

Influence of Normal Component Composition of Ruminant Fluid on Level of Biosensor Luminescence Level

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Abstract: During the study of differential biological toxicity of rumen fluid and its individual components the following reporter strains of *E.coli fabA:: lux*, *E.coli ibpA:: lux*, *E.coli recA:: lux* and *E.coli cda:: lux* were used. Measurement of luminescence was carried out with illuminometer in kinetic mode for 120 min. Bioluminescence level of bacterial strains was expressed in bioluminescent index (BLI). In the course of studies it was found that the real (native) luminal fluid and model mixture gave similar effects of bioluminescence suppression level in the beginning of the experiment with toxic effect not expressed. These data confirm the possibility of using the created model mixture to determine the influence of separate components of luminal fluid on bioluminescence of the recombinant strain *E. coli*. Components of model luminal fluid had not any expressed toxic effect on sensory luminescent strain *E. coli*; and the effect of bacterial bioluminescence suppressing in the initial stage of the experiment can be explained, in our opinion, by the influence of ammonia and acetic acid, which can be considered as a manifestation of moderate toxicity.

Key words: Bioluminescence • Biototoxicity • Strain *E.coli* • Substrate

INTRODUCTION

The current problem of impact of feed containing toxins on organism of herbivorous animals requires the search of identification ways, including in the rumen of animals. Microorganisms in the rumen of herbivorous animals can more often neutralize or reduce the toxicity of these feeds in the process of metabolism, at the same time microbial biotransformation of certain substances may enhance their toxicity [1,2]. Recent researches demonstrated that, for example, plant tanning substances (tannin) have depressing effect on the development of rumen microorganisms, reduce the digestibility of nutrients and minerals [3-7], violate functions of cell walls of microorganisms, as well as the catalytic activity of extracellular enzymes [8]. In this connection, to evaluate the toxic effect of the feed components on rumen microorganism's bioluminescence method can be used.

The high degree of conjugation of bioluminescence with major energy flows in bacterial cell [9] was a prerequisite for the use of luminous organisms during testing different natural environments; their biotoxicity

may be integrally evaluated through the change in bioluminescence intensity [10]. On the basis of these assumptions and based on freeze-dried luminescent bacteria and luciferase enzyme system liberated from them a number of methods of express bioluminescent analysis are proposed.

MATERIALS AND METHODS

A series of experiments to evaluate the integral biotoxicity of rumen fluid and its separate components was carried out on the basis of the microbiology laboratory of All-Russian Research Institute of beef cattle breeding and the Department of Microbiology of the Orenburg State University.

To study the differential biotoxicity of rumen fluid and its separate components reporter strains of *E.coli fabA:: lux*, *E.coli ibpA:: lux*, *E.coli recA:: lux* and *E.coli cda:: lux* were used, while a day-old culture grown on LB-agar supplemented with ampicillin in concentration of 100 mkg/ml was resuspended in LB- broth and grown for 2 hours at 37°C, after this the strains were washed three

times in normal saline. Further, mixture was formed in plate wells consisting of 225 mkl of tested agent and 25 mkl of bacteria. Luminescence was measured using illuminometer in kinetic mode for 120 min.

Level of bioluminescence of bacterial strains was expressed in units of bioluminescent index (BLI), calculated by the formula:

$$BLI = \frac{I_n^{test} \cdot I_0^{control}}{I_0^{test} \cdot I_n^{control}}$$

Where

I_n^{test} – bioluminescence level of tested sample at n-minute;

I_0^{test} – Bioluminescence level of tested sample at 0-minute;

$I_n^{control}$ – Bioluminescence level of tested sample at n-minute;

$I_0^{control}$ – Bioluminescence level of tested sample at 0-minute.

As basic equipment used for research “Artificial scar” KPL-01 (Popov, 1983), illuminometer LM-01T (Immunotech, Czech Republic), pH-meter-ionometer Expert 001 (Econix-Expert, Russia), laboratory centrifuge CM-6M (Elmi, Russia) were used.

The following research objects were used: phosphate buffer (pH from 6.0 to 8.0), based on propionic, lactic, acetic, butyric acids, glucose, an aqueous solution of 10 % ammonia, which were mixed together in physiological concentrations (model mixture), luminal fluid taken from beef cattle (Kazakh white-headed breed). Whole luminal digest and fluid were selected before feeding with fodder with a help of Janet’s syringe and gavages.

Upon completion of research the obtained results were analyzed with methods of statistical analysis using PC software («Excel», «Statistical»).

RESULTS AND DISCUSSION

According to the stated objective in the course of the first experiment the formation of possible biotoxicity of native luminal fluid was assessed. As a control, the suspension of luminescent recombinant strain *E. coli* K12 TG1 with cloned luxCDABE genes - genes of natural marine luminescent microorganism *P. leiognathi* in normal was taken saline (Figure 1).

