*Microbiology, Vol. 74, No. 2, 2005, pp. 236–243. Translated from Mikrobiologiya, Vol. 74, No. 2, 2005, pp. 278–286.*


**EXPERIMENTAL ARTICLES**

# A Database on Natural and Transgenic Luminous Microorganisms: BiolumBase

**S. E. Medvedeva[1], A. N. Boyandin, Yu. P. Lankin, D. A. Kotov, T. V. Kargatova, E. K. Rodicheva, and L. Yu. Popova**

*Institute of Biophysics, Siberian Division, Russian Academy of Sciences, Krasnoyarsk, 660036 Russia*

Received October 14, 2003; in final form, April 1, 2004

**Abstract**—The database BiolumBase was designed for the collection and systematization of available information on microorganisms containing bioluminescent systems; it includes two sections: natural and transgenic luminous microorganisms. By now, logic schemes of these sections have been developed, classification of the objects has been performed, ways of presentation of characteristics and structure of fields for input of information have been elaborated, and the necessary program modules have been developed. The database is filled on the basis of published data and our own experimental results; subsequent linkage of the database to the Internet is envisaged. Users will be able to obtain not only catalogues of strains but also information concerning the properties and functions of the known species of luminous bacteria, the structure, regulatory mechanisms, and application of bioluminescent systems and genetically engineered constructions with *lux* genes, as well as to find references and to search strains by using any set of attributes. The database will provide information that is of interest for the development of microbial ecology and biotechnology, in particular, for the prediction of biological hazard from the application of transgenic strains.

*Key words*: database, bioluminescence, natural and transgenic luminous microorganisms.

The studies on marine bioluminescence carried out for many years at the Institute of Biophysics, Siberian Division, Russian Academy of Sciences, resulted in the development of a unique culture collection (CCIBSO N836), which contains over 700 strains of luminous marine bacteria isolated from various regions of the oceans. In recent years, bacterial strains carrying vectors with luminescent genes were added to this collection [1].

Bioluminescence, first of all bacterial, is a widely applied and useful marker that makes natural and transgenic strains of luminous microorganisms a promising tool of modern biology. Bioluminescent reactions can be easily measured; moreover, genes encoding bioluminescence can be inserted into various metabolic pathways of microorganisms not previously exhibiting this property, with the aim to study the functioning of these routes under changed environmental conditions. Promoters and regulatory genes from other, nonluminescent operons are used in cloning; *lux* genes from various microorganisms, including eukaryotes, can be cloned completely or partly. Measuring the intensity and dynamics of bioluminescence makes it possible to evaluate the regulation, level, and stability of the expression of both luminescence-encoding genes and (indirectly) other genes under various conditions, which, in the case of application of other methods, would require time-consuming and expensive analyses.

At present, there is a great body of information concerning the application of the bacterial bioluminescent system for cloning in various species of nonluminous bacteria. Methods were developed for cloning of *lux* genes in various vectors and construction of transgenic strains [2, 3]. There are abundant data on the characteristics of luminous microbial strains and peculiarities of the luminescence expression, as well as publications concerning methods of strain storage in collections and recommendations on strain application [3–8]. However, such information is scattered and distributed among numerous scientific papers and monographs, which hinders its retrieval and use. Wide and versatile application of the reporter *lux* genes poses the problem of putting the information in order with respect to the structural peculiarities of bioluminescent systems in natural and transgenic microorganisms and closely allied characteristics. The basis for the solution of this problem is the employment of modern technologies for the processing and storage of information, primarily databases (DBs) [9], including web-based DBs. There is a great deal of web-based DBs on culture collections, particularly on the collections of genetically modified microorganisms and microbial vectors [10], that most often provide only a list of microbial strains and vectors. Recently, DBs on meta-

[1] Corresponding author. E-mail: ccibso@ibp.ru; lubg@ibp.ru

0026-2617/05/7402-0236 

bolic functions and genetic regulation in some microbial species were developed [11, 12].

The aim of the development of the BiolumBase DB was the collection and analysis of all information concerning microorganisms with bioluminescent systems in order to provide the possibility of searching various strains and vectors carrying *lux* genes and of comparing them in terms of their suitability for the solution of particular fundamental and practical problems.

### *The Software Applied*

The information system was developed according to client/server technology, which is the most promising for projects of such type and allows effective interaction of users with the server for network applications, including the Internet and local networks. Sybase Adaptive Server Anywhere is used as the DB control system (DBCS). The system was designed with the use of the object-oriented Computer Associates ERrwin 4.0 data modeler [13]. User software was developed by using two software products, Sybase Power Builder 7.0 and Borland C++ Builder 6.0.

### *Requirements for the BiolumBase DB*

The following requirements for the DB were formulated: (1) cooperative processing of common data with different levels of data access; (2) high speed of query processing despite the large information volume (high speed of acquisition of necessary information); (3) high fault-tolerance and recoverability in the case of critical situations; (4) record retrieval according to the given characteristics of microorganisms and vectors.

