Research progress on the antibacterial mechanisms of carvacrol: a mini review

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ABSTRACT

Background: Carvacrol is an aromatic phenolic terpenoid widely existing in the volatile oils of thyme, oregano, and some other aromatic plants. Recent studies have found that carvacrol possesses excellent antibacterial activity. Thus, in order to provide an updated information about the antibacterial potentials of carvacrol we summarized recent publications about the antibacterial activity of carvacrol, with special attention paid to its antibacterial molecular mechanisms, including disrupting cell membrane, depleting intracellular ATP, inducing reactive oxygen species, inhibiting efflux pumps, as well as suppressing two important virulence factors: biofilm and quorum sensing. In conclusion, carvacrol is a promising natural antibacterial compound with potential application in food preservation and infection.

Keywords: Carvacrol, antibacterial mechanisms, biofilm, quorum sensing
INTRODUCTION

Antibiotics are one of the greatest discoveries of the 20th century and have been used to treat infectious diseases and promote the growth of animals as food additives for a long time [1, 2]. However, the abuse of antibiotics has led to severe problems, such as the emergence of drug-resistant bacteria, antibiotic residues, and environmental pollution. Therefore, increasing attention has been paid to seeking for desirable antibiotic alternatives.

Plant essential oils are attractive antibiotic substitutes, since they are natural, have no residue, and difficult to induce drug resistance [3]. Among them, oregano (*Origanum vulgare*) essential oil has been widely used as food preservatives and feed additives for antiseptic and antibacterial effects in Europe and the United States due to its quite high antibacterial activity [4]. Carvacrol (Figure 1), also known as 5-isopropyl-2-methylphenol, is an oxygenated monoterpenoid. Carvacrol is the major bioactive compound in oregano essential oil, accounting for 70%-80%. Carvacrol has a broad antibacterial spectrum against *Escherichia (E.) coli* [5], *Staphylococcus (S.) aureus* [6], *Listeria (L.) monocytogenes* [7], and *Salmonella (S.) typhimurium* [8]. Significantly, carvacrol has been reported not to influence or even positively increase the growth of beneficial intestinal bacteria, including *Lactobacillus*, *Bifidobacteria*, and *Firmicutes*, at the concentration of *Clostridium* inhibition, providing theoretical evidence for its use in feed additives and clinical applications [9-12]. To provide a better understanding of the antibacterial potential of carvacrol, we summarize and discuss its antibacterial mechanisms.

**Figure 1.** The chemical structure of carvacrol.

ANTIBACTERIAL MECHANISMS

*The disruption of cell membranes*

Bacterial cell membranes form barriers against the permeation of antibiotics and chemical fungicides and avoid the phagocytosis of the body's immune system [13]. Studies have suggested that the disruption of cytoplasmic membrane is an important mechanism for the antibacterial activity of carvacrol (Figure 2), seriously influencing the fluidity, integrity, and functionality of
Wang et al. found that the content of membrane unbranched fatty acids (UBFAs) in Gram-positive *S. aureus* ATCC 43300 rose from 34.9% to 62.37%, compensating for the increased fluidizing effects caused by carvacrol at 0.77 mM. More serious membrane morphological damage, such as destruction of integrity could be observed in the presence of higher concentrations (from 1.03 to 4.12 mM) of carvacrol [16]. Additionally, another study also revealed that exposure of *S. aureus* to the sub-lethal concentration of carvacrol (0.6 µL/mL) resulted in the increased absorbance at 260 nm, indicating that membrane damage caused the leakage of intracellular substances, and the subsequent dysregulation of membrane-related protein secretion system further accelerated the bacterial death [17]. Additionally, it was reported that compounds with the octanol/water partition coefficient (log P) value higher than 3 had intrinsic hydrophobic properties, showing high affinity to cell membranes and causing physical changes, such as the order and stability of membrane phospholipid bilayer [18] and the log P value of carvacrol is 3.62 [19]. The hydrophobic nature of carvacrol should play an important role in the loss of bacterial membrane integrity and influence the proteins embedded, like proteins that form efflux pumps, disrupting the normal physiological function of related organelle [20]. Moreover, carvacrol has been suggested to have a stronger antibacterial effect on gram-positive bacteria than gram-negative bacteria, probably due to the later rich in hydrophilic lipopolysaccharide in the cell membrane [21]. Therefore, the findings above support that carvacrol can target bacterial cell membranes and destroy membrane-related functions.

