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Influence of Normal Component Composition of Ruminal Fluid on Level of Biosensor Luminiscence Level

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Abstract: To 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 luminometer 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) ruminal 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 ruminal fluid on bioluminescence of the recombinant strain *E. coli*. Components of model ruminal 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 · Biotoxicity · Strain *E. coli* · Substrate

INTRODUCTION

The problem 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 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, 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 respect, to evaluate the toxic effect of the feed components on rumen microorganisms 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. Articles studying the action of different antibiotics on luminescence of biluminiscent phenotype *Escherichia coli* [11] and also resistance of *Escherichia coli* to them and role in physiology of digestion and contraction of a disease [12-14] are well-known.

MATERIALS AND METHODS

A series of experiments to evaluate the integral biotoxicity of rumen fluid of young beef cattle (the Kazakh white-headed breed) 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.

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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 luminometer 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 a 0-minute.

As basic equipment used for research "Artificial scar" KPL-01 (Popov, 1983), luminometer LM-01T (Immunotech, Czech Republic), pH-meter-ionomer 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), ruminal fluid taken from beef cattle (Kazakh white-headed breed). Whole ruminal digesta and fluid were selected before feeding with fodder with a help of Janet's syringe and gavage.

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

RESULTS AND DISCUSSION

According to the stated objective in the course of the first experiment the formation of possible biotoxicity of native ruminal 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).

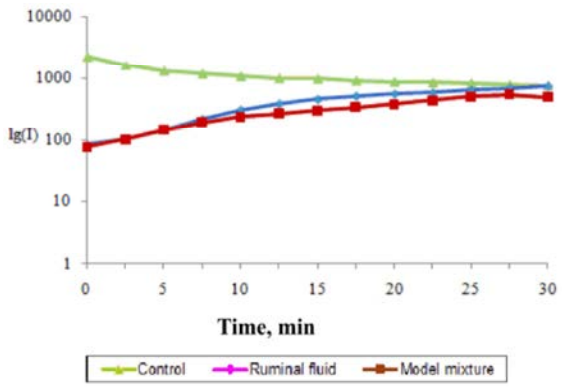

Fig. 1: Influence of ruminal fluid on bioluminescence of recombinant strain *E. coli*

In the beginning of experiment to study the influence of native rumimal fluid on bioluminescence of reporter strain of *E. coli*, induction of bacterial bioluminescence was observed; it was starting from the middle of the experiment. As a result first 30 minutes of existence of the mixture biosensors were characterized by the same level of luminescence as control group had. This effect is probably connected with a sharp change of the proton strength of medium due to the acidity of the analyzed fluid [15]. Besides ruminal fluid is a colored solution that also can influence on the nature of the registered luminescence [16].

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 [17], in order to further clarify the influence of individual components on the actual ruminal fluid on reporter strains of *E. coli*. Thus, model ruminal fluid was obtained in such a way; it was analyzed by means of sensor strain *E. coli*, the same as in case with native ruminal fluid. 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 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 conclusion may be made that simple organic acids and inorganic compounds (acetic acid, ammonia) caused significant quenching of luminescence up to 34±3.05 % and 44.6±1.76 % as compared to the control group respectively. That particular substances probably determine sharp quenching of luminescence of sensor strain upon contact with ruminal fluid. On the other hand, acids with longer carbon skeleton did not express the properties of luminescence inhibitors and glucose even caused the increase of luminescence acting as available substrate.

In the next series of experiments reporter strains that allow to identify different damaging effects on bacterial cells were used [18]. In this case development of damage is detected according to the increase of luminescence intensity in comparison with background, that is the reflection of activation of relevant genes under stress. Analysis of activity of each component allow to characterize principle of the used components of model ruminal fluid. In this case induction of glucose did not trigger luminiscence as important cellular "fuel" and does not cause damaging effect.

Butyric acid had no toxic effect on any used reporter strain, except for *E. coli recA::lux*, which BLI in the end point increased to 3,8 relative units, that attested to its influence on nucleic acids of cell. This acid did not cause any membrane or protein damages, as it is formed in the rumen of ruminants as a as a by-product of bacteria that digest carbohydrates, sugars and polysaccharides [19]. The similar situation was observed with propionic and lactic acids, however the responce intensity of *E. coli recA::lux* was less expressed. Propionic acid is also biologically obtained during metabolic degrading of fatty acids containing an odd number of carbon atoms and after degrading 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 forestomach of herbivores [19].

On the other hand, noci-influence of ammonia and acetic acid were detected, sensor strains were under the highest inhibitive influence. So, beginning from 15th minute and up to the end of assessment BLI increased from 0.6 to 2.3 in reporter strain *E.ñoli* with the vector plasmid *pfabA::luxCDABE* upon contact with ammonia. Acetic acid caused the increase of luminescence of this strain up to BLI 4,1, thus confirming the idea about the influence on membrane cell constituents. Moreover, the same effect was identified in strain *E. coli recA::lux*, ammonia caused the increase of luminescence up to 4,35 units and acetic acid - up to 12,4. The received data attest to the additional effect damaging DNA upon contact of these substances with bacterial cells.

In a whole, use of model ruminal fluid, formed from above described components did not cause significant activiation of reporter strains, except for the enhancement of luminescence of *E. coli fabA::lux* up to 2,1 units and *E. coli recA::lux* up to 6,4, that is the result of integral action of the used components. In other cases model mixture did not cause luminescence, that attest to the fact that ruminal fluid is not toxic environment for microbial flora of rumen.

CONCLUSION

Thus, in comparison with actual ruminal 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 ruminal fluid on bioluminescence of the recombinant strain *E. coli*.

In addition, none of the components of the model ruminal fluid had no 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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