Particular emphasis was placed on the provision of users with the substantial and useful information on the known strains of natural and transgenic microorganisms carrying luminescent genes, as well as on the conditions of *lux* gene expression. The structure of the designed DB on luminous microorganisms allowed the storage of both typical easily formalizable data and functional information on bacteria and cloned elements. Designations of characteristics and their types were standardized, which made it possible to avoid bad errors and facilitate the reorganization of the DB structure in the case of DB change. The designed DB provides not only text information but also graphic documentation if necessary.

### *Principle of DB Design*

The DB is relational since it is based on a system of related tables; each of them describes certain informational category. Tables contain a set of fields (attributes) providing information on individual properties of the objects described or special information concerning intertable relations. Each table contains a unique digital field, identifier, which is the primary key; for example, the table that describes species (“Species”) contains the following fields: “ID species,” “species name,” “author name,” “year,” “flagellum type,” “capsule formation,” etc., where the key field is “ID species” (Fig. 1). To establish relations between tables, two linkage types were used; for example, one species may include many strains, but a particular strain may belong to only one species. Links of the type “one relates to many” were realized by the introduction of a special field “Foreign Key” (FK) into the daughter table, which refers to the primary key field of the parent table. One such example is the linkage between tables “Species” and “Strain” in Fig. 1. On the other hand, bacteria of the same species may be grown on numerous media and one medium may be used for cultivation of microorganisms belonging to different species. That is why linkage of the type “many relate to many” was established between the tables “Species” and “Nutrient Media”; this was realized via a special interface table “Species–Medium,” which includes two fields referring to the primary key fields of the first and second linked tables, respectively.

Software for the DBCS was designed in such a way that additional adjustment of the programs for work with the DB is virtually not required and is limited to a number of standard adjustments. To accelerate record retrieval, special forms were introduced that make it possible to view the block of records in the required table and to find the necessary record by using not only its full name but also its fragments, if the user does not remember the full name. The software for the correct deletion of any record from any table of the DB was developed; it integrates all the information on the structure of the intertable links in the DB, which allows correction of all references to the given record before its deletion. In this case, there is a possibility of calling any form that displays tables with references to the deleted record. The adjustment of links between tables is realized through the choice of a linking record of the type “one relates to many” or “many relate to many” by calling an additional form. The possibility of editing linked tables by calling their forms from the current form is provided. Checkout programs were developed for testing the DBCS and for detection and correction of errors occurring at the design phase, as well as for the correction of links between tables at the level of the program interface.

### *Structure of the DB*

The BiolumBase DB on bioluminescent organisms has been developed for two different types of luminous microorganisms, natural and transgenic, which are related by the presence of the *lux* systems (natural or cloned) responsible for bioluminescence (Fig. 2). We developed a unified classification of the common characteristics of the two types of bioluminescent systems (native and cloned) and common approaches to the collection and input of information, as well as to unification of experimental and published data applicable to the designed information system.

