

## Review

# Antimicrobial Effect of Tea Polyphenols against Foodborne Pathogens: A Review

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## ABSTRACT

Food contamination by foodborne pathogens is still widespread in many countries around the world, and food safety is a major global public health issue. Therefore, novel preservatives that can guarantee safer food are in high demand. Contrary to artificial food preservatives, tea polyphenols (TPs) are getting wide attention as food additives for being “green,” “safe,” and “healthy.” TPs come from many sources, and the purification technology is sophisticated. Compared with other natural antibacterial agents, the antibacterial effect of TPs is more stable, making them excellent natural antibacterial agents. This review includes a systematic summary of the important chemical components of TPs and the antibacterial mechanisms of TPs against various foodborne pathogens. The potential applications of TPs are also discussed. These data provide a theoretical basis for the in-depth study of TPs.

## HIGHLIGHTS

- TPs are food additives considered “green,” “safe,” and “healthy.”
- The antibacterial mechanisms of TPs against foodborne pathogens are described.
- The potential application areas of TPs are highlighted.

Key words: Antimicrobial mechanism; Chemical properties; Food; Foodborne pathogens; Tea polyphenols

In recent years, the increasing incidence of foodborne illnesses has become a serious threat to public health, leading to huge economic losses. Foodborne pathogens, including *Staphylococcus aureus*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella*, *Campylobacter*, *Bacillus cereus*, and *Aspergillus flavus*, are the major cause of foodborne illnesses (11, 16, 25, 26, 36, 39, 50, 70, 75, 89). These pathogens can contaminate food and produce toxins that seriously endanger human health (33). According to the World Health Organization, 582 million cases of 22 different foodborne enteric diseases resulted in 351,000 deaths in 2010 (4). In 2011, an outbreak caused by Shiga toxin-producing *E. coli* resulted in serious damage to the German industrial economy, with a loss of US\$1.3 billion, which forced the country to seek emergency assistance of US\$236 million from the 22 EU Member States (81). These data alone signify that the loss caused by *E. coli* contamination cannot be underestimated (4). *Salmonella* is the most common foodborne pathogen and is found widely in the intestines and feces of animals. In the United States, >1 million cases of *Salmonella* infection occur every year,

of which nearly 20,000 result in hospitalization and 400 result in death, indicating the seriousness of the health threat from this pathogen (3, 36).

Natural polyphenols, such as tea polyphenols (TPs; extracted from black or green tea) have antibacterial, antioxidant, and anticancer effects (52). Compared with chemicals or artificially prepared food additives, TPs are safer for human consumption and popular among consumers (51, 94). TPs have broad-spectrum antibacterial activity, especially their inhibitory effects on *S. aureus* (86). Castillo et al. (11) found that TPs inhibited the movement and biofilm formation of *Campylobacter jejuni*. Zhao et al. (93) found that TPs can inhibit drug-resistant *S. aureus* and reduce its drug resistance when used synergistically. Thus, TPs have been recognized as natural antibiotics, have attracted great attention for their antibacterial properties, and are widely used in foods, cosmetics (e.g., mouthwash), and medicines (88). Chemical agents are the most common means of reducing harm from foodborne pathogens. However, the abuse of these agents can increase the drug resistance of pathogenic bacteria and it may have adverse effects on the human body (84). In contrast, natural TPs have the advantages of safety, lack of drug resistance effects, and strong antibacterial activity. In the food industry, TPs are used to preserve pastry, dairy products,

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beverages, and meat products (49). Therefore, their antibacterial mechanism(s) must be studied in detail. Here, we review the chemical and antibacterial properties of TPs and their potential applications in food, medicines, and cosmetics.

### PROPERTIES OF TPs

TPs, which are polyhydroxyphenols, are flavonoid compounds with a basic structure of  $\alpha$ -phenyl-benzopyran. Dried tea extract contains 25 to 40% polyphenols (7). The molecular structure of TPs and their key active molecules determine the color, fragrance, health benefits, and pharmacological effects of tea (80). TPs comprise about 30 phenols, with 30 to 42% catechins, 5 to 10% flavonols, and 2 to 4% other flavonoids. The four types of catechin molecules are epicatechin, epigallocatechin, epicatechin gallate, and epigallocatechin gallate (EGCG), which differ in their molecular characteristics (24, 61, 65, 66). EGCG is the most abundant antioxidant catechin and can promote skin health (61).

