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Probing putative carcinogenic potential of processed and unprocessed meat using bioluminescent bacterial bioreporters

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ABSTRACT

Consumption of meat is increasing worldwide and to prolong both it's shelf-life and safety companies add various chemicals, some of which are under evaluation to assess their responsible cancer induction. The International Agency of research on Cancer classified red meat and processed meat as probably carcinogenic (Group 2A) and carcinogenic (Group 1) respectively for humans. The aim of this study was to propose a biological sensor able to detect DNA mutations that could lead to cancer induction in beef, and chicken as well as processed meats. Genetically engineered *E. coli* DPD2794 strain is able to produce bioluminescence in the presence of damages in the DNA molecule, allowing for the screening and discrimination between different meats in terms of potential carcinogenic induction. Unprocessed beef and chicken induced low amounts of bioluminescence, whereas processed beef and chicken induced values above the cut-off limit established. These values where low in comparison to the bioluminescence produced by a strong activator used as our positive reference, leading us to the conclusion that processed meat a low carcinogenic effect.

1. Introduction

In the last 20 years, a lot of interest has been drawn by studies that relate cancer induction to diet, including of plant or animal origin meat, including processed meat, the latter are both widely consumed as they make an important contribution to nutrient intake in a balanced diet, contributing valuable bioavailable nutrients that are beneficial to health, such as proteins, long-chain *n*-3 fatty acids, iron, zinc, selenium, vitamin D and vitamin B12, that are essential for both growth and development [1]. The International Agency for Research on Cancer (IARC), and the cancer agency of the World Health Organization (WHO) carcinogenic studies in meat suggest that small increases in the risk of several cancers may be associated with the high consumption of red meat or processed meat [2–4]. IARC classified the consumption of red meat as probably carcinogenic to humans (Group 2A), mostly for colorectal cancer, although associations were also observed for pancreatic and prostate cancers. Processed meat (including hot dogs, ham, sausages, corned beef, beef jerky and canned meat) was classified as carcinogenic to humans (Group 1) as meat eaten daily increases the risk of colorectal cancer by 18% [5]. These risks are important for public health because the consumption of meat is increasing worldwide and in low- and middle-income countries [6].

Processing meat offers the opportunity to add value, reduce price, improve food safety and extend its shelf-life [7], which is done by adding various compounds to meat products to improve colour, taste and shelf-life, while little has been studied to estimate their possible carcinogenic toxicity in humans. Substances like Nitrite and N-Nitroso compounds (NOCs) are alkylating agents used in meat processing to inhibit bacterial growth, set the meat's colour and improve its aroma.

Humans can be exposed by endogenous exposure to NOCs in relation to the amount of meat ingested with the diet, and although the concentrations of these compounds in foods are small, their long term effect in people cannot be ignored [8]. According to European Food Safety Authority (EFSA) [9] the acceptable daily intake (ADI) for nitrite is 0.07 mg/kg bw/day. In Europe the limit for nitrite/nitrate to add to meat is 150 mg/kg. The rate of formation of nitrosamines is proportional to the square of the nitrite concentra-

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tion, thus reduction of nitrite concentration is important to reduce the amount of nitrosamine formed. The identification of substances capable of inducing DNA mutations has become an important procedure in food safety assessment in the last years. Based on reports that relate chemicals used for meat processing to liver and colon cancers, interest has been focused on the possibility to develop a bioassay that can distinguish between cancerogenic and non cancerogenic substances on a toxicological basis. Bioreporting bacteria as sensing elements started with the bacterial mutation assays developed by Aimes in 1971 using *Salmonella* [10,11], and more recently in the construction of bioreporter bacteria able to translate a chemical signal into a quantifiable reporter signal [12]. Despite obvious differences between prokaryotic and eucaryotic metabolisms there are correlations or extrapolations that can be considered between carcinogenic (i.e., genotoxic) effects on bacteria and those on mammalian cells. Thus bacterial cells can be used as a simple screen for mutagenicity for either environmental samples [13]. One such test that is, sensitive and easy to use, is based on the luminescence of bacterial strains which are triggered to glow from a mutagenic action by certain chemicals. In these strains a "SOS-repair" dependent promoter is fused to lux-operon (*luxCDABE*) and after activation by mutagenic compounds they produce light measurable signals. Using such strains genotoxicity of water and air samples or even antibiotic action were successfully evaluated [14–17]. A comprehensive and thorough study made in China further confirmed the need to study the effects of meat in the diet [18]. We therefore embarked on evaluating chicken and beef with and without the addition of preservatives, and their ability to trigger genotoxicity in an engineered *Escherichia coli* DPD2794 strain sensitive to DNA damages. The tests were conducted by adding extracts obtained both from nonprocessed and processed beef and chicken into a growth medium of *Escherichia coli* DPD2794. This bioreporter strain, is expected to produce bioluminescence whenever it is in presence of DNA damaging agents.