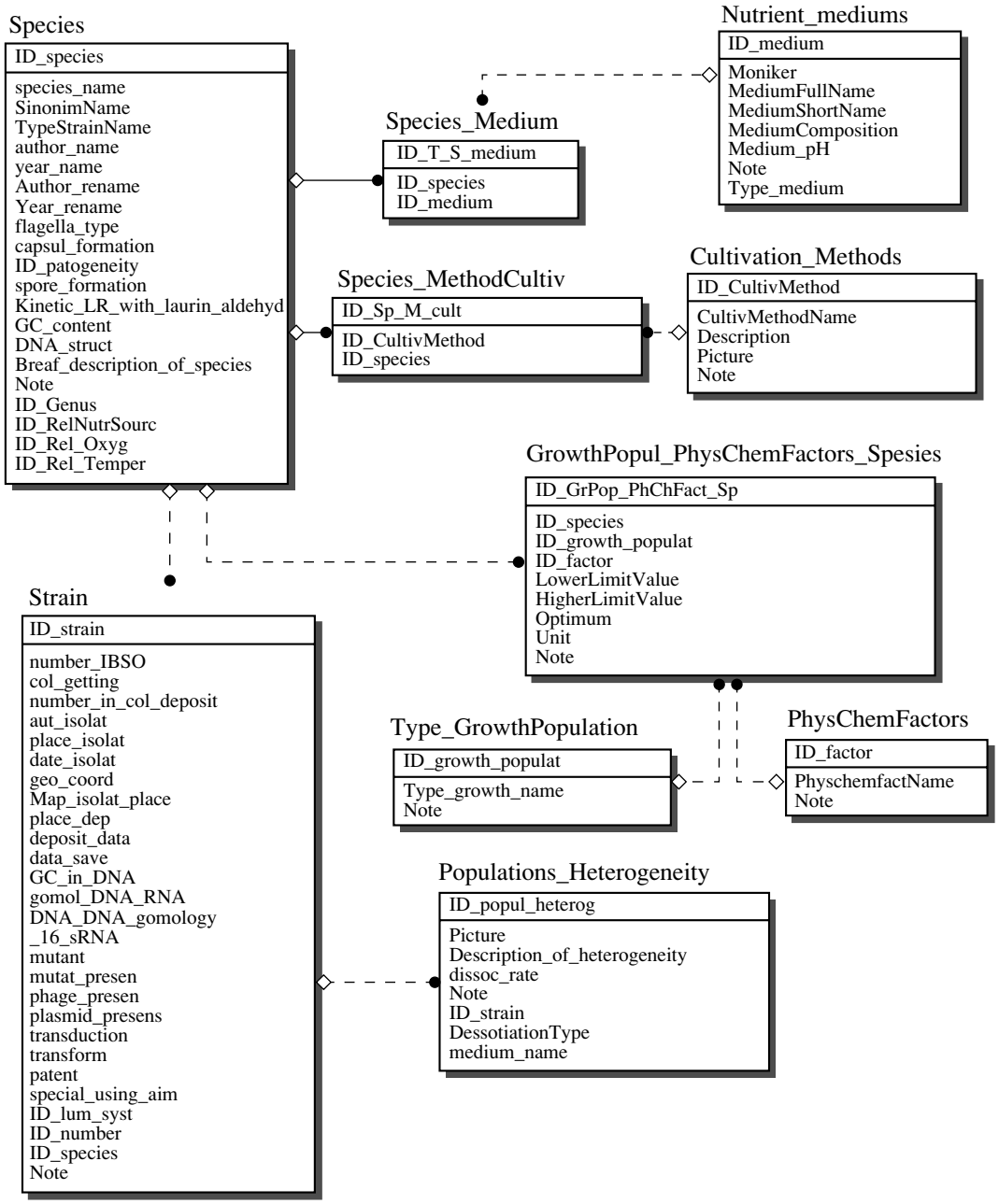


**Fig. 1.** Example of linkages between tables containing information on the properties of a population (from the division "Natural Luminous Microorganisms").

Several blocks (subdivisions) can be logically recognized in the DB structure; each represents a set of tables with related subjects (Fig. 2). The descriptions of systematic position of microorganisms and peculiarities of their metabolism, cultivation, and storage are kept in the blocks "Species" and "Strain"; the list of relevant publications is stored in the block "Publications"; the fields of possible application are presented in the block "Areas of Use." Each block contains certain functional information that may be needed by users.

The connecting blocks "Luminescent System of Bacteria" from the division "Natural Luminous Microorganisms" and "Operon" from the division "Transgenic Luminous Microorganisms" form the central block of the whole DB (Figs. 2, 3). They contain information on bioluminescence of natural and transgenic luminous microorganisms, luminescent systems, and various constructions carrying *lux* genes. The block "Luminescent System of Bacteria" contains versatile information concerning luminescent system of luminous bacteria: phenomenology, structure, kinetic char-

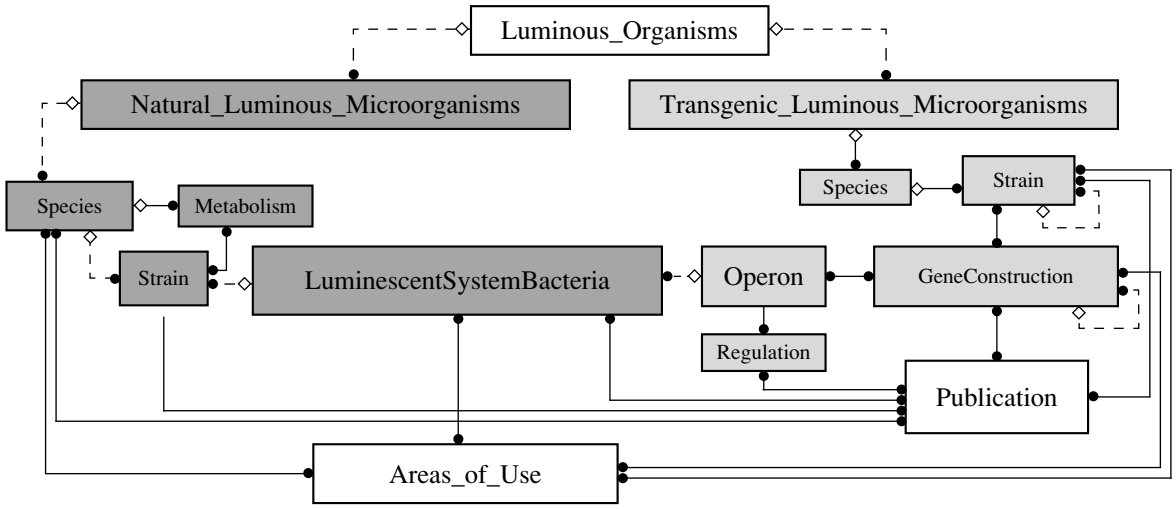


**Fig. 2.** Structure of the BiolumBase DB. Tables from the divisions "Natural Luminous Microorganisms" and "Transgenic Luminous Microorganisms" are differently colored. Common blocks are "Publication" and "Areas of Use."

