Polish Journal of Microbiology
2007, Vol. 56, No 4, 227–231

MINIREVIEW

The Use of Marine Bacteria in Mutagenicity Assays

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Received 15 August 2007, revised 25 September 2007, accepted 2 October 2007

Abstract

Mutagenic pollution of environment is a global and important problem. This includes marine environment. Although many mutagenicity assays have been developed, there are specific problems with testing marine water and sediments for mutagenic contamination. One of them is the fact that most of genetically modified strains used in commonly available microbiological mutagenicity assays, like *Escherichia coli* or *Salmonella*, survive relatively poorly in marine waters, especially those of higher salinity. Thus, alternative assays have been developed, in which bacteria occurring naturally in marine habitats are employed. These assays, reviewed in this article, appear to be useful in testing not only marine samples but also can be used in other approaches, which involve detection and estimation of the amount of mutagenic compounds.

Key words: bioluminescence, marine bacteria , mutagenicity assays, mutagenic pollution of environment

Introduction

The presence of mutagenic compounds in different habitats appears to be a common phenomenon rather than an exception (Davey, 1999). The list of known mutagenic chemicals is very long. For example, Genetic Toxicology Data Bank (GENE-TOX), a part of Toxicology Data Network (TOXNET), includes peer-reviewed genetic toxicology test data for over 3000 chemicals (http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?GENETOX). Thus, mutagenic pollution of the natural environment is undoubtedly a serious and general problem.

Mutagenic chemicals can induce serious diseases, including cancer, due to their genotoxic (mutagenic) activities (El-Bayoumy, 1992; Depledge, 1998; Au *et al.*, 2001; Martin, 2001; Tornqvist and Ehrenberg, 2001; Barton *et al.*, 2005). The germ line of higher organisms may be also affected by these compounds, which may lead to fertility problems and to negative genetic changes in future generations (Shelby *et al*., 1993). Currently, mutagenic pollutants appear in the environment mostly as side effects of industrial processes (Heddle *et al.*, 1999; Goldman and Shields, 2003; Vargas, 2003; Jha, 2004).

Since chemical mutagens elicit deleterious effects on living organisms, their detection in the environment is very important. However, as there are thousands of known mutagens that occur in natural habitats, there are no simple chemical procedures which might be employed for testing the presence of such compounds. One should note that mutagens usually reveal genotoxic effects at very low concentrations. Thus, to perform an analysis for the presence of mutagenic compounds in an environmental sample, for example a water sample, material from a few hundred litres must be concentrated before an actual analysis. Moreover, chemical methods are useful mainly in assays for particular chemicals. Therefore, it appears that for preliminary and rapid detection of mutagenic activities in environmental samples, biological assays are more useful than chemical analyses. Although no currently available biological test can provide detailed and precise information about the chemical nature of detected mutagens, such tests provide a possibility to answer the question whether examined samples contain mutagens at levels potentially dangerous for organisms. Therefore, it seems that the most reasonable strategy for testing environmental samples is to use a biological assay as a preliminary test to detect

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the presence of mutagenic compounds. Then, if necessary, subsequent detailed chemical analysis can be performed to determine the specific type of mutagen(s).

Among biological mutagenicity assays, microbiological tests are commonly used as they are relatively simple (Węgrzyn and Czyż, 2003). However, there are also some problems with the commonly used microbiological mutagenicity assays, especially when environmental samples should be tested. This concerns particularly marine waters. The commonly used Ames test is simple, sensitive, and was shown to be an excellent tool for laboratory studies on various chemicals (Ames, 1971; Ames *et al.*, 1973, 1975). However, to obtain results of measurements, one must usually wait as long as two days since sample withdrawal, which is usually too long for efficient monitoring and assessment of marine environment (Mortelmans and Zeiger, 2000). Moreover, survival of *Salmonella enterica* serovar Typhimurium strains (used in the Ames test) in marine water is poor (Czyż *et al*., 2000), which makes the use of this test for assessing marine water samples problematic. This concerns also other bacteria for which marine environment is a natural habitat. Therefore, efforts have been performed to construct alternative tests, which should be more rapid and more applicable for testing samples of marine water and sediments. For this purpose, either genes of marine bacteria or strains of marine vibrios have been employed to construct useful tools, applicable for monitoring of mutagenic pollution of marine environment as well as for other purposes which require rapid and sensitive methods of estimation of mutagenicity of tested samples.

