Journal of Photochemistry and Photobiology B: Biology 130 (2014) 140–145

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology B: Biology

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

Strain dependent UV degradation of *Escherichia coli* DNA monitored by Fourier transform infrared spectroscopy

Cristina M. Muntean [a], Alexandra Lapusan [b], Liora Mihaiu [c], Razvan Stefan [b,*]

[a] National Institute for Research & Development of Isotopic and Molecular Technologies, 65-103 Donath Str., 400293 Cluj-Napoca, Romania
[b] University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, 3-5 Calea Manastur Str., 400372 Cluj-Napoca, Romania
[c] Iuliu Haţieganu University of Medicine and Pharmacy, Faculty of Pharmacy, 8 Victor Babeş Str., 400012 Cluj-Napoca, Romania

ARTICLE INFO

Article history:
Received 24 July 2013
Received in revised form 25 October 2013
Accepted 12 November 2013
Available online 23 November 2013

Keywords:
DNA structure
Escherichia coli strains
Drug susceptibility
UV radiation
FT-IR spectroscopy

ABSTRACT

In this work we present a method for detection of DNA isolated from nonpathogenic *Escherichia coli* strains, respectively. Untreated and UV irradiated bacterial DNAs were analyzed by FT-IR spectroscopy, to investigate their screening characteristic features and their structural radiotolerance at 253.7 nm.

FT-IR spectra, providing a high molecular structural information, have been analyzed in the wavenumber range 800–1800 cm^{-1}. FT-IR signatures, spectroscopic band assignments and structural interpretations of these DNAs are reported.

Also, UV damage at the DNA molecular level is of interest. Strain dependent UV degradation of DNA from *E. coli* has been observed. Particularly, alterations in nucleic acid bases, base pairing and base stacking have been found. Also changes in the DNA conformation and deoxyribose were detected.

Based on this work, specific *E. coli* DNA-ligand interactions, drug development and vaccine design for a better understanding of the infection mechanism caused by an interference between pathogenic and nonpathogenic bacteria and for a better control of disease, respectively, might be further investigated using Fourier transform infrared spectroscopy. Besides, understanding the pathways for UV damaged DNA response, like nucleic acids repair mechanisms is appreciated.

1. Introduction

Analysis of bacterial deoxyribonucleic acid (DNA) is fundamental in human diagnostics, food science, biodefense studies and antimicrobial research. Particularly, information on biomolecular structural changes can provide new insights into biophysical and biochemical processes and a deeper understanding of the development of diseases at the molecular level [1,2].

Escherichia coli (abbreviated as *E. coli*) are a large and diverse group of bacteria (rod-shaped). Although most strains of *E. coli* are harmless, others can make one sick. The characterization of nucleic acids from these and other bacteria may yield a better understanding of the mechanisms of infection and information useful for vaccine design ([2] and references therein).

The *E. coli* chromosome is a circular DNA molecule that is ~1000 times compacted in the living cell, where it occupies ~15% of the cellular volume. The genome is organized in a way that facilitates chromosome maintenance and processing [3].

Particularly, the interaction between a nonpathogenic and a pathogenic strain was shown to synergistically enhance extraintestinal virulence in *E. coli* [4]. This work reported that a bacterial infection cannot be reduced to a population of genetically identical organisms infecting a host. Different organisms from the same species can interact within the host. Many aspects of bacterial virulence still need to be examined. These encompass bacteria-host and also bacteria-bacteria interactions. In an era of increasing antibiotic resistance, disrupting these fundamental interactions could lead to new and interesting ways to fight against infections [4].

Besides, the food-borne pathogen, *E. coli* O157:H7, has been associated with gastrointestinal disease and the life-threatening sequela hemolytic uremic syndrome. The genes for the virulence factor, Shiga toxin 2 (Stx2), in *E. coli* O157:H7 are encoded on a temperate bacteriophage under the regulation of the late gene promoter. The hypothesis that nonpathogenic *E. coli* could amplify Stx2 production if infected with the toxin-encoding phage was investigated [5].

Also, evaluation of recombinant invasive, nonpathogenic *E. coli* as a vaccine vector against the intracellular pathogen, *Brucella* was reported [6].

Nucleic acids of every size (short oligonucleotides, long native DNA, etc.) might be studied by infrared and Raman spectroscopy. Vibrational spectroscopy can solve the problem of resolving the long fragments structure of DNA strands ([2] and references therein). Particularly, FT-IR spectra reflect the total chemical composition of DNA and some of the spectral bands can be assigned to

* Corresponding author. Tel.: +40 264 596384/207.
E-mail address: rstefan@usamvcluj.ro (R. Stefan).

1011-1344/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jphotobiol.2013.11.009

distinct functional groups or chemical substructures ([7] and references therein). The information content of an IR spectrum is high [8]. Characteristic IR marker bands are a function of base composition, DNA conformation and the presence of an H_2O environment [9].

Particularly, the molecular mechanisms of the biological action of UV radiation on DNA have not yet been completely clarified [10]. It is well accepted that DNA is one of the principal macromolecular targets for radiation damage and that double-stranded breaks are the most lethal, resulting in loss of essential genetic information [7].

Several damages to structural units of DNA from *Pantoea agglomerans* were detected by FT-IR spectroscopy at various doses of γ-irradiation, to investigate the radiotolerance of this bacterium [7]. Besides, the effects of UV synchrotron radiation on calf thymus DNA cast films have been systematically investigated by vacuum ultraviolet and infrared spectrophotometry as a function of irradiation time [11].

