Journal of Microbiological Methods 114 (2015) 1–8

Contents lists available at ScienceDirect

Journal of Microbiological Methods

journal homepage: www.elsevier.com/locate/jmicmeth

Isolation of a conjugative F-like plasmid from a multidrug-resistant *Escherichia coli* strain CM6 using tandem shock wave-mediated transformation

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ARTICLE INFO

Article history:
Received 11 April 2015
Accepted 20 April 2015
Available online 23 April 2015

Keywords:
F-like plasmid
Escherichia coli
Multidrug-resistant bacteria
Transformation
Tandem shock waves

ABSTRACT

Genetic characterization of plasmids from bacterial strains provides insight about multidrug resistance. Ten wild type *Escherichia coli* (*E. coli*) strains isolated from cow fecal samples were characterized by their antibiotic resistance profile, plasmid patterns and three different identification methods. From one of the strains, a fertility factor-like plasmid was replicated using tandem shock wave-mediated transformation. Underwater shock waves with a positive pressure peak of up to approximately 40 MPa, followed by a pressure trough of approximately −19 MPa were generated using an experimental piezoelectric shock wave source. Three different shock wave energies and a fixed delay of 750 μs were used to study the relationship between energy and transformation efficiency (TE), as well as the influence of shock wave energy on the integrity of the plasmid. Our results showed that the mean shock wave-mediated TE and the integrity of the large plasmid (~70 kb) were reduced significantly at the energy levels tested. The sequencing analysis of the plasmid revealed a high identity to the pHK17a plasmid, including the replication system, which was similar to the plasmid incompatibility group FII. It also showed that it carried an extended spectrum beta-lactamase gene, ctx-m-14. Furthermore, diverse genes for the conjugative mechanism were identified. Our results may be helpful in improving methodologies for conjugative plasmid transfer and directly selecting the most interesting plasmids from environmental samples.

1. Introduction

Bacteria may contain additional genetic information physically separate from the chromosomal deoxyribonucleic acid (DNA) in the form of plasmids. These double-stranded DNA molecules encode a large variety of functions that are not essential for the bacteria host or the plasmid but sometimes increase fitness in atypical niches (Francia et al., 2004). In addition to other resistance properties, plasmids protect bacteria hosts from the toxic effects of heavy metals and free-radicals or carry genes with a large metabolic repertoire, including enzymes for the synthesis of bioactive molecules, such as colicins i.e., bacteriocins produced by some strains of *Escherichia coli* (*E. coli*) that can be toxic to other *E. coli* strains. Some host lifestyle-modifying factors, such as biological products that confer pathogenicity by encoding toxins or the colonization of antigens, are also present. Plasmids have been considered ancient vectors of genetic mobilization by transduction, transformation and conjugation in populations of the same or different species or even being capable of transfer between bacteria and yeast (Couturier et al., 1988). This available pool of genetic information has important effects on bacterial evolution. For a plasmid it is crucial to persist inside its host, where the mechanism of replication, inheritance and dissemination is essential for its survival. Autonomous replication is an important characteristic for maintaining plasmids within their host, and it could potentially be regulated at the level of initiation, elongation or termination (Couturier et al., 1988). In many plasmids, the basic replicon is represented in a 1–3 kb region. Some small plasmids typically contain a single basic replicon, but many large plasmids may have multiple

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http://dx.doi.org/10.1016/j.mimet.2015.04.007
0167-7012/

replicons (Couturier et al., 1988). Plasmids with multiple replicons increase the host's range of dissemination and persistence, including an interaction control for incompatibility with a resident plasmid. For example, the activation of an origin of replication has been observed in species ranging from *E. coli* to *Proteus mirabilis* and from *Bacillus cereus* to *Bacillus anthracis* (Couturier et al., 1988).

Plasmids play a crucial evolutionary role in diverse bacterial environments by providing a massive reservoir of genetic information. Because of this fact, genetic characterization provides an important insight into understanding the repertoire of genes in plasmids isolated from a multidrug-resistant bacterial strain. Plasmid persistence in a host strain requires not only physical mechanisms for DNA transfer but also activation of transcriptional factors and promoters for the expression of plasmid-encoded genes. The essential encoded information includes those for replication, maintenance and proliferation, which confer some factors modifying host lifestyle, such as antibiotic resistance and pathogenicity. To minimize the potential risk of contamination by pathogenic *E. coli* strains during manipulation in the laboratory and to study plasmid characteristics, our aim was to only use the plasmid and replicate it in a non-pathogenic strain using shock wave-mediated transformation i.e., DNA transfer into shock wave-exposed bacteria.