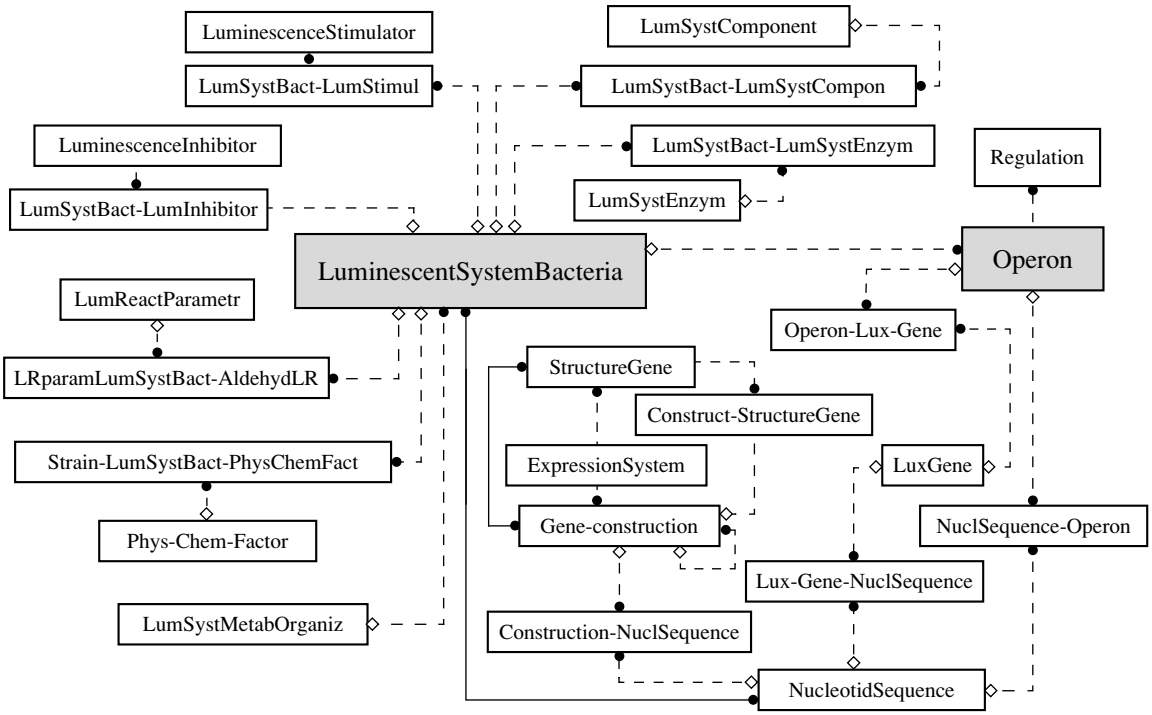


**Fig. 3.** Structure of the central block "Luminescent System" of the BiolumBase DB.

acteristics, relation to the general cellular metabolism, effect of physicochemical factors on luminescence, and regulation and control of the *lux* gene expression (Fig. 3). The other important subdivision of the central block, "Operon," includes various characteristics of the *lux* operon concerning, primarily, the structural organization of the known *lux* operons of luminous bacteria and peculiarities of their regulation in both host cells and novel recombinant constructions. Interrelations between the characteristics of microorganisms attributed to the central block were elaborated. New elements were introduced that characterize functional interrelations between bioluminescence, general metabolism of the cells carrying *lux* genes, and environmental factors. Thus, the central block offers types of bioluminescent systems of the known species of luminous bacteria, which are used as reporter systems in recombinant constructions, as well as mechanisms of the bioluminescent reaction and regulation of the expression of the *lux* operons.

Part of the information stored in two DB divisions differs somewhat in its structure due to the data specificity and some differences in priorities in the descriptions of natural and transgenic strains. In particular, a detailed description of the properties of natural microorganisms is important for their identification, storage, and application, whereas peculiarities of cloning of *lux* systems and structure and functioning of the genetically engineered constructions (such as plasmids), population resistance, and expression of new genes are more important for transgenic microorganisms. That is why in the division "Natural Luminous Microorganisms," the block "Metabolism," describing metabolic features of luminous bacteria at the level of species and strains, is of particular importance. It presents properties that are necessary for the determination of the taxonomic status of the strain and its practical application. Tables of this block present the main physiological and biochemical characteristics of strains, including specific features of free-living and associative bacteria, and data on the production of vitamins, exoenzymes, and reserve compounds, which may be needed for biotechnology. At the same time, the division "Transgenic Luminous *Microorganisms* contains the block "Genetically Engineered Constructions," in which vectors with cloned *lux* genes are described.

### *The Content of DB*

The information on natural and transgenic luminous microorganisms is kept in tables of corresponding DB blocks, whereas the information on the features of functioning of their luminescent systems is stored in the tables of the central block. Provision was made for the storage of 140 characteristics of natural and 150 characteristics of transgenic luminous microorganisms. Currently, the DB contains information on 6 type strains and over 20 collection strains of natural and 30 strains of transgenic luminous bacteria.