In this work we present a method for detection of DNA extracted from nonpathogenic *E. coli* strains, respectively. Also, the influence of UV radiation (253.7 nm) on these genomic DNAs is reported. Characterization of untreated and UV irradiated DNAs from *E. coli* bacteria, was undertaken by use of Fourier transform infrared (FT-IR) spectroscopy.

All FT-IR spectra were presented in the wavenumber range 800–1800 cm^{-1}. FT-IR signatures, spectroscopic band assignments and structural interpretations for these DNAs are reported.

Based on these results, specific *E. coli* DNA-ligand interactions, drug development and vaccine design for a better understanding of the infection mechanism and for a better control of disease, respectively, might be further investigated using Fourier transform infrared spectroscopy. Besides, understanding the pathways for repair of UV-induced DNA damage is appreciated.

In microbiology field, *E. coli* DNA might be used to explore the interaction between DNA and small molecules, which is important in connection with probing the accurate local structure of DNA and with understanding the natural DNA-mediated biological mechanisms ([12,13] and references therein).

2. Materials and methods

2.1. Isolation of nonpathogenic E. coli colonies

The isolation protocol followed the steps recommended by the International Organization for Standardization, Geneve, Switzerland (ISO) (ISO-16649: 2000). Briefly, the meat samples (25 g) were previously homogenized in buffered peptone water (225 ml) with a laboratory blender (Stomacher 400, England) for approximately 2 min. After incubation for 18–24 h at 37 °C, 0.1 ml was inoculated in 10 ml EC Broth (Oxoid, England) and incubated for 18–24 h at 42 °C. From the enrichment broth obtained 1 ml was streaked onto Tryptone, bile, X-glucoronide (TBX) agar (Madrid, Spain). Following the incubation at 37 °C for 24 h, presumptive *E. coli* colonies were characterized by their biochemical properties through slide agglutination using standard protocols. The positive colonies were then identified as *E. coli* using the Sensititre Automated Microbiology System Aris 2X (Thermo Scientific, England) following the protocol stated by the producer. Nonpathogenic *E. coli* A strain was considered as reference for bacteria.

One *E. coli* strain (*E. coli* E) was isolated from human excrement.

2.2. DNA extraction from E. coli bacteria

The bacterial DNA extraction followed the basic steps previously described by Yang et al. [14] with a few particularities. Briefly, 150 μl of Pure Water Grade (Sigma, Germany) was added in Eppendorf tubes (1.5 ml) (RatioLab). The tubes were subjected to UV sterilization in a Microbiological Laminal Flow – Class II (Bioquell) so as to remove any possible contaminants from the manipulation performed earlier. 1–2 colonies were harvested with a sterile microbiological loop and immersed in the DNA free water. The following extraction temperatures were used: 57 °C – 30 min; 94 °C – 5 min. The last step included a high speed centrifugation (14,000 rot/min) for one minute. Four DNA samples (*E. coli* A1, *E. coli* B, *E. coli* C, *E. coli* D) were extracted in duplicate. One of the DNA sets was to UV for 30 min in a Microbiological Laminal Flow – Class II (Bioquell) (UV radiation 253.7 nm) (ISO-16654: 2001) [14]. Besides, two independently extracted DNA samples (*E. coli* E, *E. coli* A2) were isolated in quadruplicate. The two DNA sets were exposed to 253.7 nm UV radiation for 30 min, 1 h and 1 h 30 min, respectively. Isolated nucleic acids were dried and used in solid state.

2.3. Drug susceptibility testing

A number of 11 antimicrobials, generally used in animal and human therapy, were tested on the isolated strains of *E. coli* using the classical disk diffusion method, according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) (2006). The isolates were tested against ampicillin (AMP, 10 μg), cefotaxime (CTX, 30 μg), ceftazidime (CAZ, 30 μg), chloramphenicol (CHL, 30 μg), ciprofloxacin (CIP, 5 μg), gentamicin (GEN, 10 μg), nalidixic acid (NA, 30 μg), streptomycin (S, 10 μg), sulfamethoxazole (SMX, 300 μg) trimethoprim/sulfamethoxazole (SXT, 1.25/23.75 μg), and tetracycline (TET, 30 μg). The CLSI breakpoints were applied for the interpretation of the results.

E. coli antibiograms:

E. coli A: R – Tetracycline; trimethoprim/sulfamethoxazole; nalidixic acid;
E. coli B: R – Nalidixic acid, streptomycin, sulfamethoxazole, tetracycline;
E. coli C: R – Ciprofloxacin, nalidixic acid, tetracycline;
E. coli D: R – Streptomycin; cholramphenicol, ciprofloxacin;
E. coli E: R – Ciprofloxacin, nalidixic acid, intermediary tetracycline.

In *E. coli* isolates the highest rate of resistance was against nalidixic acid, tetracycline, ciprofloxacin. Isolates showing resistance to three or more antimicrobials were classified as multidrug resistant (MDR). The findings showed that *E. coli* isolates were MDR in all cases.

2.4. FT-IR spectroscopy

FT-IR measurements were performed in the absorbance with a spectrophotometer FT-IR-4100 Jasco, using the KBr pellet technique. The spectra were obtained in the wavenumber range 400–4000 cm^{-1}. Spectral resolution was set at 4 cm^{-1} and all spectra were acquired over 256 scans.

3. Results and discussion

FT-IR spectral profiles of bacterial DNAs are presented in the region 800–1800 cm^{-1} (Fig. 1) and also between 1800–4000 cm^{-1} as Supporting information [15]. No significant spectral information was found in the wavenumber range 400–800 cm^{-1}.

In the case of Fig. 1, spectra of DNAs extracted from different strains of *E. coli* bacteria, respectively, are presented. Untreated