Biological characteristics of luminescent *Lactococcus lactis* transformed with *lux* genes

Aimin Jiang a, Haifeng Wang b, Nancy Lee c, Gongming Yang a, Mansel W. Griffiths b,c,*

a College of Food Science, South China Agricultural University, Washan, Tianhe District, Guangzhou, China
b Canadian Research Institute for Food Safety (CRIFS), University of Guelph, 43 McGilvray Street, Guelph, Ont., Canada N1G 2W1
c Department of Food Science, University of Guelph, Guelph, Ont., Canada N1G 2W1

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Abstract

Until now there has been no effective method to measure the adhesion of probiotics to the intestinal tract because it is very difficult to determine the ability of cells to attach to intestinal epithelium in vivo. Methods for construction of luminescent *Lactococcus* sp. transformed with *lux* genes and the biological characteristics of these transformants were investigated so as to lay a foundation for in vivo studies of the distribution and survival of probiotics, and their mechanisms to benefit human health. The results showed that the plasmid pMG36e is a suitable vector for expressing *lux* genes derived from a gram negative bacterium in the gram positive bacterium, *Lactococcus lactis*. Experiments revealed that *luxAB* genes in pMG36e can be inherited stably in *Lactococcus* even without selective pressure. The introduction of *lux* genes into *Lactococcus* did not affect its growth or acid production. Luminescence was affected by all factors tested, including *lux* gene size, media components and pH.

Keywords: Luminescent *Lactococcus lactis*; *lux* genes; Probiotic

1. Introduction

The immunostimulating effects of “bioactive” fermented dairy products are attributed to not only the bioactive compounds they contain, but also to the probiotic properties of bacteria including their ability to adhere to and colonize the intestinal tract. However, most studies have been observational rather than mechanistic, and the processes responsible for many probiotic phenomena are seldom explained. Research results are often contradictory, because it is very difficult to determine the ability of cells to attach to intestinal epithelium in vivo (Pestka, Ha, Warner, Lee, & Ustunol, 2001; Prassad, Gill, Smart, & Gopal, 1998; Ranalli, Chiavari, & Nanni, 2000; Saavedra & Tschernia, 2002; Shu & Gill, 2001; Shu et al., 2000).

The *lux* gene from *Vibrio harveyi* was cloned and successfully expressed in *Escherichia coli* in 1982 (Belas et al., 1982; Cohn et al., 1983; Hastings, 1996; Hastings, Portikus, Gupta, Kurfurst, & Makemson, 1985; Hill, Rees, Winson, & Stewart, 1993). The bioluminescence reaction involves an intracellular, luciferase-catalyzed, oxidation of a long-chain fatty aldehyde and reduced flavin mononucleotide (FMNH$_2$) by molecular oxygen (Hastings et al., 1985; Stewart & Williams, 1992). The bacterial luciferase (heterodimeric enzyme with MW 76 kDa) contains two non-identical subunits, $\alpha$ (40 kDa) and $\beta$ (36 kDa), which are encoded by the *luxA* and *luxB* genes, respectively (Meighen, 1990). The fatty acid reductase complex, reducing fatty acid to fatty aldehyde, is encoded by *luxC*, *luxD* and *luxE*. However, no genes encoding luciferase and fatty acid reductase exist in the chromosome of the vast majority of microorganisms (Meighen, 1988, 1990; Ulitzur & Kuhn, 1987). In order for non-luminescent microorganisms to acquire a luminescent phenotype, it is necessary to transform...
them with \textit{lux}A and \textit{lux}B genes (controlling synthesis of luciferase) and \textit{lux}C, \textit{lux}D and \textit{lux}E genes (controlling synthesis of fatty acid reductase). If only \textit{lux}AB genes are transformed into bacteria it is necessary to add a long-chain fatty aldehyde to the medium.

