

The dynamics and mechanism of the antimicrobial activity of tea tree oil against bacteria and fungi

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Abstract Tea tree oil (TTO) is a yellow liquid extracted from *Melaleuca alternifolia*. Although the antimicrobial activity of TTO has been known for a long time, its specific antimicrobial effects and mechanism underlying these remain poorly characterized. The present study investigated the chemical composition of TTO and the dynamics and mechanism of its antimicrobial activities in two bacterial and two fungal strains. Gas chromatography–mass spectrometry analysis identified alkenes and alcohols as the main constituents of TTO. Terpinen-4-ol was the most abundant individual component, accounting for approximately 23 % of the TTO. Poisoned food technique assessment showed that the minimum inhibitory concentrations of TTO for bacterial strains (*Escherichia coli* and *Staphylococcus aureus*) and fungal strains (*Candida albicans* and *Aspergillus niger*) were 1.08 and 2.17 mg/mL, respectively. Antimicrobial dynamic curves showed that with increasing concentrations of TTO, the rate of cell killing and the duration of growth lag phase increased correspondingly. These data indicated that TTO produced concentration and time-dependent antimicrobial effects. The minimum bactericidal and fungicidal concentrations of TTO were 2.17, 4.34, and 4.34 against *E. coli*, *S. aureus*, and *C. albicans*, respectively. However, *A. niger* conidia were not completely

eradicated, even after 3 days in the presence of 17.34 mg/mL TTO. Transmission electron microscopy images indicated that TTO penetrated the cell wall and cytoplasmic membrane of all the tested bacterial and fungal strains. TTO may also penetrate fungal organelle membrane. These findings indicated that TTO maybe exerts its antimicrobial effects by compromising the cell membrane, resulting in loss of the cytoplasm and organelle damage, which ultimate leads to cell death.

Keywords Tea tree oil · *Escherichia coli* · *Staphylococcus aureus* · *Candida albicans* · *Aspergillus niger*

Introduction

Tea tree oil (TTO) is a yellow liquid extracted from *Melaleuca alternifolia*, an evergreen native Australian tree commonly referred to as the “tea tree” or “paperbark” (Homer et al. 2000). TTO has been used in medicine for at least 80 years (Carson and Riley 1993; Hammer et al. 1996; Hammer et al. 1999; Arweiler et al. 2000). The European Pharmacopeia (EDQM 2006) and the International standard (ISO 4730) (1996) require TTO to be obtained from the foliage and terminal branchlets of *M. alternifolia* Cheel by steam distillation. TTO consists of about 100 different compounds, and the major components are terpenes and sesquiterpenes, such as terpinen-4-ol, α - and γ -terpinene, 1,8-cineole, and terpinolene (Brophy et al. 1989; Kwieciński et al. 2009; Hammer et al. 2012). The concentrations of the components of TTO can vary significantly between different preparations, and this may influence their antimicrobial activities (Arweiler et al. 2000). Moreover, there is natural variation in the TTO content of *M. alternifolia* Cheel. Six distinct oil chemotypes were identified based on TTO compositions and yields from 615 trees. One chemotype is dominated by terpinen-4-ol, one by 1,8-cineole, and one by terpinolene;

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the remaining three chemotypes are all dominated by 1,8-cineole and differ in either terpinen-4-ol or terpinolene content (Homer et al. 2000). ISO 4730 (1996) requires commercial tea tree oil to have a minimum content of 30 % terpinen-4-ol and a maximum content of 15 % 1,8-cineole. Terpinen-4-ol is the major TTO component, and this has shown strong antimicrobial and anti-inflammatory properties (Kwieciński et al. 2009; Mondello et al. 2006).

The efficacy of TTO against bacteria (Carson et al. 1995; Nelson 1997; Natalie et al., 2013; Lim and Hammer 2015; Shi et al. 2016), fungi (Hammer et al. 1997; Hammer et al. 1998; Hammer et al. 2000; Li et al., 2016a, b), virus (Bishop 1995; Garozzo et al. 2011; Evgeny et al. 2013), and protozoa (Campi et al. 2012; James and Callander 2012; Pazinato et al. 2014) has gained the attention of scientists, physicians, and consumers. TTO efficiently killed all tested clinical strains of *Staphylococcus aureus*, both as planktonic cells and as biofilms, and its effective concentration was never higher than 1 % v/v (Kwieciński et al. 2009). In an in vivo assay, TTO showed antifungal activity against *Trichophyton equinum* (Pisseri et al. 2009), with a minimum inhibitory concentration (MIC) of 2.5 % (v/v) while 4 % (v/v) yielded a fungicidal effect (Nardoni et al. 2010). TTO and its nanoparticles were active against both bacteria and fungi, with MICs ranging from 0.002 to 2.5 % (Souza et al. 2014). TTO showed strong antiviral activity against the influenza A virus and the *Escherichia coli* phage, M13, and was capable of inactivating model viruses with an efficiency of more than 95 % within 5–15 min of exposure (Usachev et al. 2013).