2. Material and methods

2.1. Reagents

Luria Bertani (LB) medium (Sigma, St. Louis, MO, USA) was prepared by adding 2.5 g LB broth powder into 100 mL double distilled water (25 g/L of LB powder).

2.2. Bacteria

Escherichia coli DPD2794 strain [19] containing the promoter of DNA damage sensitive *recA* gene, the operon of *luxCDABE* gene, fused in the plasmid of an *E. coli* strain was used as the bioluminescent reporter. The engineered strain produces a luminous signal via the expression of the l*uxCDABE* gene as a response to the presence of genotox compounds that cause DNA damage, as shown in Fig. 1. The *E. coli* DPD2794 suspension was stored at −20 °C in a LB broth containing 40%, glycerol (v/v) till use.

2.3. Meat samples

Four types of meat were used as DNA stressing agents. 1) unprocessed beef, fresh muscle of adult bovine; 2) processed beef, curls of pastrami smoked beef shoulder (beef shoulder 80%, water, processed starch (E 1142), salt, sugar, amplifiers of taste (monosodium glutamate and yeast extract), preservatives (E 326 potassium lactate, E261 potassium acetate, E 250 sodium nitrite), thickeners (E 407 carrageenan, E 508 potassium chloride), a blend of spice, oxidation inhibitor (E 316 sodium erythrobate or E 301 sodium ascorbate); 3) unprocessed chicken, fresh chicken breast; 4) processed chicken, chicken breast 73%, water, maltodextrine, salt, modified starch (E 1142), starch, stabilizers (carrageenan, phosphate), acidity regulators (potassium and calcium lactate), spices, dietary fiber, flavors for flavors and fragrance, preservatives (E 250 sodium nitrite), food color (annatto, caraway seeds), oxidation inhibitor (E 316 sodium erythrobate or E 301 sodium ascorbate). All samples used in this work have been purchased from a supermarket.

An amount of 9 g of meat sample was weighted in a sterile Stomacher bag and then, an amount of 9 mL of double distilled water was added to reach a concentration of 1 g of meat protein per mL. The Stomacher bag with the sample was placed into a Stomacher (AES laboratory, FRANCE) for 1 min to homogenize the sample. The meat extracts obtained were used for serial dilutions till a final concentration of 1 μg meat extract/mL was obtained.

2.4. Growth of Escherichia coli *DPD2794*

Escherichia coli DPD2794 growth was performed in 10 mL of LB supplemented with 10 μL ampicillin (100 mg/μL, Sigma, St. Louis, MO, USA) at 37 °C for about 12 h in a shaker incubator (Thermo Scientific™ MaxQ™ 4450 Benchtop Orbital Shakers, USA). A volume of 200 μL of overnight culture was used to inoculate 20 mL LB broth without any antibiotics and incubated at 30 °C for 1–3 h (Hy mini incubator, Hylabs, Israel) until a value of 0.2 OD (optical density) at 600 nm corresponding to a bacterial exponential growth phase (10^8 cells/mL).

2.5. Bioluminescence measurements for Escherichia coli *DPD2794*

All bioluminescence measurements were performed using opaque 96 wells micro titer plates (Costar, Coaring Incorporated, Tewkbury, MA, USA) containing 90 μL of the bacterial culture at 0.2 OD and 10 μL of meat extract dilutions to obtain the following concentrations of the meat extract in the microtiter plate: 100 mg/mL, 10 mg/mL, 1 mg/mL, 100 μg/mL, 10 μg/mL, 1 μg/mL, 0.1 μg/mL. Negative controls were prepared loading 100 μL of standardized bacterial culture without the addition of any meat extract solution.

A test to evaluate the production of bioluminescence from *E. coli* DPD2794 using sodium nitrite at 1 mg/mL, 0.1 mg/mL, 0.01 mg/mL, 0.001 mg/mL, and 0.0001 mg/mL was performed.

A luminometer type Luminoskan Ascent (Thermo Fisher Scientific USA) was used for measurements. Each well was inoculated in triplicate and the result obtained was the average of three experiments. The set-up of bacterial growth and bioluminescence is shown in Fig. 2.

Responses from *E. coli* DPD2794 were acquired at 26 °C [19] every 5 min for 20 h. Data values on bioluminescence consisted of induction factor (F_i), ratio of Relative Light/Luminescence Unit (RLU) value of treated sample and RLU value of the untreated sample [20]. To evaluate the DNA damaging activity of the meat extracts a value of F_i at 2 was used as a cut off value, when the induction factor doubles the value of the background [21]. Values over the cut-off were considered having a genotoxic activity on DNA in *E. coli* DPD 2794 [22]. Positive and negative control tests were conducted to verify the ability of *E. coli* DPD2794 to detect DNA damaging substances. As positive control, mitomycin C (MMC), an intercaling agent causing DNA damage, at the concentration of 80 ppb was used [19,23,24]. As negative control ethanol (EtOH) at a concentration of 2% v/v, was used as it does not cause any DNA damage [20]. All experiments were performed in triplicate.