The application of the \textit{lux} gene as a reporter was explored in the 1980s, and has since received much attention for monitoring bacteria of importance to food quality and safety (Hill et al., 1993; Stewart & Williams, 1992; Walker, Jassim, Holah, Denyer, & Stewart, 1992). Luminescent phenotypes of pathogenic and non-pathogenic \textit{E. coli} (Gu, Dhurjati, Van Dyk, & LaRossa, 1996; Gu, Gil, & Kim, 1999; Hudson, Chen, Hill, & Griffiths, 1997; Rocchetta, Boylan, Foley, & Iversen, 2001), \textit{Salmonella} (Baumstark-Khan, Rode, Retberg, & Horneck, 2001; Chen, Clarke, & Griffiths, 1996; Ramsaran, Chen, Brunke, Hill, & Griffiths, 1998), \textit{Campylobacter jejuni} (Allen & Griffiths, 2001; Kelana & Griffiths, 2003) and \textit{Listeria monocytogenes} (Loessner, Rees, Stewart, & Scherer, 1996; Ramsaran et al., 1998) are among the bacteria that have been used. However, there have been very few reports in the literature describing the transformation of lactic acid bacteria with \textit{lux} genes, and even fewer that have investigated the physiological characteristics of these strains (Ahmad & Stewart, 1991; Breslaw & Kleyn, 1973; Griffiths, 1993).

The objective of this research was to develop a method for the transformation of lactic acid bacteria with \textit{lux} genes to produce strains with a luminescent phenotype. At the same time, the physiological characteristics, including growth, acid production and tolerance, as well as environmental effects on luminescence of these strains were investigated. These strains will enable the spatial and temporal distribution of probiotic bacteria in a wide variety of samples including food to be investigated, and provide a tool for in vivo research of mechanisms of probiotic action.

2. Materials and methods

2.1. Strain and media

\textit{Lactococcus lactis} subsp. Cremoris MG1363 (kindly provided by Dr. Maarten van de Guchte, Department of Genetics, University of Groningen, Haren, The Netherlands) was cultivated at 30 °C with shaking at 200 rpm in M17 broth or agar (Difco, Mississauga, ON, Canada) supplemented by 0.5% (w/v) glucose (GM17). MRS broth (Difco, Mississauga, ON, Canada) was used to determine pH effects on luminescence. Erythromycin (Sigma–Aldrich, Oakville, ON, Canada) was used in concentrations of 15 μg/ml when used in media when it was needed.

Plasmid pSB377 (Winson et al., 1998) was obtained from Dr. Simon Swift, School of Biological Sciences, University of Nottingham, UK, and used as the template for amplifying \textit{lux} genes. Plasmid pMG36e (from Dr. Kok, University of Groningen, The Netherlands) is 3.6 kb in size, contains a promoter p32, pWV01 origin of replication, and an erythromycin resistance gene, which has been reported to be functional in a wide range of bacteria (Chikindas, Venema, Ledeboer, Venema, & Kok, 1995; van de Guchte, van der Vossen, Kok, & Venema, 1989).

2.2. Construction of recombinant plasmids pMG36e-lux

To construct luminescent \textit{L. lactis}, \textit{luxAB} or \textit{luxCDABE} genes were cloned into the multiple cloning site of pMG36e. The \textit{lux} gene clusters were amplified by PCR using high-fidelity Pwo DNA polymerase (Roche Diagnostics, Laval, QC, Canada), and the plasmid pSB377 as the template. The primers used to amplify the \textit{lux} gene clusters are listed in Table 1. PCR reaction mixture (50 μl) containing 2.5 mM Mg²⁺, 50 mM KCl, 200 μM dNTPs, 1 μM primers, 1.25 units of Pwo polymerase and 100 ng of plasmid DNA, was prepared in a 0.25 ml PCR tube. The PCR was performed using a GeneAmp® PCR System 9700 (Applied Biosystems, Foster, CA).

The PCR conditions for \textit{luxAB} were 94 °C for 3 min, 10 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min; 25 cycles of 94 °C for 30 s, 64 °C for 30 s, and 72 °C for 1 min; finalized by 7 min at 72 °C. The PCR thermal cycles for \textit{luxCDABE} were 94 °C for 3 min, 10 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 4 min; 25 cycles of 94 °C for 30 s, 64 °C for 30 s, and 72 °C for 4 min; finalized by 7 min at 72 °C.

The PCR product of \textit{luxAB} or \textit{luxCDABE} genes was purified by QIAquick PCR Purification Kit (QIAGEN, Mississauga, ON, Canada) using a protocol provided by the manufacturer. The \textit{luxAB} gene fragment was digested by 10 units of \textit{XbaI} and \textit{PstI} in SuRE buffer H at 37 °C for 4 h, and ligated to the vector, pMG36e digested by the same restriction enzymes under the same conditions. The \textit{luxCDABE} gene fragment was digested by 10 units of \textit{SacI} and \textit{PstI} in SuRE buffer M at 37 °C for 4 h, and ligated to the vector, pMG36e digested by the same restriction enzymes under the same conditions. The ligation was performed in 1x ligation buffer with 2 units of T4 DNA ligase (all enzymes and buffers from Roche, Mississauga, ON, Canada) at 16 °C overnight.

