

Effect of Carvacrol on O157 and non-O157 Shiga Toxin Producing *Escherichia coli*

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Abstract

Shiga toxin *Escherichia coli* (STEC) strains are important foodborne bacteria linked to diarrhea, enteritis, hemolytic-uremic syndrome and in some cases death. *E. coli* O157:H7 is the most common strain amongst STECs however non-O157 STECs have been connected with several outbreaks on an international level. The use of natural plant extracts to reduce the risk from foodborne pathogens is gaining increasing importance. Therefore in this study, we tested the antibacterial effect of carvacrol, a major component of oregano essential oil, on *E. coli* serogroups O157, O26, O45, O103, O111, O121, O145 as well as serogroup O104 responsible for the massive outbreak in Germany in 2011. Carvacrol showed antibacterial effect on all strains tested. The relative electric conductivity was assessed in order to investigate the changes in membrane permeability and thus to investigate the antimicrobial mechanism of carvacrol. Results showed that the relative conductivity increased with increasing concentrations of carvacrol which showed that there was an increasing leakage of electrolytes due to disruption of the cell membrane. The data presented here revealed that carvacrol has the potential to be used as a natural antimicrobial against STECs.

Keywords: antibacterial, carvacrol, non-O157 *E. coli*, O 157 *E. coli*, permeability.

1. Introduction

Shiga toxin producing *Escherichia coli* (STEC) strains are zoonotic pathogens that are found in the lower gastrointestinal tracts of cattle and other ruminants. STEC strains are shed in the faeces of these animals, which serve as reservoirs and major sources of foodborne illness [1]. Faecal contamination of crops and water systems as well as direct contact with animals, are also considered as a cause of infection [2]. STEC strains can be associated with human diseases, ranging from uncomplicated diarrhea to hemorrhagic colitis and

hemolytic-uremic syndrome [3]. *Escherichia coli* O157:H7 is the most common serogroup associated with illnesses and deaths in humans [4]. Nevertheless, other serogroups (e.g. O104) have been associated with outbreaks and sporadic cases [5]. Processors, food safety researchers, and regulatory agencies have been increasingly concerned with the growing number of foodborne illness outbreaks caused by these pathogens [4,5]. The increasing antibiotic resistance of *E. coli* pathogens that are associated with food-borne illness is an additional issue [6]. Therefore, there has been growing interest in the development of new, effective and non-toxic antimicrobial compounds. Plant essential oils are an alternative source of antimicrobial compounds [7].

The objectives of this study were to test the antimicrobial effect of carvacrol on 8 different serogroups of STECS and to measure the electric

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conductivity in order to assess the changes in membrane permeability and thus to investigate the antimicrobial mechanism of carvacrol.

2. Materials and methods

2.1. Bacterial strains

All *E. coli* strains (O157, O26, O45, O103, O111, O121, O145, O104) were cultured in Muller-Hinton broth at 37°C for 24 hours. Carvacrol was obtained from Sigma-Aldrich (UK) and carvacrol solutions at different concentrations were made in Muller-Hinton broth.

2.2. Agar diffusion assay

The standard agar diffusion method was used for the determination of antibacterial efficacy of carvacrol against all strains [8]. Tryptic soy agar with 0.6% yeast extract plates were inoculated with 0.1 mL of standardized inoculum containing 10^7 CFU/mL bacterial suspension and uniformly spread, and allowed to dry for 5 min. Sterile filter paper discs (6 mm diameter) were impregnated with different concentrations of carvacrol (0, 0.03 and 0.06%) and placed on the plates. The plates were incubated at 37 °C for 24 h. Antibacterial activity was evaluated by measuring the diameters of inhibition zones against the tested strains. Each assay in this experiment was performed in triplicate.

2.3. Bacterial Cell Membrane Permeability

The permeability of the bacterial cell membrane was determined according to Kong et al. [9], with minor modifications. All strains were incubated at 37°C overnight in Muller Hinton broth. Subsequently, cells were separated by centrifugation at $4000 \times g$ for 10 min. Cells were washed with 5% (w/v) glucose solution until their electrical conductivities were near to that of 5% glucose, and they were the case for isotonic bacteria. Carvacrol was added to glucose (5%) at two different concentrations after thorough mixing the electrical conductivity was measured (L1). Consequently, different concentrations of carvacrol were added into the isotonic bacterial solution. After mixing, the samples were incubated at 37°C and conductivities were measured at different time intervals (2, 4, 6, 8 and 18 h) (L2). Also, the conductivity of glucose (5%)

treated bacterial cells in boiling water for 5 min was determined (Lo). The changes in bacterial cell membrane permeability was assessed in terms of the relative electric conductivity by using the following Equation: Relative electric conductivity (%) = $((L2 - L1)/Lo) \times 100$. Each assay was performed in triplicate.

2.4. Statistical analysis

All data were expressed as the mean \pm standard deviation. One way analysis of variance and the Tukey's test was used to express the significance of differences between means ($P < 0.05$).