**Figure 2.** The schematic diagram of carvacrol destroying bacterial cell membrane

- Carvacrol

### Depletion of intracellular ATP

The existing free hydroxyl and delocalized electronic systems of carvacrol have also been proven to be essential for its antibacterial activity (Figure 3) [18]. When carvacrol enters the cytoplasm of bacteria, the delocalized lone electron pair on the hydroxyl oxygen of carvacrol can form a p-π

![Diagram of carvacrol destroying bacterial cell membrane](image-url)
conjugate with its benzene ring, promoting the release of proton on the phenolic hydroxyl group into the bacterial cytoplasm, thereby increasing the acidity of bacterial cytoplasm. However, the normal cytoplasmic pH is generally close to neutral. In order to maintain the appropriate pH inside the cell, bacteria have to transport excessive proton out of the cell accompanied with the consumption of ATP, finally depleting the intracellular ATP pool and leading to the cell death [22]. Therefore, depletion of intracellular ATP is an important antibacterial mechanism of carvacrol.

**Induction of Reactive oxygen species (ROS)**

Previous studies found that limonene and some bactericidal antibiotics shared similar antibacterial mechanisms by inducing ROS, such as hydroxyl radicals [23, 24]. In addition to fluorine in nature, hydroxyl radicals demonstrate high oxidizing power, which can easily destroy proteins, lipid membranes, and DNA, leading to cell death [25].

Carvacrol has been reported to induce ROS production in bacteria (Figure 3). At a concentration of 450 µg/mL, carvacrol was reported to have elevated ROS levels in *E. coli* by fluorescence imaging [26]. Additionally, Chueca et al. found that 100 µL/L of carvacrol reduced the amount of *E. coli* by over 2.5 logs in 3 hr under aerobic conditions, but the reduction could be controlled within 1 log by adding a ROS scavenger thiourea. Compared with the control, the ΔrecA mutant of *E. coli* was found to be more sensitive to carvacrol, indicating that carvacrol caused oxidative damage to DNA because RecA could induce SOS responses in normal strains with DNA damage [27]. In the presence of carvacrol at 15 µg/mL, increased oxidative stress was also found in *Streptococcus* *mutans*, verified by the downregulated expression of superoxide dismutase gene [28]. Overall, ROS plays an important role in carvacrol-induced cell damage, even cell death.

**Inhibition of efflux pump**

The efflux pump is a chromosome-encoded translocator that is located on the plasma membrane of bacteria. Many bacteria can extrude the antibacterial drugs to the extracellular environment through the efflux pump system, thereby reducing the intracellular drug concentration and causing the antibiotic resistance of bacteria [29]. Plant products are natural sources of the efflux pump inhibitor (EPI). Recent studies found that carvacrol can be a potential EPI of bacteria (Figure 3).

Miladi et al. demonstrated that carvacrol inhibited the efflux of ethidium bromide (EB) in the food-borne pathogens *S. aureus* ATCC 25923 and *E. coli* ATCC 35218 in a dose-dependent manner; the combination of carvacrol and tetracycline at sub-inhibitory concentrations also increased the sensitivity of the test bacteria to antibiotics [30]. Additionally, by using the DNA microarray-based transcriptomics assay, the transcriptional levels of multidrug efflux pump
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regulatory genes, such as acrA and mdtM, were significantly altered in E. coli MG1655 treated with 100 mg/L of carvacrol [31]. The results above indicate that carvacrol can be used as an EPI to increase the sensitivity of the test strain to antibacterial agents and made the bacteria more easily cleared.

**Figure 3.** The schematic diagram of carvacrol depleting intracellular ATP (a), inducing reactive oxygen species (b), and inhibiting efflux pump (c).

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Inhibition of bacterial biofilm

Many bacteria have the ability to adhere to biotic or abiotic surfaces and then proliferate, differentiate, and secrete a series of polysaccharide-protein complexes, which envelop the bacterial community to form a membrane of organized aggregates of bacteria, called biofilm. The biofilm is highly resistant to antibiotics and the host immune defense systems [23].

More recently, the antibiofilm activity of carvacrol has been reported in many bacteria. These bacteria include Gram-positive bacteria like S. aureus [32], Streptococcus mutans [28], and L. monocytogenes [33], in addition to Gram-negative bacteria like S. typhimurium [34], Pseudomonas (P) aeruginosa [35], and E. coli [36]. The diagram of this mechanism is displayed in Figure 4. The inhibitory effect of carvacrol on the growth of the biofilm is related to its intrinsic characteristics. The solubility of carvacrol in water is 800 ± 10 mL/L and the relative hydrophilicity allows carvacrol to diffuse through the polar polysaccharide matrix of the biofilm. Additionally, the general hydrophobicity promotes carvacrol to specifically interact with cell membranes, affecting their structures and functions [22, 37].