2.3. Transformation of \textit{L. lactis}

The ligation mix was directly used for transformation by electroporation as described by Holo and Nes (1989) with transformation efficiency of 3.6 kb in size, contains a promoter p32, pWV01 origin of replication, and an erythromycin resistance gene, which has been reported to be functional in a wide range of bacteria (Chikindas, Venema, Ledeboer, Venema, & Kok, 1995; van de Guchte, van der Vossen, Kok, & Venema, 1989).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Primers for amplifying \textit{lux} gene clusters from pSB377 by PCR</th>
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<tr>
<td>\textit{luxAB}</td>
<td>Forward AGCGTCTGAGATGAAATTGGAAATTTGCCTTTACATACCA</td>
</tr>
<tr>
<td></td>
<td>Reverse AGCGTCTGAGATGAAATTGGAAATTTGCCTTTACATACCA</td>
</tr>
<tr>
<td>\textit{luxCDABE}</td>
<td>Forward AGCGAGGCTCATGACTAAAAAATTTCATTCATTATACGGCC</td>
</tr>
<tr>
<td></td>
<td>Reverse AGCGTCTGAGATGAAATTGGAAATTTGCCTTTACATACCA</td>
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The primers are presented from 5' to 3' ends. The introduced restriction sites are underlined.
minor modifications. MG1363 strain was first grown in GM17 to an OD_{600 nm} of 0.5–0.8, and then diluted 100-fold in SGM17 (GM17 supplemented by 0.5 M sucrose and 1% glycerol). The incubation at 30 °C was continued until OD_{600 nm} of 0.5–0.7. The cells were harvested at 4 °C by centrifugation at 5000g. After two washings with ice-cold washing buffer (0.5 M sucrose containing 10% glycerol), the cells were suspended in 1/100 culture volume of the washing buffer. Cell suspensions (50 μl) were mixed with 4 μl of the ligation mix on ice, and transferred into an ice-cooled electroporation cuvette with 2-mm gap (Bio-Rad, ON, Canada). The pulse was delivered by a Gene Pulser Xcell (Bio-Rad, Mississauga, ON, Canada), set at 25 μF, 12.5 kV, and 200 Ω. Upon discharge, 450 μl SGM17MC (SGM17 containing 20 mM MgCl₂ and 2 mM CaCl₂) was added into the cuvette. The cell suspension was transferred into a 1.5-ml eppendorff tube, and incubated at 30 °C for 2 h. Luminescent colonies of L. lactis were selected on GM17 plates supplemented by 15 μg/ml erythromycin (stock solution is 15 mg/ml erythromycin in ethanol) after incubation at 30 °C for 48 h using a NightOWL Molecular Imager (EG&G Berthold, Bad Wildbad, Germany).

Other DNA molecule manipulations and agarose gel electrophoresis were performed as described in Sambrook and Russell, 2001.

2.4. Plasmid stability

The stability of the recombinant plasmids pMG36e-lux-AB and pMG36e-luxCDABE in L. lactis MG1363 was examined by transferring the luminescent MG1363 cells to antibiotic-free M17 or GM17 broth. After 7 h incubation at 30 °C, the cell suspension was serially diluted, and 100 μl of the appropriate dilution was spread on plates prepared with the same medium with or without erythromycin. The plates were incubated at 30 °C overnight. The colonies on both plates were numerated. Only cells containing the plasmid can form colonies on the plates with erythromycin. Colonies formed on the plates without erythromycin include all cells regardless of the presence of the plasmid. The ratio of colony number from plates with antibiotic to that from plates without antibiotic was used as an indicator of plasmid stability.

2.5. Monitoring growth of L. lactis

Growth of L. lactis was determined by optical density at 600 nm (OD_{600 nm}). OD_{600 nm} was measured using a Beckman DU520 General Purpose UV/Vis Spectrophotometer. Luminescence of L. lactis on plate surface was observed using the NightOWL. Luminescence of L. lactis in liquid medium (1 ml) was measured with an Optocomb Lumimeter (MGM Instruments, Inc. Hamden, CT, USA) and expressed as relative light units (RLU). Acid production was determined by measuring pH values at the end of incubation of the culture. The pH was measured using an AB15 pH meter (Fisher Scientific, ON, Canada).