Although many studies have reported the in vitro antimicrobial activity and in vivo efficacy of TTO, little is known about its antimicrobial dynamics and mechanisms underlying these. This has limited the wider application of TTO as an antimicrobial agent and further research into these aspects is therefore urgent. The present study explored these aspects of TTO activity using four test microorganism strains (two bacterial and two fungal): *E. coli* ATCC 8739, *S. aureus* ATCC 6538, *Candida albicans* ATCC 10231, and *Aspergillus niger* ATCC 16404.

Materials and methods

Chemical reagents, microorganisms, mediums, and cultivation

TTO, extracted from the foliage and terminal branchlets of *M. alternifolia* Cheel, was provided by Guangdong Fuyang Biotechnology Company Ltd. (Heyuan city, Guangdong province, China). This TTO was nearly 100 % in purity, with a density of 0.867 g/mL. (–)-Terpinen-4-ol was purchased from Aldrich (purity >95 %, #BCBM5331V), with a density of 0.934 g/mL. The microorganism strains (*E. coli* ATCC

8739, *S. aureus* ATCC 6538, *C. albicans* ATCC 10231, and *A. niger* ATCC 16404) were purchased from the American Type Culture Collection (ATCC) and stored in our laboratory. The media used to culture these strains were described previously (Li et al. 2010, 2013, 2016a, b). These were Mueller-Hinton (MH) medium and Mueller-Hinton Agar (MHA) medium for aerobic culture of *E. coli* and *S. aureus*, Sabouraud dextrose broth (SDB) medium and Sabouraud dextrose agar (SDA) medium for the aerobic culture of *C. albicans*, and potato dextrose water (PDW) medium and potato dextrose agar (PDA) medium for aerobic culture of *A. niger*. All solvents and reagents were of analytical grade.

Gas chromatography-mass spectrometry (GC-MS) analyses of TTO

The constituents of TTO were analyzed using a similar approach to that employed in our previous studies (Li et al. 2013, 2014a). A Thermo Finnigan Trace GC ultra-gas chromatograph with a DB 5-ms column was used (30 m length × 0.25 mm ID × 0.25 µm particle diameter), equipped with a Finnigan Trace DSQ mass spectrometer. Briefly, the operating conditions were programmed from 60 to 220 °C at 10 °C/min, with an injection port temperature of 70–250 °C. Compounds were tentatively identified by comparing their mass spectra with those of authentic samples in the NIST MS library.

Antimicrobial activities of TTO and terpinen-4-ol

Antimicrobial activities were measured using the poisoned food technique described in our previous study, with slight modification (Li et al. 2013, 2014b). The test concentrations of TTO were 0 (as control), 0.14, 0.27, 0.54, 1.08, 2.17, or 4.34 mg/mL. The test concentrations of terpinen-4-ol were 0 (as control), 0.15, 0.29, 0.58, 1.17, 2.34, or 4.67 mg/mL. Around 10⁶ colony-forming units (CFU) of *E. coli* or *S. aureus* were employed/plate or around 10⁵ CFU of *C. albicans* cells or *A. niger* conidia/plate. The cell concentrations of *E. coli*, *S. aureus*, and *C. albicans* were determined by measuring optical density (OD) at 600 nm; an OD₆₀₀ of 0.1 corresponded to 10⁸ CFU/mL of the two bacterial strains, or 10⁶ CFU/mL of *C. albicans*. The concentration of *A. niger* conidia was determined by hemocytometer. The bacterial and fungal plates were sealed using Parafilm and incubated at 37 or 28 °C, respectively, in an incubator for either 7 or 14 days. The MIC values for TTO against these bacterial and fungal strains were determined as those that showed no visible growth after incubated for 1 or 7 days separately. Two separate experiments were carried out, each with triplicate evaluations.