Employing Genes of Marine Bacteria in Mutagenicity Assays

Since using the Ames test one requires two days to obtain results of the mutagenicity assay, several groups aimed to construct more rapid and possibly more sensitive tests. Many species of marine bacteria are luminescent, and the luminescence appears to be an easy parameter to be detected and quantified. Thus, genes coding for bacterial luminescent systems have been employed for construction of *Salmonella* strains, which can emit light in response to the contact with mutagenic compounds. In the VITOTOX test (van der Leile *et al*., 1997), the *Vibrio fischeri luxCDABE* operon has been placed under control of the *recN* promoter and its derivatives. Thus, strains bearing such constructs emit light after induction of the SOS response (the *recN* gene belongs to the SOS regulon), which is activated upon cellular DNA damage. This assay was found to be very sensitive, several hundred times more sensitive than the Ames test (in the case of some compounds) (van der Leile *et al*., 1997; Merilainen and Lampinen, 2004). Moreover, using VITOTOX, the results can be obtained in a few hours (usually 1–4 h) (van der Leile *et al*., 1997). Apart from these advantages, a disadvantage of VITOTOX for studies of marine waters is the use of *Salmonella* strains that survive poorly in samples of these waters. Another problem with this assay is that it detects only agents inducing the SOS response. Although all chemicals that cause DNA damage, and thus induce the SOS response, are mutagenic, there are many mutagens (*e.g.* base analogues) that do not disturb DNA integrity. Such compounds cannot be detected by VITOTOX.

A battery of similar to VITOTOX, but modified, assays has been constructed. Examples of such assays are: (i) the test based on an *E. coli* strain bearing the *recA*::*luxCDABE* fusion (Min *et al*., 1999) and its derivatives, in which a *tolC* mutant was used, the fusion was incorporated into the chromosome, the *lux* genes from *Photorhabdus luminescens* rather than from *Vibrio fischeri* were used, and the host bacterium was *S. enterica* serovar Thyphimurium rather than *E. coli* (Davidov *et al*., 2000), (ii) the test employing an *E. coli* host bearing the *V. fischeri luxCDABE* operon fused to *grpE, katG* or *fabA* (Gu *et al*., 2002; Min *et al.*, 2003), and (iii) an assay, in which two *Salmonella* strains bearing the *umu-luxCDABE* (from *V. fischeri*) are used (Taguchi *et al.*, 2004). All these tests are relatively quick (one can get results in about 4 h), but have the same disadvantage as VITOTOX.

Strains of Marine Bacteria as Testers of the Presence of Toxicants and Mutagens

One way to overcome the problems with salt-sensitive bacteria (like *Salmonella*) used in testing marine samples is the use of species naturally occurring in marine habitats. *V. fischeri* and *V. harveyi* are examples of such species. They occur in virtually all seas and oceans and have a great tolerance to salinity. Both species are bioluminescent, and this feature has been employed in three out of four assays described below.

Microtox

In the Microtox test, changes in the light output of luminescent bacteria (*V. fischeri*) are measured by a temperature controlled photometric device (Bulich and Isenberg, 1981). Toxic agents cause a decrease in bacterial luminescence, which indicates their presence in the tested sample. It is, however, important to distinguish toxicity assays from mutagenicity tests. This is because toxic chemicals are not always mutagens, and mutagenic agents are often toxic only at relatively high concentrations. Since many mutagens

occur in natural environments at concentrations too low to provoke serious toxic effects in bacterial cells, the assay described above can be used for detection of toxic substances rather than mutagenic agents. Nevertheless, Microtox is a very quick test (30 min) and it was described as one of the most sensitive biological toxicology assays (Richardson, 1996; Davoren and Fogarty, 2004).

Mutatox

The Mutatox test is a mutagenicity assay, in which a "dark" strain of *V. fischeri* is used (Ulitzur *et al*., 1980; Ulitzur and Weiser, 1981). This strain (named M169) is a mutant with a lesion in the regulatory luminescence system rather than in one of coding sequences of the *lux* structural genes. Light production is restored in this strain in the presence of relatively low concentrations of mutagens. Using Mutatox, different kinds of mutagens can be detected, including those causing base substitution, insertions and deletions, DNA synthesis inhibition or DNA damage.

The sensitivity of Mutatox was reported to be comparable to that of the Ames test for both pure compounds and environmental samples. In fact, Mutatox was employed in many environmental studies (see for example Sun and Stahr, 1993; Arfsten *et al*., 1994; Richardson, 1996). Twenty four hours are required to obtain results with this assay, which is an advantage relative to the Ames test (in which 48 h are necessary) but such a time is still too long for rapid monitoring of marine environment.

Vibrio harveyi neomycin-resistance mutagenicity assay

Apart from *V. fischeri* (used in Microtox and Mutatox), another marine luminescent bacterium, *V. harveyi* has been used to construct mutagenicity assays. The first such an assay was constructed by Czyż *et al.* (2000) and it is similar to the Ames test. However, in this *V. harveyi* assay, a set of genetically modified, neomycin-sensitive strains is used. Mutants resistant to this antibiotic can be easily isolated and the frequency of the appearance of such mutants increases in the presence of mutagens (Czyż *et al*., 2000). To enhance sensitivity of the assay, a transposon mutant, very sensitive to mutagenic factors, was isolated.