At room temperature, TPs are white amorphous crystalline compounds. During the extraction process, oxidation of catechins generates quinones, which determine the color of the tea extract (yellow to brown) (71). TPs are astringent and slightly hygroscopic and easily dissolve in hot water, ethanol, methanol, acetone, and other solvents but only slightly soluble in grease, benzene, and chloroform (74). The pH of water solutions of TPs is 3.0 to 4.0. Hong et al. (31) found that several factors, including pH, protein concentration, antioxidant concentration, and the presence of metal ions, affect the stability of EGCG; pH is the most critical factor. TP solutions are stable under acidic conditions but readily decompose under alkaline and neutral conditions (90). Natural polyphenols have strong metal complexation ability. For example, TPs form complexes with iron, copper, and zinc (19). In an analysis of wine production, a significant relationship was found between metals and polyphenols. Many researchers have found that the metal-polyphenol relationship can affect their presence (38, 76).

Polyphenol-rich foods and beverages improve the antioxidant capacity of plasma. Thus, consumption of polyphenols may result in production of reductive polyphenols or metabolites that absorb oxidizing food components (62). Polyphenols also can scavenge free radicals, regulate nitric oxide, inhibit cell proliferation, and promote angiogenesis (30, 55), which can be beneficial in patients with cancer or cardiovascular diseases (6). EGCG can alter the activity of DNA methyltransferase in esophageal, oral, skin, lung, and prostate cancer cells (7, 41, 63). Fang et al. (22) suggested that TPs could inhibit DNA methyltransferase activity and promote methylation silencing of genes in cancer cells. Nandakumar et al. (53) found that EGCG upregulated DNA methylation in human epidermal cancer cells.

Natural bioactive food supplements may be useful for countering the effects of many diseases. For instance, TPs, specifically the catechins, can have an effect in patients with atherosclerosis and cancer (8). Therefore, TPs are believed to be one of the most beneficial substances in tea (30, 42).

### ANTIMICROBIAL ACTIVITY OF TPs

From the time of their discovery, TPs have attracted much attention due to their antioxidant properties. TPs also have inhibitory effects on various microorganisms such as *E. coli*, *S. aureus*, and fungi. In many studies, TPs have been shown to have broad-spectrum antibacterial properties and were effective both in vivo and in vitro (1, 2, 5, 32, 34, 46, 54, 56–58, 68, 73, 85). Sitheequ et al. (68) evaluated the effects of four concentrations of catechins and theaflavins on *Candida glabrata*, *Candida parapsilosis*, *Candida albicans*, *Candida krusei*, and *Candida tropicalis* by using an agar diffusion assay. Both polyphenols had anti-*Candida* activity against all tested strains; *C. glabrata* was the most sensitive species. TPs also can promote the growth of *Lactobacillus*, which suggests a high level of selectivity (29). The antimicrobial activity of TPs against various pathogens is summarized in Table 1.

### ANTIMICROBIAL MECHANISM OF TPs

The antibacterial mechanism of TPs is not completely understood. However, results of previous studies indicate that the antibacterial mechanism of polyphenols has multiple aspects (Fig. 1).

**Destruction of cell membrane structure.** TPs can directly bind to and damage bacterial cell membranes. Gram-positive bacteria are more sensitive to TPs than are gram-negative bacteria (15) because EGCG can easily bind to the peptide polyphenols exposed on the surface of gram-positive bacteria such as *S. aureus*. In gram-negative bacteria, although the peptidoglycan layer is thin (one or two sheets), it is overlaid by an outer membrane mainly composed of lipopolysaccharides. A combination of EGCG and peptidoglycan causes cell membrane damage. Methicillin-resistant and methicillin-sensitive *S. aureus* have reduced tolerance to high ion concentrations and low osmotic pressure in the presence of EGCG, which supports the assumption of direct cell wall damage (93).