Antimicrobial dynamics of TTO

The antimicrobial dynamics of TTO were determined as described in our previous study (Li et al. 2013), with slight modification. The test concentrations of TTO were 0 (as control), 0.14, 0.27, 0.54, 1.08, 2.17, or 4.34 mg/mL. The cell concentrations of *E. coli* or *S. aureus* were both about 10^6 CFU/mL, and the cell or conidia concentrations of *C. albicans* or *A. niger* were both about 10^5 CFU/mL. The bacterial and fungal preparations were incubated with shaking at 150 rpm in water baths at 37 or 28 °C, respectively. Samples of bacteria were taken after incubation for 0, 2, 4, 6, 8, 10, 12, 24, 48, and 72 h; gradient dilutions of these samples were then cultured on MHA Petri dishes for 1 or 2 days at 37 °C. The bacterial colonies were counted after this incubation period, and the total number of live cells per milliliter was calculated. Similarly, fungal samples were taken and cultured on SDA and PDA Petri dishes for 2 or 3 days at 28 °C. The fungal colonies were counted after this incubation period, and the total number of live cells per milliliter was calculated. The antimicrobial dynamic curves for TTO against these four strains were developed using these data. The experiments were carried out in triplicate.

Transmission electron microscopy (TEM) of bacterial and fungal strains exposed to TTO

This was investigated as described in our previous study (Li et al. 2011). The test concentrations of TTO were 0 (as control) or 2.17 mg/mL. The bacterial and fungal cell concentrations were 10^8 and 10^7 CFU/mL, respectively. Before adding TTO, *A. niger* had been incubated at 28 °C for 15 h and the mycelium was well established. TTO or an equal volume of phosphate-buffered saline was then added into the TTO treatment cultures or control cultures, respectively. These were incubated with shaking at 150 rpm in water baths at 37 or 28 °C, as appropriate, for 5 h. The cell cultures were then sampled and prepared for TEM (Hitachi H-7650). These experiments were carried out in triplicate.

Results

The main constituents of TTO

The major TTO constituents identified by GC-MS are presented in Table 1 and their chemical formulae are shown in Fig. 1. The main chemical components of TTO identified in the present study were alkenes and alcohols. Terpenes (γ -terpinene, α -terpinene, terpinolene, β -pinene, α -pinene, and β -thujene) and terpene alcohols (terpinen-4-ol, carveol, and α -terpineol) were the first and second major categories of constituents,

Table 1 Chemical constituents of tea tree oil analyzed by GC-MS

No.	RT/min	Area%	Name
1	4.722	2.03	β -Thujene
2	4.873	3.44	α -Pinene
3	5.524	2.18	β -Pinene
4	5.715	1.97	β -Pinene
5	6.369	11.35	α -Terpinene
6	6.498	6.54	Carveol
7	7.119	13.01	γ -Terpinene
8	7.488	4.55	Terpinolene
9	9.244	22.78	(-)-Terpinen-4-ol
10	9.421	2.83	α -Terpineol
11	11.574	1.03	Isolatedene
12	12.032	1.00	α -Gurjunene
13	12.172	1.00	Caryophyllene
14	12.467	3.62	Alloaromadendrene
15	12.717	1.04	Alloaromadendrene
16	13.178	4.39	Ledene
17	13.466	3.31	δ -Cadinene
18	14.238	2.11	Globulol
19	14.349	1.32	Viridifloro
20	14.689	1.45	Cubenol
21		9.05	others

accounting for approximately 38.5 and 32.2 %, respectively, of the TTO. The level of terpinen-4-ol was particularly high, representing approximately 23 % of the whole. The third major constituent category was sesquiterpenes (isolatedene, α -gurjunene, caryophyllene, alloaromadendrene, ledene, and δ -cadinene), followed by sesquiterpene alcohol (globulol, viridifloro, and cubenol); these accounted for approximately 15.4 and 4.9 %, respectively, of the TTO.

The activity of TTO against four microbe strains

Images of the poisoned food determinations of antimicrobial activity are shown in Fig. 2. *E. coli* (Fig. 2 (A)), colonies were formed in the presence of 0, 0.14, 0.27, or 0.54 mg/mL TTO after incubation for 1 day, but no colonies were found in the presence of 1.08 or 2.17 mg/mL TTO. These results were almost unchanged after incubation for 7 days. For *S. aureus* (Fig. 2 (B)), many colonies were formed after incubation for 1 day in the presence of 0, 0.14, or 0.27 mg/mL TTO, while dozens of colonies developed in the presence of 0.54 mg/mL TTO, and none were observed in the plates with 1.08 or 2.17 mg/mL TTO. This pattern was also observed after the 7-day incubation. Therefore, the MIC of TTO against both *E. coli* and *S. aureus* was 1.08 mg/mL (1.25 μ L/mL). For the fungal strains, numerous *C. albicans* (Fig. 2 (C)) colonies developed, in the presence of 0, 0.14, 0.27, or 0.54 mg/mL