The second modification was the introduction of a plasmid bearing *mucA* and *mucB* genes, coding for proteins involved in an error-prone DNA repair. The assay consists of detection of neomycin-resistant mutants on plates either containing a mutagen in the solid medium or after incubation of bacterial cultures in a liquid medium, in the presence of tested compounds or environmental samples. Neomycin is an aminoglycoside antibiotic that interferes with decoding at the ribosomal A site during translation (Dahlberg, 1989). Resistance to this antibiotic occurs as a result of various rRNA modifications in the decoding site. Therefore, a large spectrum of mutagenic agents, causing different types of mutations, may lead to the appearance of neomycin-resistant mutants, which can be detected.

It was demonstrated that for some compounds, the *V. harveyi* assay is more sensitive than the Ames test (Czyż *et al*., 2002). The *V. harveyi* test has been found useful in environmental studies as detection of mutagens in marine water samples from different geographical regions was possible (Czyż *et al*., 2003). This assay was further optimized by using various cultivation conditions of the tester strains (Podgórska *et al*., 2005).

Although one needs a relatively long time to perform this assay (48 hours, like in the case of the Ames test) there are some advantages of its use, apart from a possibility to test marine water samples. Namely, *V. harveyi* is not pathogenic to humans, and thus, it is completely safe to work with. Furthermore, this bacterium is more sensitive to mutagens than *E. coli*, and its LPS is significantly more permeable for large molecules than LPS of *Salmonella* (Czyż *et al*., 2000). Interestingly, the *V. harveyi* neomycin-resistance mutagenicity assay was used not only in testing marine samples, but also in laboratory studies on mutagenicity of certain compounds and antimutagenic activities of other substances (Piosik *et al*., 2003, 2005; Ulanowska *et al*., 2005, 2007). In one case, the problem of contradictory conclusions on mutagenicity of one compound, presented by different authors who use the same assay (the Ames test), was resolved by employment of the *V. harveyi* test (Ulanowska and Węgrzyn, 2006).

Vibrio harveyi luminescence mutagenicity assay

Because of some disadvantages of the *V. harveyi* neomycin-resistance mutagenicity assay, mentioned in the previous subsection, its significantly modified version has been developed (Podgórska and Węgrzyn, 2006). This novel assay is based on the use of the *V. harveyi* mutant in the *luxE* gene. This mutant is dim, but upon contact with mutagens, fully luminescent revertants or pseudorevertants appear, thus luminescence of a bacterial culture became significantly increased. It was demonstrated that this increase in luminescence is effective and easily measurable after just a few (2–4) hours of treatment with various mutagenic agents, revealing a dose-response correlation (Podgórska and Węgrzyn, 2006). Although this assays resembles Mutatox, its advantage is a short time of the analysis (a few hours in this assay, relative to 24 hours in Mutatox).

Usefulness of the *V. harveyi* luminescence mutagenicity assay in testing environmental samples has been

demonstrated for different materials alone, as well as in combination with some other methods. As mentioned above (see Introduction) mutagens occur in natural environment usually at low concentrations, while still having marked biological activities. Triolein-containing semipermeable membrane devices (SPMDs) provide a method for concentration of hydrophobic organic contaminants, including a large fraction of mutagens (Lu *et al*., 2002). A procedure based on direct addition of tester (*V. harveyi luxE*) bacterial cultures into SPMD has been proposed. This procedure was found to be rapid and sensitive, and potentially useful in monitoring marine waters for mutagenic contamination (Chęć *et al*., 2006).

The *V. harveyi* luminescence mutagenicity assay has been demonstrated to be suitable for testing samples of marine water (Podgórska *et al*., 2007a), plant tissue extracts including plants from marine habitats (Podgórska *et al*., 2007b) and marine sediments (Podgórska *et al*., 2007c). Moreover, the *V. harveyi luxE* mutant can be used in assessment of accumulation of mutagenic compounds in animal tissues, which was demonstrated in studies on extracts of mussel tissue (Chęć *et al*., 2007). Apart from demonstrating usefulness of the *V. harveyi* luminescence mutagenicity assay, the above listed investigations provide some information regarding levels of mutagenic pollution of certain regions of Baltic Sea (Podgórska *et al*., 2007a, 2007b, 2007c).

Concluding Remarks

The use of marine bacteria in toxicity and mutagenicity assays (Microtox, Mutatox, *V. harveyi* neomycin-resistance mutagenicity assay and *V. harveyi* lumincesce mutagenicity assay) provide important advantages relative to commonly used tests based on the use of *E. coli* and *Salmonella* strains. First, assays employing marine bacteria allow direct testing of marine samples. Second, marine vibrios used in the assays are not pathogenic to humans, and thus, they are completely safe to work with. Third, these bacteria are more sensitive to mutagenic agents than *E. coli*, partly because LPS of vibrios is significantly more permeable for large molecules than LPS of *E. coli* and *Salmonella*. Fourth, three of four tests based on marine vibrios are significantly more rapid methods than the Ames test and similar assays. Thus, the assays described in this article have already been used in marine environment monitoring and assessment, and it is likely that such studies will be expanded significantly in near future.

Acknowledgemnts

The authors acknowledge a financial support from the Institute of Oceanology of the Polish Academy of Sciences (task grant No. IV.3.2).

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