The properties of the *lux* systems determined by the metabolic activity of luminous bacteria (phenomenology, structure, kinetic characteristics, association with the general cellular metabolism, characteristics of light emission, effect of physicochemical factors on luminescence, regulation and control of the *lux* gene expression) are described in the tables of the subdivision "Luminescent System of Bacteria" in the central block of the DB (Fig. 4). Peculiarities of individual species and strains are determined by the enzyme luciferase and the substrates of the bioluminescent reaction, as well as by the type of luminescence regulation at the level of genome, which is reflected in the dynamics of luminescence during cell growth at the maximum light intensity. In the subdivision "Operon" of the central block, various characteristics of the *lux* operon are described; they are related mainly to the structural organization of the known *lux* operons of luminous bacteria and peculiarities of their regulations in both host cells and new recombinant constructions. For transgenic microorganisms carrying reporter bioluminescent system from marine luminous bacteria, common characteristics of cloned *lux* systems include the source of the *lux* system and mechanisms responsible for its expression in the strain. Of much importance is the variability of the *lux* gene expression in relation to the construction of the cloned system, applied vector, promoter type, and the type of metabolism of the new host cell (Fig. 5).

The DB contains figures illustrating structural organization of bioluminescent systems from various luminous microorganisms and their fragments applied for cloning. To make the DB more informative, it was supplied with a map of the distribution of luminous bacteria in the oceans (Fig. 6) and photographs of luminescence in various natural and transgenic microbial strains and growth of colonies, as well as with microphotographs of morphology and ultrastructure of cells. Of great interest to users is the illustration of the structural organization of the sequences of DNA and proteins associated with bioluminescence. Comparative data on the sequences of the bioluminescent genetic systems of marine luminous bacteria, including those applied for cloning, will undoubtedly be in large demand.

The DB of luminous microorganisms also envisages input of data on the relations between the induction and development of luminescence depending on the environmental factors. The effect of biotic, abiotic, and anthropogenic factors on bioluminescent genetic systems of transgenic and natural luminous bacteria is a key aspect in both DB divisions. This information is of great importance for the development of fundamental investigations and practical application of the reporter *lux* genes.

Both DB divisions were supplied with bibliographies concerning taxonomy, morphology, physiology, and biochemistry of luminous microorganisms, as well as structure, functioning, and regulation of their bioluminescent systems; expression and structure of the *lux* genes; and their application in various fields of science, ecology, medicine, and biotechnology. The DB allows incorporation of extended publications (annotated references and papers in pdf format) and Web references, including Web pages of world collections of microorganisms and vectors.

## CONCLUSIONS

Bioluminescence in the visible spectrum is a unique phenomenon; it is typical of living organisms belonging to various evolutionary branches; however, the majority of luminous microorganisms occur in aquatic ecosystems. In recent years, nonluminous microorganisms with cloned reporter bioluminescent systems have been of greater practical significance than natural luminous organisms. Among other reasons, this is explained by the well-studied physiological and biochemical characteristics and genome structure of the traditional