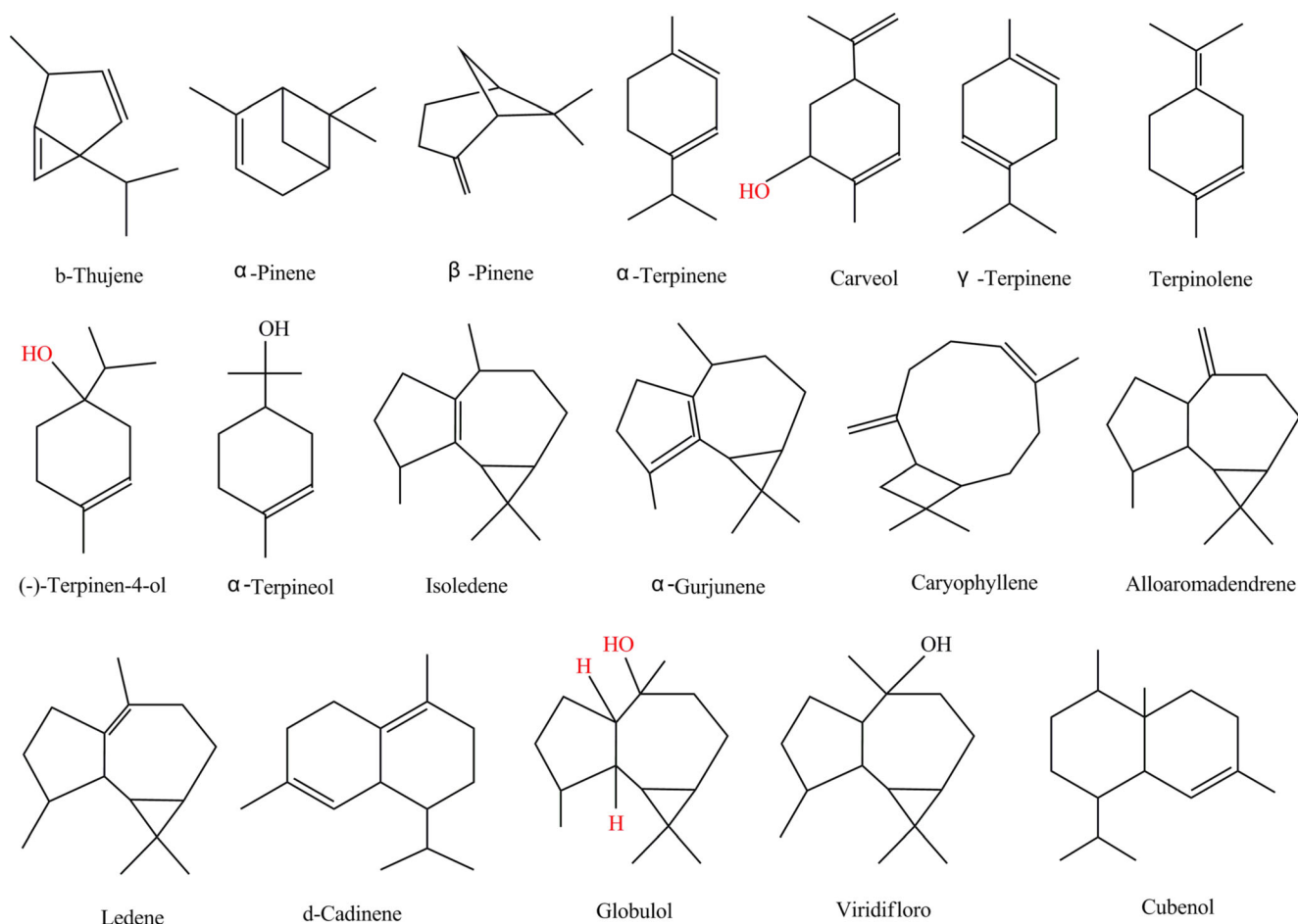


Fig. 1 Chemical formulae of TTO constituents identified by GC-MS

TTO after incubation for 2 days, while none developed in the plates containing 1.08 or 2.17 mg/mL TTO. However, after incubation for 7 days, many colonies grew in the plates with 1.08 mg/mL TTO, while none developed in the presence of 2.17 mg/mL TTO. This indicated that the MIC of TTO against *C. albicans* was 2.17 mg/mL (2.5 μ L/mL). The plates containing 0, 0.27, or 0.54 mg/mL TTO were full of white mycelium colonies of *A. niger* (Fig. 2 (D)) after 1 day, while none developed in the presence of 1.08, 2.17, or 4.34 mg/mL TTO. After 7 days, the 0, 0.27, 0.54, or 1.08 mg/mL TTO plates were all full of mycelia and were covered with black *A. niger* conidia, while none developed in the 2.17 or 4.34 mg/mL TTO plates. After incubation for 14 days, the plates containing 2.17 mg/mL TTO were also full of mycelia and had small quantities of black conidia. These findings indicated that the MIC of TTO against *A. niger* conidia was 2.17 mg/mL (2.5 μ L/mL) for a 7-day incubation.