Lee and Tan (46) found that EGCG can also cause damage by binding to the cell membrane phospholipid bilayer. These authors cultured *Enterococcus faecalis* biofilms on dentine disks and exposed these biofilms to medium alone or EGCG. The control group cells grew rapidly, but the growth of EGCG-treated cells was significantly inhibited after exposure for 7 days. Lee and Tan also used bacteriological culture to test the viability of these biofilms and found that exposure to EGCG significantly reduced bacterial levels compared with untreated biofilms. The destruction of the bacterial cell membrane weakens the cell and inhibits bacterial aggregation and the formation of biofilms. EGCG can also inhibit bacterial growth by suppressing the expression of specific genes related to virulence and biofilm formation (46).

The formation of biofilms is closely related to bacterial pathogenicity and drug resistance (46). Zhang et al. (91), using in situ analysis with confocal laser scanning microscopy, found that *Rosa rugosa* tea polyphenol treatment decreased the thickness of bacterial biofilms and

TABLE 1. Antimicrobial activity of tea polyphenols

Pathogen	Inhibition zone diameter (mm) or MIC (µg/mL)	Reference
<b>Gram-negative bacteria</b>		
<i>Salmonella</i> Typhimurium TSA-2121	NP	73
<i>Salmonella</i> Enteritidis 87-350	NP	73
<i>Vibrio parahaemolyticus</i>	NP	73
<i>Escherichia coli</i>	NP	58
<i>Salmonella</i> Typhi	NP	86
<i>Pseudomonas aeruginosa</i>	200–400 mm	32
<i>Prevotella intermedia</i>	27.05 ± 1.6 µg/mL	5
<i>Klebsiella pneumoniae</i>	NP	86
<i>Serratia marcescens</i>	NP	85
<i>Porphyromonas gingivalis</i>	26.65 ± 1.56 µg/mL	5
<i>Yersinia enterocolitica</i>	19.2 ± 0.2 µg/mL	31
<b>Gram-positive bacteria</b>		
<i>Staphylococcus epidermidis</i> ATCC 1228	NP	73
<i>Micrococcus luteus</i>	NP	2
<i>Clostridium difficile</i> ATCC 9689	NP	1
<i>C. perfringens</i> ATCC 13124	NP	1
<i>Streptococcus salivarius</i>	NP	58
<i>S. mutans</i>	NP	58
<i>S. aureus</i> ATCC 25923	18.970 ± 0.287 µg/mL	57
<i>Bacillus subtilis</i>	NP	59
<i>S. mutans</i>	36.3 µg/mL	5
<i>Enterococcus faecalis</i>	NP	45
<b>Fungi</b>		
<i>Candida albicans</i> ATCC 10231	0.004 µg/mL	56
<i>C. glabrata</i>	NP	68
<i>C. krusei</i>	NP	68
<i>C. parapsilosis</i>	NP	68
<i>C. tropicalis</i>	NP	68

<sup>a</sup> NP, not provided in reference.