Shock waves have been used for several years in urology, orthopedics and traumatology (Loske, 2011). The transformation of bacteria using shock waves is an emerging and interesting technique (Jagadeesh et al., 2004; Loske et al., 2011; Campos-Guillén et al., 2012). For biomedical applications, shock waves consist of a compression pulse in water with a short rise time (below 10 ns), a full width at half maximum (FWHM) of approximately 0.5 to 3 μs, and a peak pressure between approximately 20 and 120 MPa. The positive pressure peak is followed by a decompression pulse of up to −25 MPa with duration of approximately 2 to 20 μs (Fig. 1).

Shock wave-induced cavitation plays a fundamental role in cell transformation. A cloud of bubbles forms at the focus of any shock wave generator used for clinical applications. Cavitation occurs because microbubbles or cavitation nuclei previously existing in the cell suspension are compressed by the positive phase of each incoming shock wave. After shock wave passage, the bubbles expand during approximately 200 to 600 μs and violently collapse, emitting secondary shock waves of nanosecond duration and high-speed (up to 400 m/s) jet blasts of fluid (microjets), which interact with cells (Johnsen and Colonius, 2008). Bubble collapse and microjet emission depends on the initial size of the bubbles and the pressure profile. Furthermore, the larger the viscosity of the liquid, the weaker the shock wave emitted during bubble collapse. According to Ohl and Ikink (2003) microjets may act as syringes capable of injecting a volume of approximately 0.1 R_0^3, where R_0 is the initial bubble radius before the arrival of the pressure pulse. The maximum pressure produced by secondary shock waves can be extremely high; however, a major part of the shock wave energy is dissipated within approximately 100 μm from the bubble (Brujan et al., 2008). These shock waves may interact with boundaries and other cavitation bubbles. Actually, there are two types of secondary shock wave emissions after bubble collapse. In the first case, secondary shock waves are a consequence of the independent collapse of the bubbles contained in the fluid. In the second case, shock wave formation is a consequence of the interaction between the cloud collapse-induced shock wave and microbubbles situated close to the collapse site of the cloud. This phenomenon is supposed to be analogous to sonoporation by ultrasound. In aqueous solutions, ultrasound forms cavitation bubbles, which create transient pores of approximately 30 to 100 nm in bacterial membranes, enabling the transfer of molecules into bacterial cells (Liu et al., 2006). In this case, the rupture is also caused by shock waves and microjets generated by collapsing bubbles. The membrane may recover after a few seconds.

It is known that the energy of a collapsing bubble and the following microjet emission can be significantly increased if a second shock wave is sent shortly before the bubble starts to collapse. The most convenient delay between the first and second shock waves depends on the specific application. This technique has been referred to as tandem shock wave generation (Loske et al., 2002, 2011) and was used in this study to transform *E. coli*.

2. Material and methods

2.1. Shock wave generation

A Piezolith 2501 shock wave source, manufactured by Richard Wolf GmbH in Knittlingen, Germany, was used to generate underwater shock

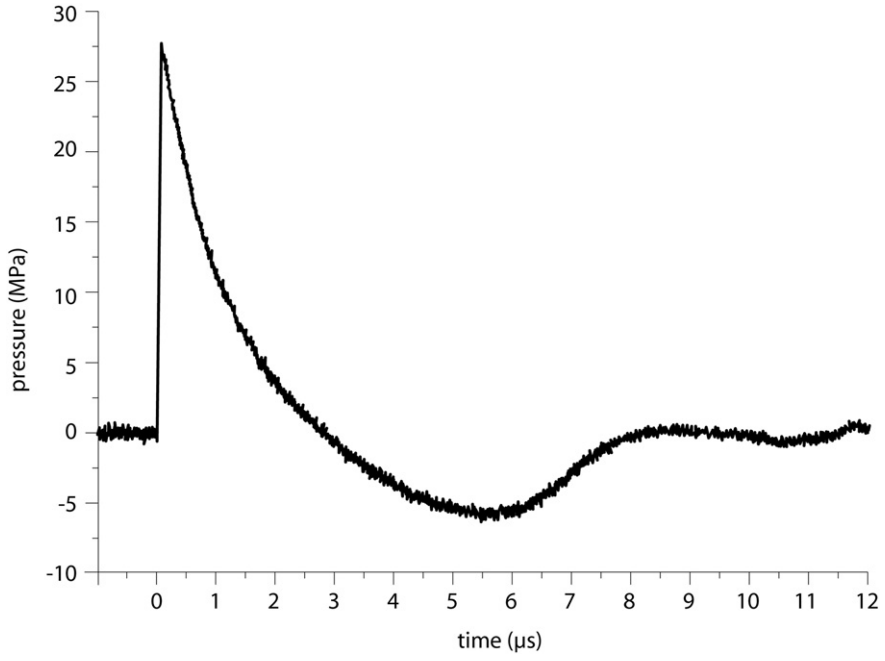

Fig. 1. Pressure profile of a single-pulse underwater shock wave emitted by the shock wave generator used in this study.