Images of the poisoned food determinations of terpinen-4-ol antimicrobial activity are shown in Fig. S1. The MICs of terpinen-4-ol were 1.17 (1.25 μ L/mL), 1.17 (1.25 μ L/mL), 2.34 (2.5 μ L/mL), and 2.34 mg/mL (2.5 μ L/mL) against *E. coli* (Fig. S1-A), *S. aureus* (Fig. S1-B), *C. albicans* (Fig. S1-C), and *A. niger* (Fig. S1-D), respectively.

The dynamics of TTO against four microbe strains

The antimicrobial dynamic curves of TTO against the four tested strains are shown in Fig. 3. Under control conditions (no TTO), *E. coli* (Fig. 3a) and *S. aureus* (Fig. 3b) strains showed typical growth curves, including a 2-h lag phase, an exponential phase, a stationary phase after 10 h, and a decline phase. *E. coli* cultured with 0.27 mg/mL TTO showed a longer lag phase (4 h) and a delayed stationary phase (12 h). In the presence of 0.54 mg/mL TTO, a sharp decline in the quantity of live *E. coli* cells was observed after 2 h and the small number of tolerant *E. coli* cells gradually entered into lag phase and reached the stationary phase after 24 h. The *E. coli* cells cultured with 1.08 mg/mL TTO showed a sharper decline after 2 h, and the growth of the few remaining cells began to grow slowly after 48 h. No live *E. coli* cells were observed in the presence of 2.17 mg/mL TTO from 2 to 72 h. As shown in Fig. 3b, *S. aureus* showed a greater tolerance to TTO than did *E. coli*. A gradual time-dependent decline in the quantity of live *S. aureus* cells, without obvious growth, was observed in the presence of 1.08, 2.17, 4.34, or 8.67 mg/mL TTO. However, no living cells were observed after incubation of *S. aureus* with 4.34 or 8.67 mg/mL TTO for 12 or 24 h.