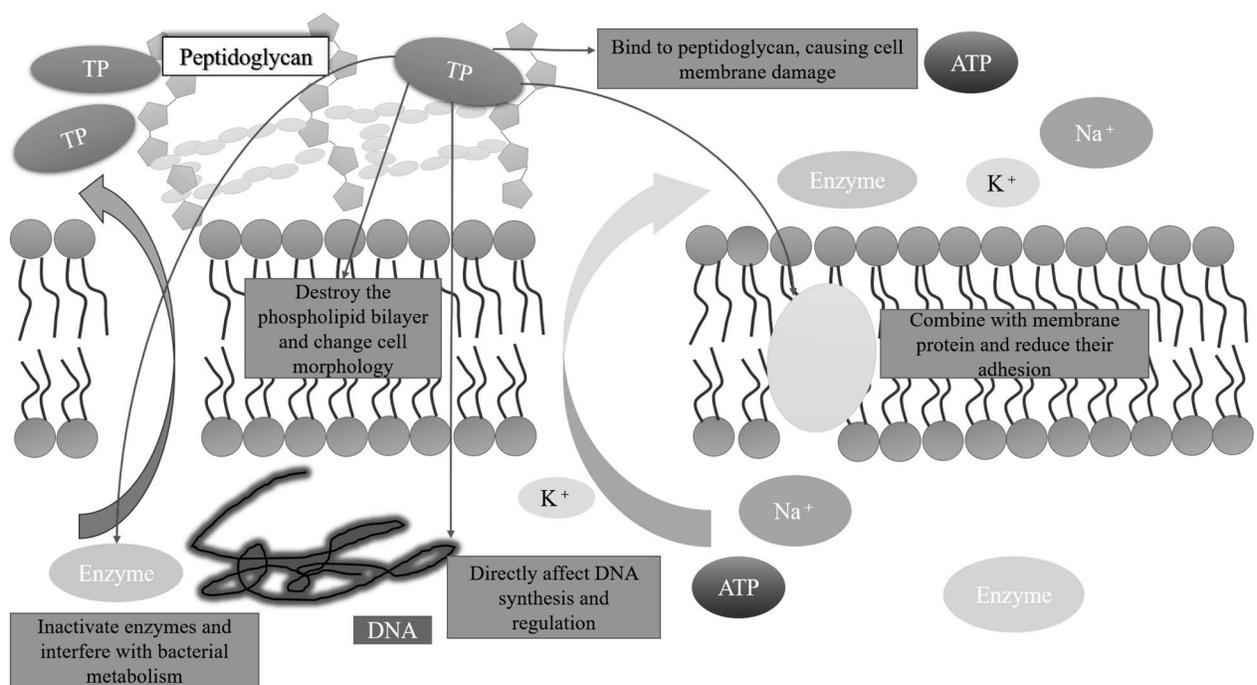


FIGURE 1. Antibacterial mechanism of tea polyphenols (TPs).

influenced the covered surface area. An increase in *S. aureus* cell membrane permeability led to the loss of cell components such as proteins (including enzymes), potassium ions, and sodium ions, which resulted in cell death.

**Change in bacterial cell morphology.** Bacterial cells have relatively stable morphology and structure under normal conditions; however, irregular polymorphism occurs under adverse conditions. TPs can destroy the phospholipid bilayer, causing changes in bacterial cell morphology that interfere with cell division and normal physiological functions, ultimately resulting in cell death. Cho et al. (12) found that TPs collapsed the bacterial cell membrane structure, destroyed the cell integrity, and increased leakage of intracellular material. These authors used scanning electron micrographs to reveal changes in the morphology of *Streptococcus* spp., *S. aureus*, *Lactobacillus plantarum*, and *Neisseria meningitidis* cells treated with a TP solution. Although no leakage of cytoplasmic material occurred as was observed in TP-treated *C. albicans*, irregular protrusions were visible on the cell surface.

TP also may affect cell division, causing a change in cell shape from a short rod to a long rod. Scanning electron microscopy revealed that TP-treated bacterial cells had various degrees of aggregation and perforation, which affected the cell membrane formation and made the cells less able to attach and create an infection (64). The bactericidal effect of EGCG was attributed to membrane perturbation. Cho et al. (13) also examined the morphologic changes of *E. coli* cells treated with TPs (lethal concentrations of 10,000 µg/mL for 12 h). Cells grown in Luria-Bertani broth without TPs had a typical rod shape with a smooth surface, whereas cells treated with TPs were irregular rod forms with wrinkled surfaces that had several destructive openings in the cell envelope.

**Impact on microbial proteins and DNA.** Proteins are important structural components of cells and function as enzymes to regulate cellular homeostasis (77). TPs are polyhydroxy compounds consisting of hydroxyl groups and a hydrophobic benzene ring, which can interact with proteins via amino and carboxyl groups or hydrophobic interactions. Djuardi et al. (17) found that treatment with TPs from black tea extract induced a concentration-dependent decrease in the surface hydrophobicity, and the effect was larger than that of TPs from green tea extract. Polyphenols in black tea contain more hydroxyl groups than do polyphenols in green tea, which increases the hydrophilic interactions and significantly decreases the surface hydrophobicity of soy protein. Cho et al. (14) also found that physiological levels of 14 proteins, including the chaperone proteins DnaK and GroEL, capsular polysaccharide synthesis enzyme Cap5G, surface protein, leukocidin subunit precursor,  $\alpha$ -hemolysin precursor,  $\beta$ -hemolysin, and exotoxin15, were markedly decreased or completely inhibited after treatment with TPs. Ben Lagha et al. (10) found that EGCG and theaflavin can attenuate *Fusobacterium nucleatum* adherence to Matrigel (a well-known basement membrane model) and oral epithelial cells.