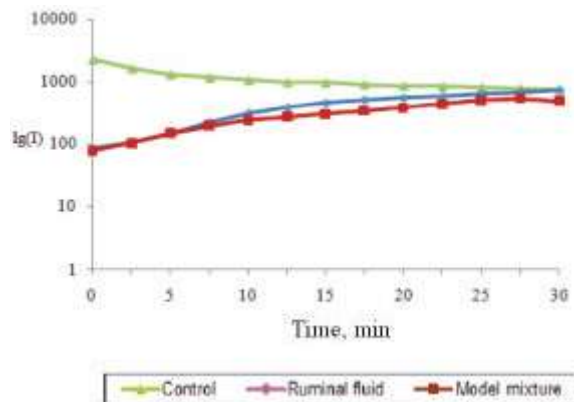


Fig. 1: Influence of ruminal fluid on bioluminescence of recombinant strain *E. coli*

In the beginning of experiment to study the influence of native ruminal fluid on bioluminescence of reporter strain of *E. coli*, induction of bacterial bioluminescence was observed; it was starting from the middle of the experience. However, this situation was leveled to the end of the experiment.

Based on the preliminary results of the experiment, we assumed that the influence of any one or more components included in the ruminal fluid on bacterial bioluminescence may be the probable cause of this phenomenon. In order to confirm or deny this assumption, at the next phase of work we created a model mixture (model ruminal fluid) based on phosphate buffer, which consisted of propionic acid, lactic acid, butyric acid, acetic acid, glucose and aqueous solution of 10% ammonia taken in physiological concentrations, in order to further clarify the influence of individual components on the actual ruminal fluid on reporter strains of *E. coli*. But at first we had to evaluate the identity of a model mixture and ruminal fluid created by us. In the whole, the findings attest to the fact that actual (native) ruminal fluid and model mixture gave similar effects of suppressing the bioluminescence level in the beginning of the experiment (had similar character), without showing, however, the toxic effect. The obtained results allow us to estimate the identity of both liquids and, therefore, to study the influence of separate components on the recombinant strain of *E. coli*.

Analyzing data on the influence of separate components of the model ruminal fluid on a recombinant strain of *E. coli*, a preliminary conclusion may be made - none of the components had any expressed toxic effect on this strain.

Induction of glucose did not trigger luminescence as important cellular "fuel" during anaerobic energy generation is 6-carbon sugars, first of all - glucose. It serves as a source of energy for microbial activity. At the first hour no luminescence of the reporter strain was observed, but at the second hour of experiment BLI was 0.4 and on 105 minute it was 0.6.

Butyric acid within 30 minutes has no toxic effect on the strain, but in the period from 30 to 100 minute BLI increased from 0.7 to 1.5 and then decreased to 120 minute it reduced to 1.3. Butyric acid did not have membrane damaging effect, as it is formed in the rumen of ruminants as a by-product of bacteria that digest carbohydrates, sugars and polysaccharides.

Lactic acid had not expressed membrane-damaging effect, as it is a by-product of glucose decay, which, in its turn, is a provider of energy for microorganisms and support their metabolism. Up to 35 minute of experience inhibition of luminescence was observed, at this time BLI was 0.4. In the course of time, the induction of luminescence gradually increased and up to the 120 minute it was 1.5.

Model mixture did not promote luminescence of this microorganism in the initial period of time and on the 25th minute BLI was 0.6. Further, with the course of time BLI increased and on the 120th minute it was 3. Mixture had no expressed luminescence induction, so luminal fluid itself is not a toxic medium for luminal microorganisms.

Ammonia in the beginning of the experiment had no toxic effect, but within the period from 15 to 120 minute BLI increased from 0.6 to 2.3. Consequently, this component promoted minor luminescence induction of reporter strain of *E. coli* with the vector plasmid *papa::luxCDABE*. This is probably due to the fact that ammonia is the end product of nitrogen metabolism. It is formed during metabolism of proteins, amino acids and other nitrogenous compounds. Ammonia is toxic for this microorganism.

Propionic acid inhibited luminescence, but from the 25th minute BLI was 0.7 and over time began to grow, reaching maximum of 1 on the 115th minute. Therefore, this acid had no significant induction of luminescence because it is product of sugar disintegration and it is formed during fermentation of carbohydrates. Propionic acid is also biologically obtained during metabolic degrading of fatty acids containing an odd number of carbon atoms and after degrading of some amino acids. Bacteria of the genus *Propionibacterium* produce propionic acid as the end product of their anaerobic metabolism. These bacteria are often found in the proventriculus of herbivores.

CONCLUSION

Thus, in comparison with actual luminal fluid model fluid gave similar effects of suppressing bacterial bioluminescence at the initial stage of the experiment, while both fluids had not any toxic effects. Accordingly, the given data confirm possible use of a model mixture created by us to determine the influence of separate components of luminal fluid on bioluminescence of the recombinant strain *E. coli*.

In addition, none of the components of the model luminal fluid had any expressed toxic effect on sensory luminescent strain *E. coli* and the effect of suppressing bacterial bioluminescence in the initial stage of the experiment can be explained, in our opinion, by the influence of ammonia and acetic acid, which can be estimated as manifestation of moderate toxicity.

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