2.6. Statistical analysis

The statistical significance of differences between groups was determined by paired-samples T-test using SPSS (SPSS, Inc., Chicago, IL). Differences were considered statistically significant when $P < 0.01$.

3. Results and discussion

3.1. Construction of luminescent L. lactis and plasmid stability

The plasmids pMG36e-luxAB and pMG36e-luxCDABE were constructed by cloning luxAB or luxCDABE gene cluster from pSB377 containing a PCR-engineered promoterless luxCDABE cassette derived from Photorhabdus luminescens Hb (Winson et al., 1998) into a broad range host vector, pMG36e. The plasmid pMG36e, contains a replication origin, and a promoter from Lactococcus sp. functioning in both gram positive and gram negative bacteria. A multiple cloning site directly downstream of the p32 promoter allows easy cloning of foreign genes into the vector. The lux genes were amplified by PCR. Introduction of the restriction sites XbaI and PstI into the luxAB fragment, and SacI and PstI into the luxCDABE fragment facilitated the insertion of lux genes into the multiple cloning site of pMG36e.

The recombinant plasmids pMG36e-luxAB and pMG36e-luxCDABE were successfully transformed into L. lactis by electroporation. To confirm the structure of the recombinant plasmids, the luminescent Lactococcus cells were grown in GM17e (GM17 containing 15 μg/ml erythromycin) and their plasmids were extracted, enzymatically cut and analyzed with 1% agarose gel electrophoresis. The electrophoretograms showed that the luminescent L. lactis contained either the 5.9 kb luxCDABE fragment or the 2.1 kb luxAB fragment (Fig. 1).

For L. lactis MG1363 containing luxCDABE incubated in M17 to an OD_{600 nm} of 0.695, the ratio of colony number from plates with erythromycin to that from plates without antibiotic (used as an indicator of plasmid stability) was less than 5%. L. lactis MG1363 containing luxCDABE incubated in GM17 to an OD_{600 nm} of 1.41 gave a ratio of less than 0.4%. By contrast, L. lactis MG1363 containing luxAB incubated in either M17 (OD_{600 nm} = 0.711) or GM17 (OD_{600 nm} = 1.401) showed a very high stability, with the plasmid stability ratio being more than 99.5% (data not shown). Thus, the recombinant plasmid pMG36e-luxAB is very stable in L. lactis MG1363, whereas the pMG36e-luxCDABE is unstable in the absence of selective pressure in the form of the antibiotic.

3.2. Growth characteristics of luminescent L. lactis

Biological characteristics of luminescent L. lactis, including growth, luminescence, and acid production at different conditions were investigated.
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Fig. 1. Analysis of the recombinant plasmid by agarose gel electrophoresis. The recombinant plasmids pMG36e-luxAB and pMG36e-luxCDABE were digested as described in the text. DNA fragments were isolated in 1% agarose gel run under 75 V for 2 h in 1x TAE buffer. Lane1, 1 kb DNA molecular weight standard (New England Biolabs, Pickering, ON, Canada), from top to bottom, ten bands represented DNA fragments of 10.0, 8.0, 6.0, 5.0, 4.0, 3.0, 2.0, 1.5, 1.0, and 0.5 kb. Lanes 2, 3, 4 and 5, pMG36e-luxCDABE enzymatically cut by SacI and PstI produced 3.6 kb (pMG36e) and 5.9 kb (luxCDABE) DNA fragments. Lanes 6 and 7, pMG36e-luxAB enzymatically cut by XbaI and PstI gave two DNA fragment, 3.6 kb (pMG36e) and 2.1 kb (luxAB).

Growth in different media under different conditions was assessed by monitoring OD_{600 nm} at the end of the incubation period. Medium composition had the greatest effect on growth rate and maximal cell concentration (P < 0.01; Fig. 2). When incubated in GM17 broth, the final OD_{600 nm} of both parent and transformed _L. lactis_ increased to a maximum of 1.4, but when these strains were incubated in M17 broth, the OD_{600 nm} of the all _L. lactis_ only increased to a maximum of 0.691 and 0.726. The parent and strains transformed with both luxAB and luxCDABE did not show any significant difference in growth rate and final cell concentration (P > 0.05) when grown in antibiotic-free media. Whereas when M17 and GM17 have been used to grow _L. lactis_, GM17 was a better medium for the strain used in this study.