Although the exact mechanism of inhibition was not clear, it seems that TP treatment altered the bacterial cell surface charges.

Li et al. (47) used multinuclear activation of galactosidase indicator assays to determine that EGCG can act as an allosteric reverse transcriptase inhibitor. In that study, the catechins and EGCG affected reverse transcription (RT) and PCR, which in turn controlled bacterial growth. Tichopad et al. (72) found a direct effect of catechin and EGCG on the performance of RT-PCRs. Both compounds were added to real-time RT-PCRs, and the fluorescence data were fitted with a mathematical model. The results suggested that EGCG could directly inhibit virus reproduction at the RT level. In other studies, catechin derivatives, especially EGCG, had an inhibitory effect on mammalian DNA polymerase. Yoshida et al. (87) found that EGCG inhibited the proliferation of fertilized eggs, which also indicates that EGCG affects DNA polymerase.

**Metal ion complexation.** In several studies, TP components such as catechins and theaflavins formed ligands with metal ions, such as iron, copper, and zinc, that affect the bacterial enzyme activity, growth, and reproduction (20). Because several metal ions function as coenzymes, metal ion complexation with TPs affect microbial metabolism by hindering enzymatic activity (43). Ben Lagha et al. (10) suggested that chelation of iron, an essential nutrient for most bacteria, further enhances the antibacterial activity of TPs and that iron-chelating activity of EGCG from green tea extract and theaflavins was higher than that of ferrichrome, a reference siderophore produced by *Ustilago sphaerogena*. Gust and Wawer (27) reported that polyphenols can complex with trivalent iron by using a single oxygen anion and a single phenolic hydroxyl group or using two oxygen anions.

## CURRENT APPLICATIONS OF TPs

The nontoxic and excellent broad-spectrum antibacterial activity of naturally occurring TPs and their high level of efficiency make them widely applicable for the preservation of meat, aquatic, and oral product.

### Applications in fruits, vegetables, and agriculture.

The traditional methods for fruit preservation are usually refrigeration and chemical preservatives. However, the improper use of chemical preservatives may lead to food safety issues (18). Consumers are paying more attention to food safety. Therefore, safe and economical preservatives that have fewer adverse effects on human health are needed. TPs are widely used in the preservation of fruits, vegetables, and agricultural products because of their antibacterial and antioxidant properties.

Yang et al. (82) found that the incidence of nectarine spoilage by the gray mold *Botrytis cinerea* was significantly reduced after the combined treatment of TPs and tea saponin in vitro. Yang et al. (83) conducted in vivo and in vitro studies and found that TPs could control plant-specific fungal organisms that cause wheat stripe rust. Lan et al. (45)

prepared polyvinyl alcohol–TP films with volume ratios of 10:1, 9:1, 8:2, 7:3, 6:4, and 5:5 by the tape casting method and compared strawberries wrapped with these different films. Films with the 8:2 ratio significantly delayed the loss of titratable acids and soluble solids from strawberries, limited the growth of microorganisms, and extended the shelf life of these fruits. Sabaghi et al. (60) studied the effects of green tea extract and chitosan coating on walnut lipids and found that 10 g/L chitosan and 5 g/L green tea extract could effectively inhibit lipid oxidation and fungal growth during walnut storage, thus prolonging the shelf life of walnut kernels.

**Applications in meat and aquatic products.** Meat and aquatic products contain many proteins and fatty acids, are rich in nutrients, differ in taste, and are prone to spoilage. Development of new ways to extend the shelf life of these products, especially through the use of biological strategies for preservation, has become an important research topic.

TPs can be used to preserve fresh meat and fish by effectively inhibiting the growth of bacteria and delaying the oxidation of volatile nitrogen bases. Fan et al. (21) analyzed the microbiological (total viable and lactic acid bacteria), physical (Hunter color L\*, a\*, and b\* values), chemical (pH and thiobarbituric acid concentration), and sensory properties of the control sausage samples and those treated with 0.03% TP and found that TP treatment improved the quality characteristics and extended the shelf life of pork sausage. Xi et al. (78) explored the effect of TPs on pathogenic *Vibrio parahaemolyticus* and suggested that green tea could be used as a natural antimicrobial agent to inactivate *V. parahaemolyticus* in oysters, thereby extending their shelf life. Jia et al. (35) evaluated the effect of TPs on the microbial community in silver carp fillets stored at 4°C and the quality of the fillets and found that TPs can improve the quality of these fillets in cold storage and that 0.5% TP can extend the storage period of fillets by 4 days.