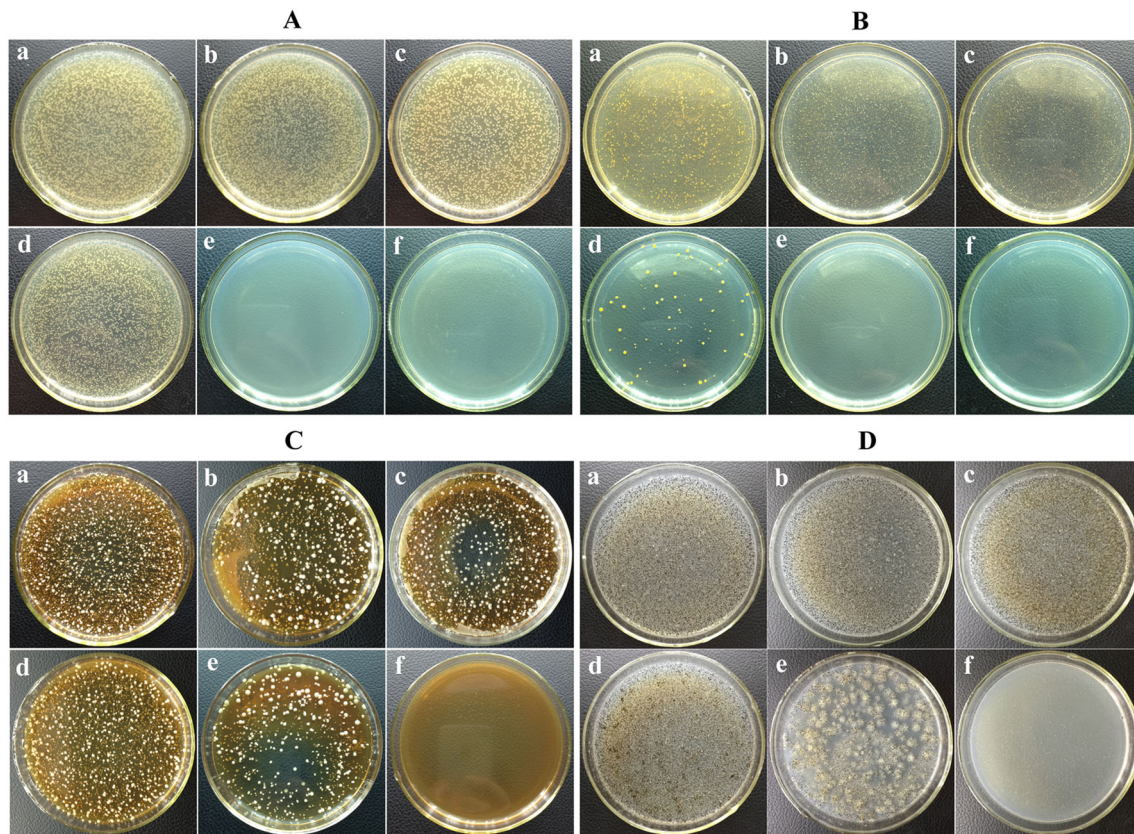


Fig. 2 Images of the poisoned food determinations of the antimicrobial activity of TTO against four microbial strains. *E. coli* (A), *S. aureus* (B), and *C. albicans* (C) are shown after treatment for 7 days with (a) no TTO (control), (b) 0.14 mg/mL TTO, (c) 0.27 mg/mL TTO, (d) 0.54 mg/mL

TTO, (e) 1.08 mg/mL TTO, (f) 2.17 mg/mL TTO; (D) *A. niger* conidia treated for 14 days with the concentrations of TTO (a) no TTO (control), (b) 0.27 mg/mL TTO, (c) 0.54 mg/mL TTO, (d) 1.08 mg/mL TTO, (e) 2.17 mg/mL TTO, (f) 4.34 mg/mL TTO

Figure 3c shows that under control condition, *C. albicans* showed a 3-h lag phase, followed by an exponential growth phase, and the stationary phase occurred after 12 h. In the presence of 0.54 mg/mL TTO, the number of surviving cells was reduced by three orders of magnitude after 3 h and reduced by two orders of magnitude after 6 h. No further decrease was observed at later time-points. The few remaining cells entered an exponential growth phase when incubated for 12 h. Cell survival declined sharply in the presence of 1.08, 2.17, or 4.34 mg/mL TTO and no surviving cells were observed after 6, 3, and 3 h, respectively. However, a small number of live cells were detected after 72-h incubation with 1.08 or 2.17 mg/mL TTO; none were identified after 72 h in the presence of 4.34 mg/mL TTO.

As shown in Fig. 3d, *A. niger* grown under control conditions showed no obvious decline in the number of conidia until 6 h. At this time-point, the conidia germinated and the mycelium grew after incubation for 9 h. The number of surviving conidia in the control group was not therefore determined after the 9-h time-point. Only minor decreases in the numbers of surviving conidia were observed after 72-h incubations with 2.17, 4.34, 8.67, or 17.34 mg/mL TTO. The survival conidia in 2.17 mg/mL TTO germinated and grew

mycelia after incubation for 48 h. The number of surviving conidia in the 2.17 mg/mL TTO group was not therefore determined after the 48-h time-point. No germination mycelia were observed after the 72-h incubation with 4.34, 8.67, or 17.34 mg/mL TTO.

The effects of TTO against four microbe strains observed by TEM

Figure 4 shows TEM images of the internal morphology of TTO-treated microbe strains. *E. coli* cells cultured under control conditions (Fig. 4 (A—a, b)) showed a typical rod shape. The cell wall, membrane, and cytoplasm were all regular and visible. The cell wall and cytoplasm membrane were close together and the cells showed homogeneous electron density. In contrast, the internal morphology of *E. coli* cells cultured with 2.17 mg/mL TTO (Fig. 4 (A—c, d)) was altered. There was a large gap between the cytoplasm membrane and the cell wall. In addition, plasmolysis and a partial disappearance of the cytoplasmic membrane were observed. Moreover, some cells appeared to be at the final stage of cell disruption, with some cytoplasm spilling out of the cells.