3. Results and discussion

Plants and their essential oils are useful sources of antimicrobial compounds and have been extensively used in food and medical research. Several studies have been published on the antimicrobial activities of plant derived compounds against many food-borne pathogens [10]. Carvacrol is the main component of oregano and thyme essential oils [11]. In the present study carvacrol showed antibacterial activity (diameter of inhibition zone) against O157, O26, O45, O103, O111, O121, O145 and O104. As low as 1% (v/v) carvacrol per disc inhibited the growth of all tested STECs with inhibition zones around 12-14 mm. The antimicrobial effect increased with increasing concentrations of carvacrol. Among the tested STECs, *E. coli* O157 was the most sensitive to carvacrol. The relative electric conductivity was determined to express the permeability changes of bacterial membranes. Results showed that the electric conductivity of all samples increased significantly in the presence of increasing amounts of carvacrol (0, 0.03 and 0.06%) during the first few hours (Fig. 1). This trend was similar for all the *E. coli* strains tested. Ion homeostasis (e.g. K^+ , Na^+ and H^+) is of great importance for cell biological functions. For this reason, changes to the permeability of membranes can negatively affect the bacterial metabolism and result in cell death [12]. In the present study it was shown that the relative electric conductivity increased with increasing concentrations of carvacrol which suggested leakage of electrolytes due to disruption of the cell membrane.

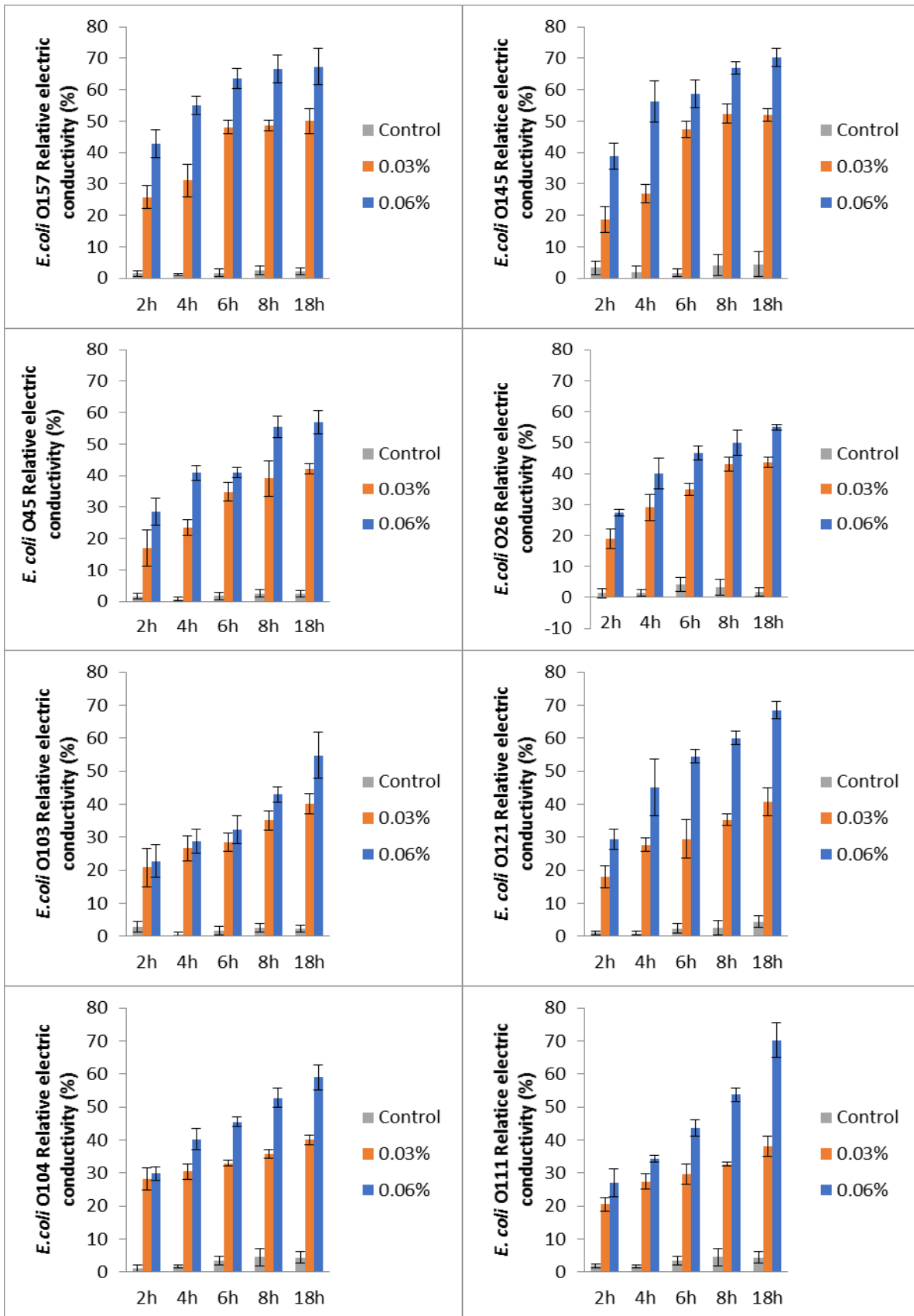


Figure 1. Effect of different concentrations of carvacrol on STECS cell membrane permeability.

4. Conclusions

The results showed that the natural antimicrobial compound carvacrol could potentially be used to improve the safety margins against the 8 relevant STECs in food and feed.

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