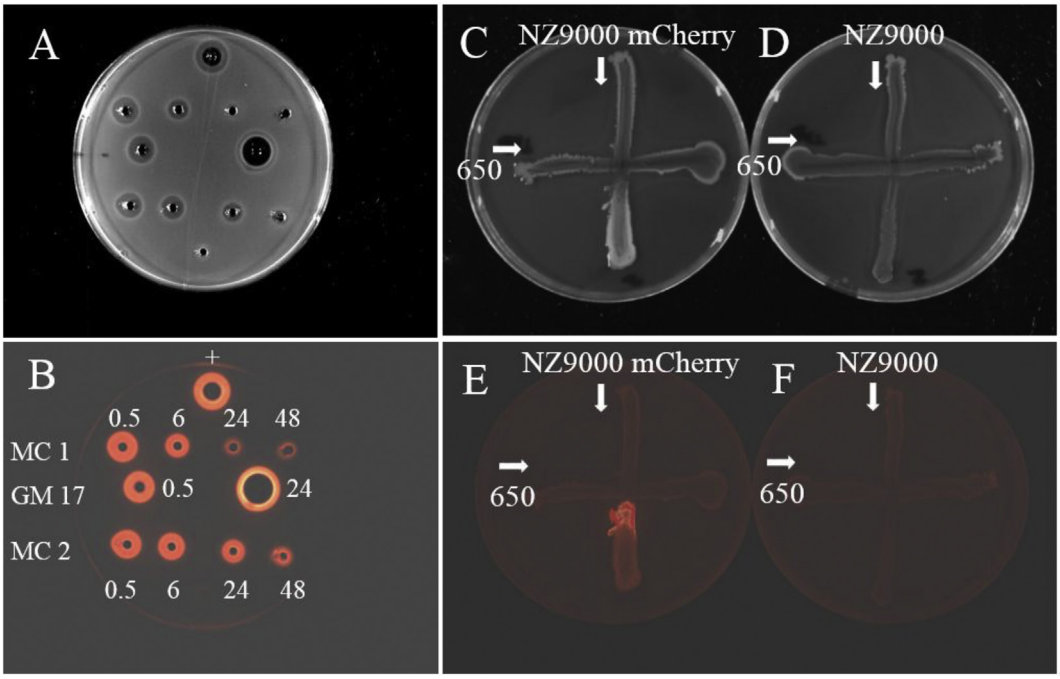

FIG. 3. Fluorescence determination of agar diffusion assays in plates inoculated with 0.1% *L. lactis* NZ9000 pNZ:Nis-mCherry (A, B), and supernatants from *L. lactis* INIA 650 incubated in colonic fermentation media in an anaerobic chamber (MC1) or at 30 °C under aerobic conditions (MC2) for 0.5, 6, 24 and 48 h. Supernatants from *L. lactis* INIA 650 incubated in GM17 media for 0.5 and 24 h, and 10 ng/ml nisin (+) were included as positive controls. Fluorescence determination of cross streak assays of *L. lactis* 650 cultures (650) with *L. lactis* NZ9000 pNZ-Nis:mCherry (NZ9000) (C, E) or its parental strain *L. lactis* NZ9000 (NZ9000 mCherry) (D, F). The filters used were: Colorimetric standard filter, white epi illumination (A, C, D) and green epi-illumination, 605:50 filter (B, E, F).

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maintained over time (22), this construction would detect the presence of nisin under anaerobic or aerobic conditions and do not need the presence of NisK/NisR as *L. lactis* NZ9000 strain contains both of them.

We first tested the behavior and nisin production of *L. lactis* INIA 650 in milk at optimal or at human colonic conditions, not observing data differences between both conditions. However, when this strain was inoculated in an *in vitro* colonic model, its counts decreased approximately 2 log units after 24 h. Despite this reduction, this strain showed a good viability under the colonic conditions tested. According to Kimoto et al. (42), the survival of *L. lactis* strains in the GIT depended on the types of carbohydrates available, and dairy products could help in that matter due to their high lactose content. In milk, fluorescence was induced in this oxygen independent biosensor by direct addition of 10 ng/ml nisin. Milk supernatants from *L. lactis* INIA 650 exhibited clear inhibition zones using *L. lactis* NZ9000 pNZ:Nis-aFP as indicator strain, while no inhibition zones or fluorescence were detected with the colonic model supernatants inoculated with the nisin-producing strain. Likewise, fluorescent colonies from *L. lactis* NZ9000 pNZ:Nis-aFP were not observed when it was coincubated with *L. lactis* INIA 650 in this colonic model. This could be due to the lack of nisin production or to the degradation of the produced nisin under colonic conditions. To look deeper into this matter, 10 ng/ml nisin was added together with the biosensor *L. lactis* NZ9000 pNZ:Nis-aFP in the colonic model, but still not fluorescence induction was detected, which supported the hypothesis that nisin was degraded or inactivated in the colonic environment. In previous studies with rat models, a reduction in nisin activity was observed in the GIT, and this reduction was higher in faecal samples than in samples from the upper part of the intestinal tract (43). This nisin inactivation can be due to a continuous degradation of nisin by proteolytic enzymes like α-chymotrypsin, or by the hydrophobic binding of nisin to the cell walls of the producer strain (44,45). Moreover, we should take into account that maybe *L. lactis* INIA 650 did not grow enough to produce an amount of nisin able to induce a detectable fluorescence in *L. lactis* NZ9000 pNZ:Nis-aFP (46,47).

After that, we developed a second construction using the plasmid pNZ8048 and the red fluorescent protein mCherry. This biosensor needs oxygen for fluorescence expression and cannot be used as *in situ* reporter system in completely anoxic environments although it would work in microaerobic intestinal conditions. Moreover, its sensibility as biosensor in agar diffusion assays was clearly superior and it was not affected by other components in the sample, even in complex matrixes like colonic samples, a frequent limitation in other reporter systems as discussed previously. Comparing the sensibility of the these two bioreporter systems using agar diffusion assays with supernatants from the colonic fermentation media, we concluded that nisin concentrations lower than 50 ng/ml are difficult to be detected by *L. lactis* NZ9000 pNZ:Nis-aFP. In contrast, *L. lactis* NZ9000 pNZ:Nis-mCherry was able to detect amounts between 1 and 10 ng/ml, as the nisin levels produced by *L. lactis* INIA 650 in the colonic environment. Moreover, supplementary experiments using *L. lactis* NZ9000 pNZ:Nis-mCherry as bioreporter has showed that there is a clear nisin degradation in the colonic media in comparison with a buffer at the same anaerobic conditions (Fig. S1). This biosensor is able to detect nisin production by *L. lactis* INIA 650 in a semi-solid colonic media under microaerobic conditions. This model mimics bacterial interactions in this complex ecosystem, where microorganisms live frequently in dense biofilms, and secretion of little amounts of bioactive compounds can have an effect in the adjacent bacteria.

In conclusion, results obtained showed that *L. lactis* NZ9000 pNZ:Nis-aFP can be considered a good nisin biosensor for nisin in milk or colonic environments at levels ≥50 ng/ml, even under anoxic conditions. On the other hand, *L. lactis* NZ9000 pNZ:Nis-mCherry was able to detect the presence of nisin at concentrations of 1 ng/ml, although it needs at least microaerobic conditions for fluorescence expression. We estimated that nisin production by *L. lactis* INIA 650 in the colonic environment reached concentrations lower than 10 ng/ml. A time dependent nisin degradation was also observed under the colonic batch fermentors conditions.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jbiosc.2019.10.011.

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