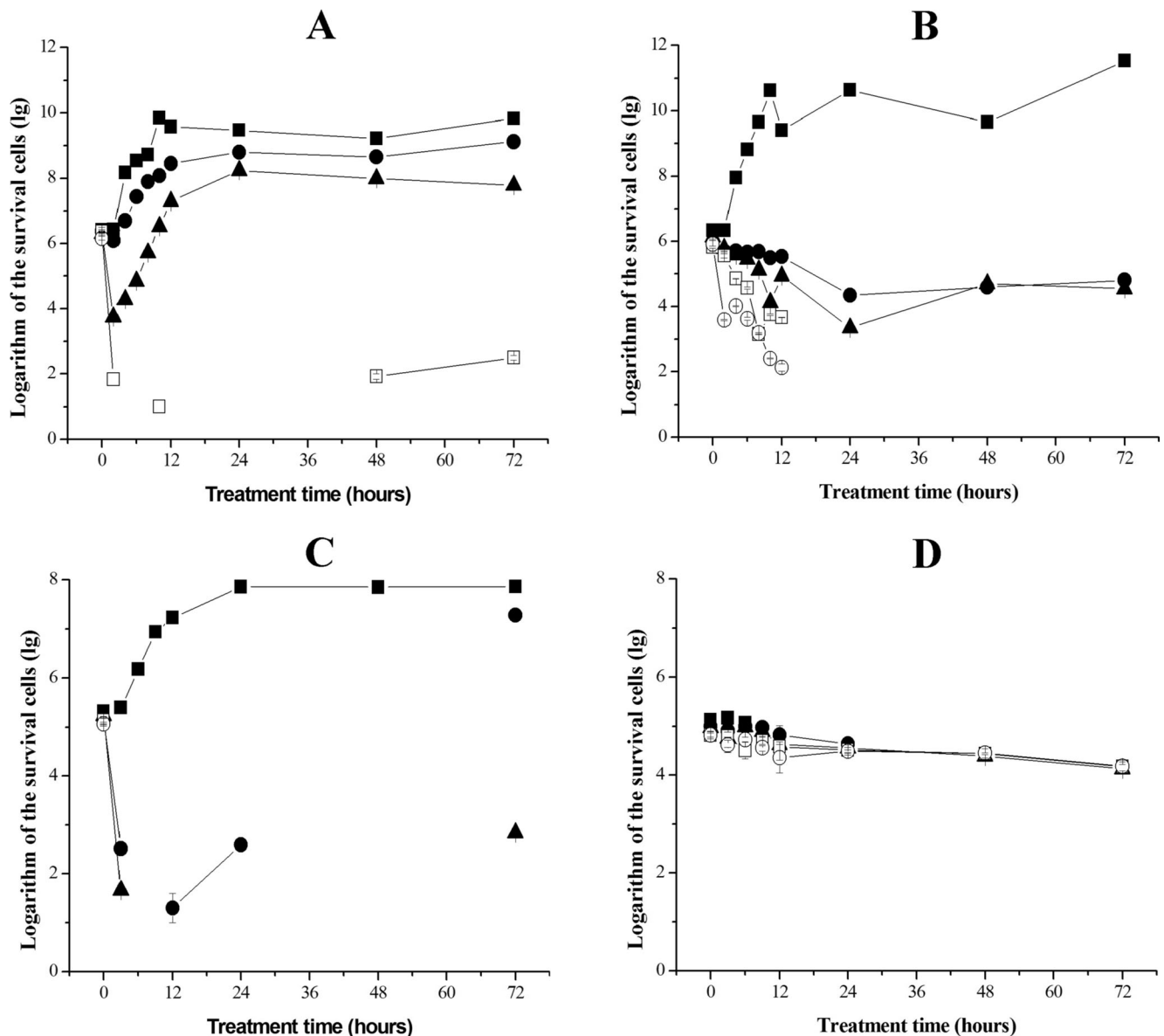


Fig. 3 The effects of TTO on the growth of four microbial strains. **a** *E. coli*, the concentrations (mg/mL) of TTO were 0 (black square), 0.27 (black circle), 0.54 (black triangle), 1.08 (white square), 2.17 (white circle); **b** *S. aureus*, the concentrations (mg/mL) of TTO were 0 (black square), 1.08 (black circle), 2.17 (black triangle), 4.34 (white square),

8.67 (white circle); **c** *C. albicans*, the concentrations (mg/mL) of TTO were 0 (black square), 0.54 (black circle), 1.08 (black triangle), 2.17 (white square), 4.34 (white circle); **d** *A. niger* conidia, the concentrations (mg/mL) of TTO were 0 (black square), 2.17 (black circle), 4.34 (black triangle), 8.67 (white square), 17.34 (white circle)

The *S. aureus* cells grown in control medium (Fig. 4 (B—a, b)) showed the normal characteristics of coccil bacteria. Their cell walls and membranes were intact, with a normal peptidoglycan layer and cytoplasmic membrane. Furthermore, the cytoplasm showed homogeneous electron density. Significant morphological changes were observed in *S. aureus* cells treated with 2.17 mg/mL TTO (Fig. 4 (B—c, d)). The cytoplasm of these cells showed heterogeneous electron density and some of it appeared to have escaped from some cells. Localized separation of the cytoplasmic membrane from the cell wall was also observed.