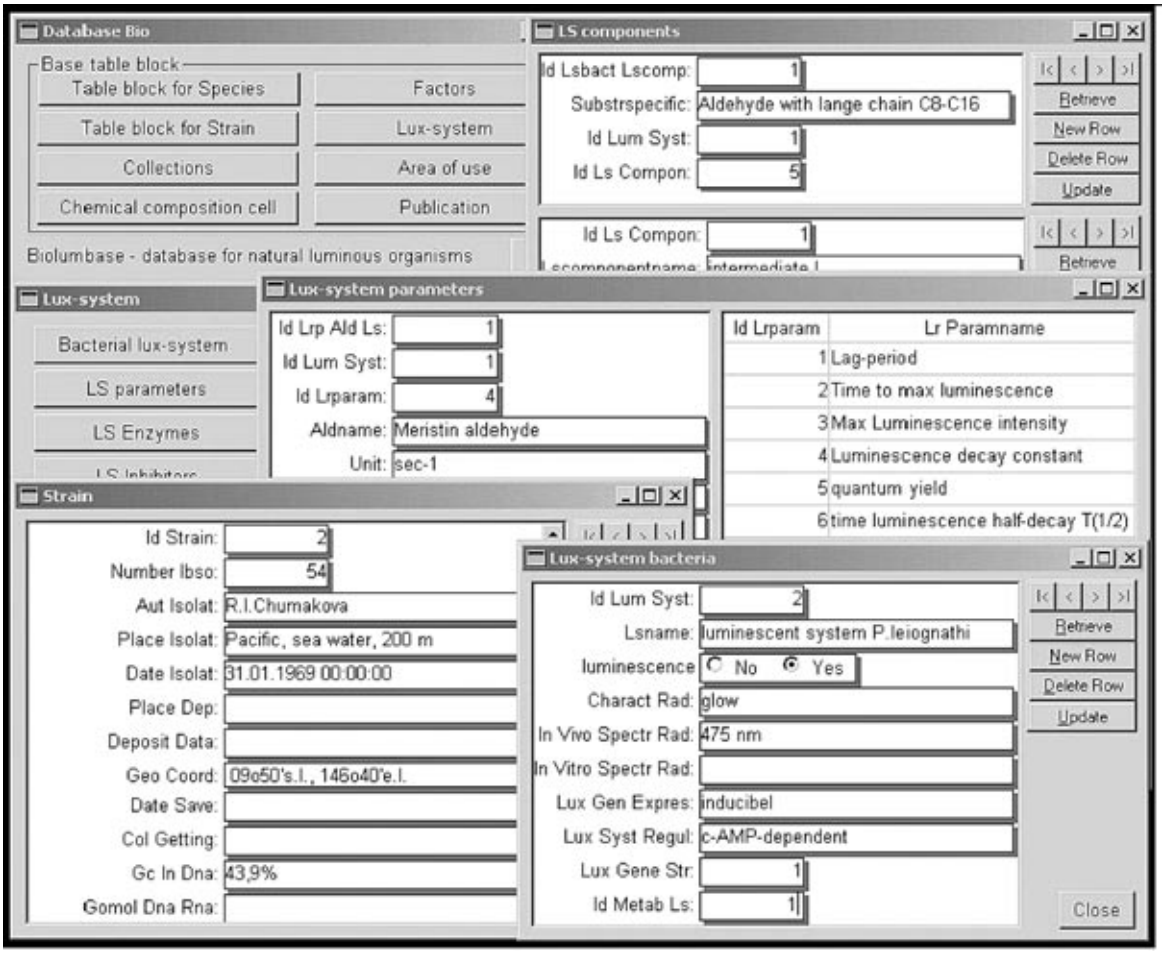


**Fig. 4.** Example of representation of the main properties of a luminescent system from natural luminous bacteria in tables of the central block of the BiolumBase DB.

objects of genetics, molecular biology, and biotechnology, such as *Escherichia coli* and bacteria of the genera *Bacillus* and *Pseudomonas.* Moreover, natural marine luminous bacteria exhibit high bioluminescence only when grown on specific media with a distinct level of salinity, which limits their application in the examination of freshwater and other environments. In addition, cloning of *lux* genes into nonluminous microorganisms is attractive because of the possibility to use various promoters whose induction or inhibition can increase or decrease the production of cloned genes, which may be determined from the changes in the intensity of luminescence. Therefore, the regulation of a certain operon can be investigated without isolation of enzymes or other proteins, which is important for the solution of fundamental problems of biology, as well as for practical application of bioluminescence in biotesting and biomonitoring.

Bioluminescent operons are nonhazardous enzyme systems providing for light emission in visible spectrum due to an oxidation–reduction reaction; the main fields of their use are ecology (monitoring of microbial cells in natural environments, evaluation of pollutant concentrations), health services (monitoring of pathogens in experimental animals evaluation of microbial contamination), molecular biology (studies of the regulation of bacterial operons), and analytical chemistry and biochemistry (development of various biotests). One of the fields of the application of bioluminescent systems is associated with the study of the fate and distribution of recombinant DNA under various conditions. Wide application of recombinant DNA in modern biotechnology is a hazard for the natural gene fund. In this connection, one of the urgent problems of microbial ecology is to evaluate the risk of accidental or the efficiency of targeted introduction of transgenic microorganisms into natural ecosystems. At present, efforts are under way in the world to develop experimental models that will reliably evaluate the efficiency of the expression of recombinant DNA and to follow its fate when its presence is undesirable. Genes of the *lux* operons cloned into nonluminous microorganisms are efficient model systems to study the consequences of accidental or targeted introduction of transgenic microorganisms into the environment. The data on the