Erythromycin was used to stabilize the plasmid in the host cell. Addition of erythromycin in the medium significantly reduced (P < 0.01) the growth rate, and the time to reach maximal OD. When incubated in broth without erythromycin, the OD of luminescent _L. lactis_ increased to a maximum at 5 h of incubation, and when incubated in broth with erythromycin, the OD of luminescent _L. lactis_ increased to a maximum at 9 h of incubation.

The size of the gene cluster used to transform _Lactococcus_ exhibited significant but much less marked effects on the cell growth than the antibiotic. The growth of luminescent _Lactococcus_ transformed with luxAB was faster than that transformed with luxCDABE (P < 0.01) only when erythromycin was present in the medium.

3.3. Light emission of luminescent _L. lactis_

pMG36e-luxCDABE was unstable in _L. lactis_ MG1363, and was eliminated in a medium without selective pressure. Only strains with pMG36e-luxAB and those with pMG36e-luxCDABE incubated in a medium supplemented with erythromycin were investigated further for luminescence.

Luminescence was measured directly from 1 ml cell culture after incubation at 30 °C by a Luminometer, and OD at 600 nm was measured at the same time. OD_{600 nm} values were converted to cell concentration in CFU/ml by a standard growth curve of CFU/ml to OD_{600 nm}. Because cell number contributed greatly to the overall luminescence of a culture, luminescence was expressed as relative light units per colony forming unit (RLU/CFU).

For all strains tested under different conditions, overall luminescence increased and reached a maximum value after 5 h incubation at 30 °C (Fig. 3). When the incubation continued, overall luminescence of the cultures decreased quickly (data not shown). _Lactococcus_ MG1363 containing luxAB incubated in M17 gave the highest luminescence (RLU/CFU). In the presence of erythromycin, cells containing luxAB showed greater luminescence than those containing luxCDABE. Though pMG36e-luxAB is very stable in the absence of erythromycin, unit luminescence was higher in the presence of the antibiotic (P < 0.01).

3.4. Characteristics of acid production

The initial pH of all the culture media was 8.2 and was unaffected by the addition of 0.5% glucose or erythromycin. Acid production was expressed as the pH value measured at the end of incubation. Several factors, including type of medium, size of transformed gene, and incubation time, influenced acid production to different extents (Fig. 4).

As determined for cell growth, medium composition had the strongest effect on acid production (P < 0.01). All
changes in pH produced by recombinant bacteria with luxAB and with luxCDABE were not significantly different. Thus, the size of the transformed gene has no effect on the ability of the transformant to produce acid ($P > 0.05$).

In summary, GM17 is a more suitable medium for *L. lactis* transformed with lux genes than M17. Addition of erythromycin only affects the rate of acid production, but does not affect the ultimate value of pH attained; which, in turn, is determined by the medium.

### 3.5. Effects of pH on light emission of luminescent *L. lactis*

The pH of cultures showed significant effects on luminescence. After luminescent *L. lactis* with pMG36e-luxAB was incubated in GM17e at 30°C for 24 h, the pH was reduced to ~4.56 and no luminescence could be detected. The luminescence was restored by neutralizing the medium with 1 M NaOH. When the pH increased from 4.56 to 5.6, the RLU increased from 12 to 1995; when the pH was raised to 6.0, the RLU increased to 13,085; the maximum RLU 216,859 (one fifth maximal RLU at 5 h in the same medium) occurred when pH was adjusted to 9.2. However, when the same strain was cultivated in MRSe (MRS supplemented with 15 μg/ml erythromycin) for 24 h (pH 4.39, RLU = 0), the maximum RLU (123,107) was restored at a pH of 7.52. When the pH was further increased, the RLU decreased again. Similar results were obtained with *L. lactis* cultures containing pMG36e-luxCDABE. There was no significant change of OD during change of pH.

To explore how pH affects luminescence of a culture, 1 M NaOH or 1 M HCl was added into a culture of luminescent *L. lactis* after incubating at 30°C for 5 h (the time at which maximum luminescence was observed). The RLU of the luminescent *L. lactis* containing luxAB was 934,069 when incubated for 5 h in GM17e (pH 6.17). At this time, if the pH of the medium was adjusted to 6.95 with 1 M NaOH, the RLU of the culture was reduced to the 909,600 (a 2.6% reduction). However, if the pH was adjusted to 7.1 by further addition of 1 M NaOH, the RLU was reduced to 474,176, corresponding to a 50% reduction of the initial luminescence.