TPs are often combined with other biological agents to prepare compound preservatives. Siripatrawan and Noipha (67) found that the incorporation of green tea extract into chitosan films enhanced the antioxidant and antibacterial properties and extended the shelf life of pork sausages. He et al. (28) used an agar dilution method to evaluate the antibacterial activity of TPs and chitosan and their combination against gram-positive and gram-negative bacteria and found that the combination of TP and chitosan had a greater inhibitory effect on the growth of spoilage microorganisms and pathogens in meat. Li et al. (48) tested the combination of TPs and rosemary extract with chitosan on the quality of large yellow croaker during refrigeration and found that two pretreatments with the combination effectively maintained the quality of these fish and prolonged the shelf life by 8 to 10 days.

TPs also can be combined with other preservation techniques, such as deposition atmosphere, radiation, and glazes, which can extend the freshness of aquatic products. Feng et al. (23) studied the effect of the combination of TPs and ozone water on the quality of black seabream and found a corresponding reduction in nucleotide decomposition,

lipid oxidation, protein decomposition, and microbial growth. This combination also improved the texture, color, and sensory properties of the fish. The combination of vacuum packaging and TPs extended the shelf life of seabass by 4 to 6 days and 3 to 4 days at 0 and 4°C, respectively, compared with the control group (37).

**Applications in medicine.** TPs are natural products with broad-spectrum antimicrobial activity, high potency, and low toxicity. In many antibacterial tests, TPs have been inhibitory or bactericidal against *Proteus*, *S. aureus*, *Staphylococcus epidermidis*, *Streptococcus mutans*, *Clostridium botulinum*, *Lactobacillus*, and *Vibrio cholerae*, especially the intestinal pathogens.

Xu et al. (79) investigated the effects of EGCG on *Streptococcus* virulence factors and *S. mutans* biofilms and found significant antibacterial activity and inhibition against specific virulence factors. Ben Lagha et al. (10) found that green tea and black tea extracts, with EGCG and theaflavin, affected the growth and virulence of *Chlamydomonas nucleoli* and can be used to treat the related diseases. Ren et al. (59) prepared highly antibacterial ultrahigh-molecular-weight polyethylene implants using TPs, which improved oxidative stability and antibacterial effects. These implants can be used in joint replacement prostheses with multiple functions.

Zhang et al. (92) investigated the combination of TPs and commonly used antibiotics (imipenem, piperacillin, piperacillin-tazobactam, cefepime, cefotaxime, and ceftazidime) and found a synergistic bactericidal effect on drug-resistant *Klebsiella pneumoniae*. Periodontitis is the sixth most prevalent disease in the world (40). The polyphenols in black tea and green tea have antiviral and anti-inflammatory effects (44), and TPs inhibited the growth and virulence of *Fusobacterium nucleatum* (9), which caused oral inflammation (10). Thus, TPs may be used to develop new treatments for oral diseases. Song et al. (69) found that EGCG can effectively inhibit the activity of *Streptococcus pneumoniae* because EGCG can block pneumococcal hemolysin-mediated hemolysis and reduce the activity of the peptidase sortase A.

## CONCLUSIONS

Foodborne illness is a major global public health issue. Although interventions for food safety have advanced, contamination with foodborne pathogens is still widespread in many countries, and the food safety situation is still very poor. This review article focused on the role of TPs in inhibiting the growth of foodborne pathogens; summarized the chemical structure, characteristics, and antibacterial mechanisms of TPs against foodborne pathogens; and provided specific cases of the use of TPs in food.

Because of the broad-spectrum antibacterial activity of TPs, they have been used in food, cosmetics, and medicine. However, TPs have some limitations, such as low absorption rate and poor stability. Researchers are looking for new ways to solve these problems. TPs can be used in conjunction with other active ingredients to prepare a variety of antibacterial products, such as oral sprays and hand sanitizers, to obtain multifunctional products. Despite

a large number of research articles and the various practical applications, more in vivo studies are needed to confirm the results that have been obtained in vitro and to provide a better understanding of TP toxicology.

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