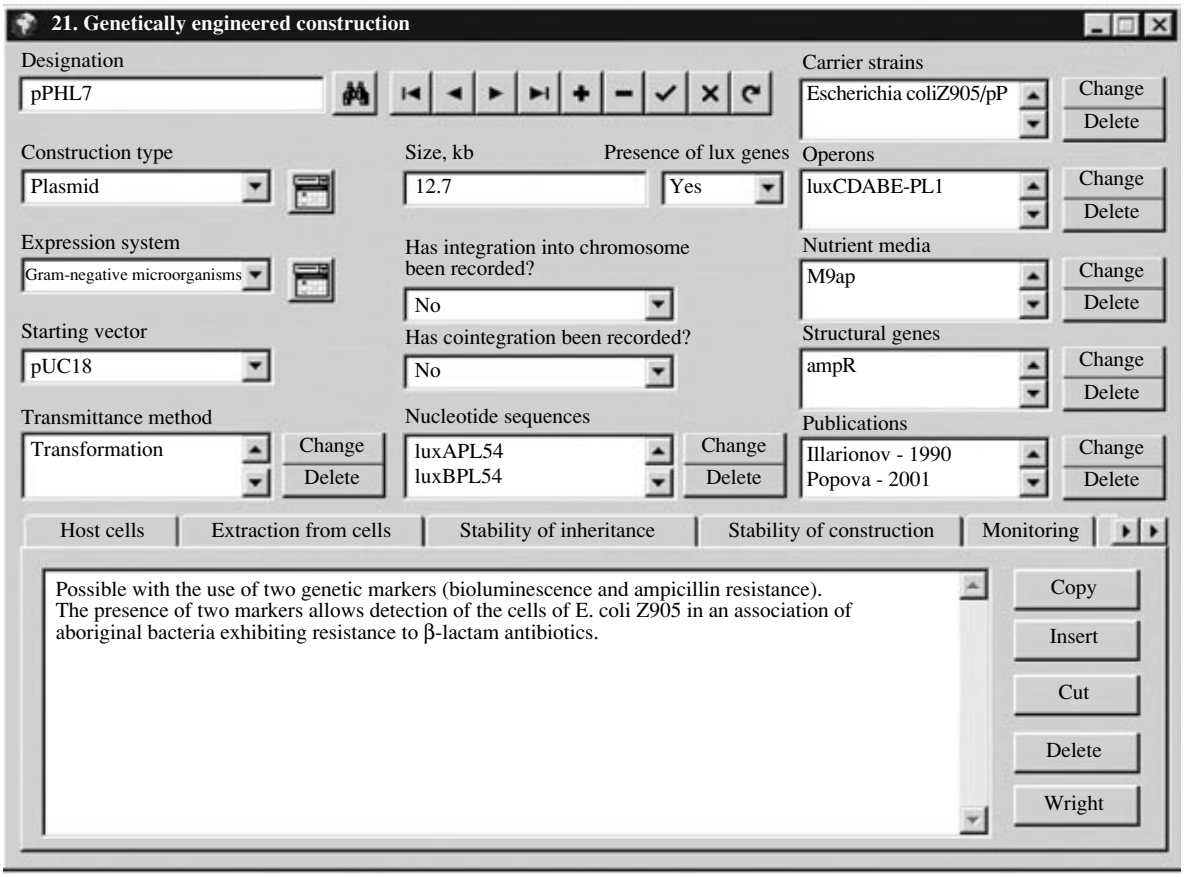


**Fig. 5.** Example of representation of the main properties of a genetically engineered construction in a table of the division "Transgenic Luminous Bacteria" of the BiolumBase DB.

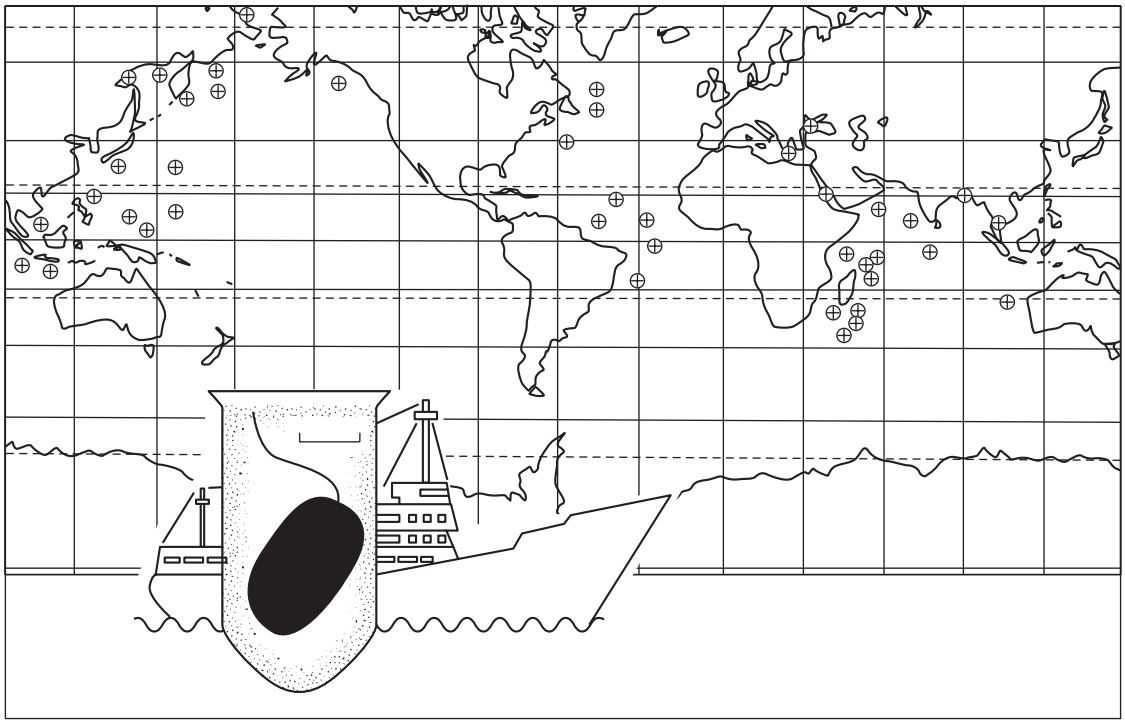

**Fig. 6.** Example of graphical information: a map of the distribution of luminous bacteria from the collection of the Institute of Biophysics, Siberian Division, Russian Academy of Sciences, in the oceans.

monitoring of transgenic microorganisms by the bioluminescent signal were confirmed by more complicated methods of molecular biology. Cloned *lux* operons or their fragments can also be used to predict stability of the expression of foreign genes in host organisms under various conditions. Such marker systems have been developed for a great number of microorganisms, higher organisms, and plants. However, in spite of the volume of information, these data are dispersed among various sources.