On the other hand, if a solution of 1 M HCl was used to adjust the pH to 2.54, the RLU of the luminescent *L. lactis* transformed with luxAB genes reduced from 934,069 to 884,114 (a 5.35% reduction). Thus, a high level of luminescence was observed at a low pH.

When luminescent *L. lactis* containing luxAB genes was grown in MRSe, the RLU was 596,911 after 5 h incubation when the pH reached 5.52. If 1 M NaOH was used to adjust the pH to 6.79, the RLU increased to 788,209 (a 32% increase). When the pH was further increased to 9.59 by the addition of 1 M NaOH, the RLU still remained high at 884,114 (2.6% increase). However, if the pH was adjusted to 7.1 by further addition of 1 M NaOH, the RLU was reduced to 474,176, corresponding to a 50% reduction of the initial luminescence.

On the other hand, if 1 M HCl was added to reduce the pH from 5.52 to 4.3, the RLU of the luminescent *L. lactis* transformed with luxAB genes reduced from 934,069 to 884,114 (a 5.35% reduction). Thus, a high level of luminescence was observed at a low pH.
From the above results, it can be concluded that media components and growth stage seemed to drastically influence the effect of pH on the luminescent *Lactococcus* culture. The GM17e medium enabled the luminescent *L. lactis* to withstand a larger pH range. This phenomenon was directly related to growth stage. When the culture was in exponential phase (5 h), it was more resistant to low pH (<2.54); when in stationary phase (24 h), it became more resistant to high pH (>9.26). The culture in MRSe showed higher resistance to high pH (>9.59) when in the exponential phase, but not to low pH regardless of growth phase. When the luminescent *L. lactis* was incubated for 5 h in GM17e, and when the pH was reduced from 6.17 to 2.54 by adding 1 M HCl, the RLU was reduced by less than 5% for the luminescent *L. lactis* with luxAB, and by 20% for the luminescent *L. lactis* with luxCDABE. By contrast, when the same strain was incubated for 5 h in MRSe, and 1 M HCl used to reduce the pH from 5.52 to 2.81, the luminescence disappeared. However, in MRSe grown cultures, when the pH was increased to 9.59 by adding 1 M NaOH, the RLU still remained 15.2% higher than the original RLU in cultures incubated at 30 °C for 5 h. The pH for maximal luminescence of luminescent *L. lactis* depended on the medium. In GM17e, the luminescent *L. lactis* containing luxAB genes exhibited maximal luminescence of 934,069 RLU at pH 6.17; in MRSe, the maximal luminescence of 788,209 RLU was observed at pH 6.79.

### 3.6. Discussion

The objective of this research was to develop a method for the transformation of lactic acid bacteria with lux genes to produce strains with a stable luminescent phenotype. At the same time, the physiological characteristics of these strains were investigated. These strains will be invaluable for future research to elucidate the behaviour of probiotic bacteria both in vitro and, more importantly, in vivo.

The recombinant plasmid pMG36e-luxCDABE and plasmid pMG36e-luxAB was successfully transformed into *L. lactis* by electroporation, and the transformants exhibited a luminescent phenotype. Thus, the plasmid pMG36e is a suitable vector to express lux genes in *L. lactis*.

Introduction of lux genes into *L. lactis* did not change its growth and acid production characteristics, and these characteristics are principally determined by medium composition. Addition of glucose (0.5%) in M17 greatly promoted growth of the organism; and inclusion of erythromycin (15 μg/ml) in the media slowed down the rates of growth and acid production, but increased luminescence of the cultures. When grown on the surface of an agar plate or in broth, the luminescent *L. lactis* transformed with luxAB genes showed significantly stronger light output than the luminescent *L. lactis* transformed with luxCDABE genes (*P* < 0.01). When grown on the surface of an agar plate or in broth supplemented with erythromycin, the luminescent *L. lactis* showed significantly stronger light than cultures grown in media without erythromycin (*P* < 0.01).

Surprisingly, media components dramatically influenced the reaction of luminescent *Lactococcus* cultures to pH. When pH was changed by adding NaOH or HCl into the luminescent *L. lactis* culture, changes in luminescence were dependent on the media. The mechanisms by which media components affected the *L. lactis* response to pH are not clear. The constructed luminescent *L. lactis* can emit strong light in a large pH range (approximately 2.5–9.5) and this increases its usefulness as a reporter in different foods and under different environmental conditions.

This method allows for transformation of lux genes into other lactic acid bacteria, and will lead to improved ways to study the survival and activities of probiotic lactic acid bacteria in vivo.

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