Significantly, carvacrol can not only inhibit the initial biofilm formation, but also eradicate pre-formed biofilms. Higher concentrations are required to remove the pre-formed biofilm since
the viscous matrix and complex three-dimensional structures of biofilm inhibit the permeation and diffusion of carvacrol in the pre-formed biofilms. The existence of dormant cells embedded in biofilm possess higher resistant ability [38, 39]. For example, the biofilm formed by *S. typhimurium* was reduced by the presence of carvacrol at sub-lethal concentrations (< 0.5 mM). However, there was little influence on pre-formed biofilm, even up to 8 mM [40]. Additionally, many studies have linked the antibiofilm ability of carvacrol to some following factors. (1) Carvacrol decreased bacterial motility and adhesion to the surface [41]. For example, the disruption of flagellin synthesis was found in *E. coli* O157:H7 treated with a sub-lethal concentration of carvacrol, coupled with decreased biofilm formation [42]. (2) Carvacrol reduced bacterial density caused by delayed cell growth in planktonic cells and subsequently reduced the quorum sensing signaling [32]. (3) Carvacrol directly killed bacteria to induce the biofilm destruction [43]. Overall, carvacrol exhibits anti-biofilm activity with diverse rationales.

**Figure 4.** Schematic diagram of carvacrol suppressing biofilm (a) and quorum sensing. (b).

**Blocking of quorum sensing**

Quorum sensing (QS) is a regulatory system of bacterial biological behavior based on population density and has received increasing attention in recent years [44]. Bacteria can synthesize and secrete numerous small molecules, which are also known as auto-inducers (AIs). With the accumulation of these signals, many bacterial behaviors can be activated, such as bioluminescence, toxin production, motility, and biofilm formation. Most Gram-negative bacteria use membrane-permeable fatty acyl homoserine lactone (AHLs) derivatives as signal molecules, which are synthesized by LuxI homologue and recognized by LuxR homologous, activating the transcription of downstream target genes [45].

In recent years, carvacrol has been highlighted as a desirable natural quorum sensing...
inhibitor (QSI) (Figure 4). In the work of Rodrigue et al., the QS-mediated phenotypes like pyocyanin production and biofilm formation in *P. aeruginosa* dose-dependently decreased by carvacrol [46]. Additionally, Janak et al. found that carvacrol formed π-π bond and hydrogen bond with phenylalanines residues in ExpI (LuxI homolog) and lysine residue in the receptor protein ExpR (LuxR homolog) respectively, inhibiting the production of signaling molecules and activation of receptors in *Pectobacterium* species, with biofilm formation and other virulence factors mediated by QS also inhibited [47]. Moreover, Myszka et al. reported that carvacrol was able to inhibit the synthesis of AHLs, finally interfering with the QS signaling [48]. Overall, carvacrol is effective in QS inhibition and its natural and nontoxic characteristics make it suitable for further applications.

**CONCLUSIONS**

In conclusion, carvacrol has a broad spectrum of antibacterial activity, with the disruption of the cell membrane, induction of ROS, and inhibition of efflux pump, biofilm, and QS system as the main antibacterial mechanisms. Therefore, it is safe to use carvacrol as antimicrobial agents in the field of medical care or animal feeding. However, most of the previous studies focused on normal foodborne pathogens, while the antibacterial efficiency and related mechanisms of carvacrol on drug-resistant bacteria have not been as investigated. Additionally, most of the previous studies used the *in vitro* experiments to test the antibacterial effects of carvacrol. Thus, its effects on animals and humans need further elucidation. Since the LD$_{50}$ of carvacrol tested *in vivo* ranges from 310 mg/kg to 2700 mg/kg, much higher than the doses used in the antibacterial filed [22], it is reasonable to speculate that carvacrol can be generally safe within its antibacterial concentration. Furthermore, the enhancement of antibacterial activity needs to be studied in the future. For example, through nanotechnology. Moreover, while the antibacterial mechanisms of carvacrol have been initially proposed, its molecular targets and regulated signaling pathways still need further identification. Overall, carvacrol exhibits promising antibacterial activity and can be good candidates as food preservatives and antibacterial agents with medical applications.

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REFERENCES


25. Chen CS, Chao HT, Pan RL, Wei YH. Hydroxyl radical-induced decline in motility and increase in lipid peroxidation and DNA modification in human sperm. Iubmb Life 2010,


37. Rodrigues JBD, de Souza NT, Alcântara JOA, de Sousa JM, Lira MC, de Figueiredo RCBQ, de Souza EL, Magnani M. Efficacy of using oregano essential oil and carvacrol to remove young and mature Staphylococcus aureus, biofilms on food-contact surfaces of stainless steel. LWT 2018, 93.