At present, the designed BiolumBase DB on natural and transgenic luminous microorganisms has no analogy in the world; it provides formalized text and graphic information on objects, as well as documents from other programs. Important properties of objects are illustrated with photographs and schemes; maps of the distribution of luminous bacteria in the oceans are provided. Bibliographies contain numerous references (including Web references) concerning the properties and fields of application of microorganisms carrying *lux* genes, isolated luminescent systems, vectors, etc. The software design is still in progress, as well as the acquisition and analysis of Web information concerning electronic culture collections and molecular DBs on nucleotide and amino acid sequences and vectors (these data may be useful for the subsequent Web integration of the designed DB). The authors have at their disposal culture collections of natural and transgenic luminous microorganisms ($lux^+$), which allows performing additional experimental studies to obtain missing important information [1, 14, 15]. The program under development envisages the publication of BiolumBase at the sites of the Institute of Biophysics, Siberian Division, Russian Academy of Sciences (http://www.ibp.ru/collection/default.htm and http://lux.ibp.ru). The data on the properties of natural luminous bacteria from the collection of the Institute of Biophysics, Siberian Division, Russian Academy of Sciences, were included in the consolidated Catalogue of Microorganisms of the All-Russia Collection of Microorganisms, which is accessible at the site (http://www.vkm.ru).

To conclude, the given description of the BiolumBase structure presents only initial stage of its design; the structure of BiolumBase implies the possibility of its further modification and development.

## ACKNOWLEDGMENTS

This work was supported by the Russian Foundation for Basic Research, project no. 00-07-90111, grant of the President of the Russian Federation no. MK-3562.2004.4 and by the Lavrent'ev project for young scientists, Siberian Division, Russian Academy of Sciences.

## REFERENCES

1. Rodicheva, E.K., Vydryakova, G.A., and Medvedeva, S.E., *Katalog kul'tur svetyashchikhsya bakterii* (Catalog of Fluorescent Bacteria), Novosibirsk: Nauka, 1997.
2. Stewart, G.S.A.B. and Williams, P., *Lux* Genes and the Application of Bacterial Bioluminescence, *J. Gen. Microbiol.*, 1992, vol. 138, pp. 1289–1300.
3. Meighen, E.A., Bacterial Bioluminescence: Organization, Regulation, and Application of the *lux* Genes, *FASEB J.*, 1993, vol. 7, no. 11, pp. 1016–1022.
4. Hill, Ph.J. and Stewart, G.S.A.B., Use of *lux* Genes in Applied Biochemistry, *J. Biolumin. Chemilumin.*, 1994, vol. 9, pp. 211–215.
5. Parveen, A., Smith, G., Salisbury, V., and Nelson, S.M., Biofilm Culture of *Pseudomonas aeruginosa* Expressing *lux* Genes As a Model To Study Susceptibility to Antimicrobials, *FEMS Microbiol. Lett.*, 2001, vol. 199, no. 1, pp. 115–118.
6. Ben-Israel, O., Ben-Israel, H., and Ulitzur, S., Identification and Quantification of Toxic Chemicals by Use of *Escherichia coli* Carrying *lux* Genes Fused to Stress Promoters, *Appl. Environ. Microbiol.*, 1998, vol. 64, pp. 4346–4352.
7. Rettberg, P., Baumstark-Khart, C., Bandel, K., Ptitsyn, L.R., and Horneck, G., Microscale Application of the SOS-LUX-TEST As Biosensor for Genotoxic Agents, *Anal. Chim. Acta*, 1999, vol. 387, no. 3, pp. 289–296.
8. Waddell, T.E. and Poppe, C., Construction of Mini-TN10luxABcam/Ptac-ATS and Its Use for Developing a Bacteriophage That Transducers Bioluminescence to *Escherichia coli* O157:H7, *FEMS Microbiol. Lett.*, 2000, vol. 182, no. 2, pp. 285–289.
9. Ulman, J.D. and Widom, J., *An Introduction to Database Systems*, Moscow: Lori, 2000 (Russian translation).
10. Baxevanis, A.D., The Molecular Biology Database Collection: 2003 Update, *Nucleic Acids Res.*, 2003, vol. 31, no. 1, pp. 1–12.
11. Akhlynin, D.S. and Gal'chenko, V.F., UNIQEM, a Microbiological Database: Problems and Prospects, *Mikrobiol*, 2000, vol. 69, no. 4, pp. 574–580.
12. Urbance, J.W., Cole, J., Saxman, P., and Tiedje, J.M., BSD: The Biodegradative Strain Database, *Nucleic Acids Res.*, 2003, vol. 31, no. 1, pp. 152–155.
13. Maklakov, S.V., *VRwin i ERwin. SASE-sredstva razrabotki informatsionnykh sistem* (WRwin and ERwin: Computer-associated Facilities for Development of Information Systems), Moscow: DIALOG-MIFI, 2000.
14. Maksimova, E.E. and Popova, L.Yu., Regulation of the *lux* Operon Cloned on a Recombinant Plasmid in *Escherichia coli* Cells: Changes over Successive Subculturing under Different Cultivation Conditions, *Mol. Biol.*, 1998, vol. 32, no. 2, pp. 371–372.
15. Rodicheva, E.K., Medvedeva, S.E., Tyulkova, N.A., Kotov, D.A., Kuznetsov, A.M., and Sukovataya, I.E., Bacterial Bioluminescent System as Visual Indicator of Metabolic and Biocatalytic Processes, in *Biological Resource Centers and the Use of Microbes—XXII ECCO Meeting Proceedings Book*, Lima, N., and Smith, D., Eds., Braga: Micoteca da Universidade do Minho, 2003, pp. 57–68.