The regular internal structure of *C. albicans* was observed in cells grown under control condition (Fig. 4 (C—a, b)). These cells showed homogeneous electron density in the cytoplasm. Their cell walls, membranes, and organelles were intact and visible. However, some irreversible alterations were observed in the internal morphology of *C. albicans* cells treated with 2.17 mg/mL TTO (Fig. 4 (C—c, d)). Some vacuoles and electron-dense areas were observed within the cells, although the cell wall and membrane appeared intact.

The hyphae of *A. niger* grown under control conditions showed a regular healthy structure (Fig. 4 (D—a, b)), with intact cell walls, homogeneous electron density in the

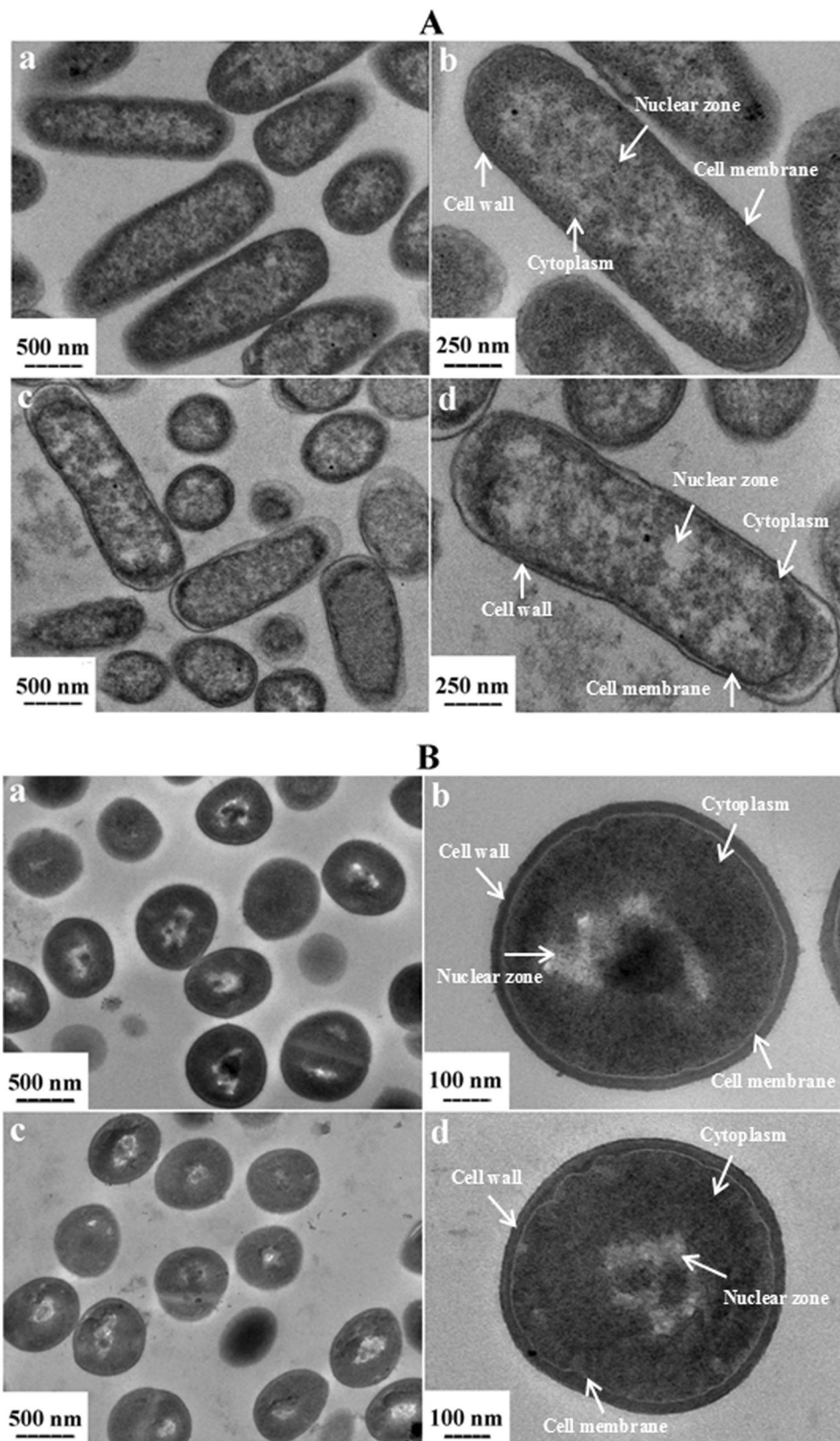


Fig. 4 TEM images of the TTO-induced morphological alterations in four microbial strains. (A) *E. coli*, (B) *S. aureus*, (C) *C. albicans*, and (D) *A. niger*; (a) and (b) control; (c) and (d) 2.17 mg/mL TTO

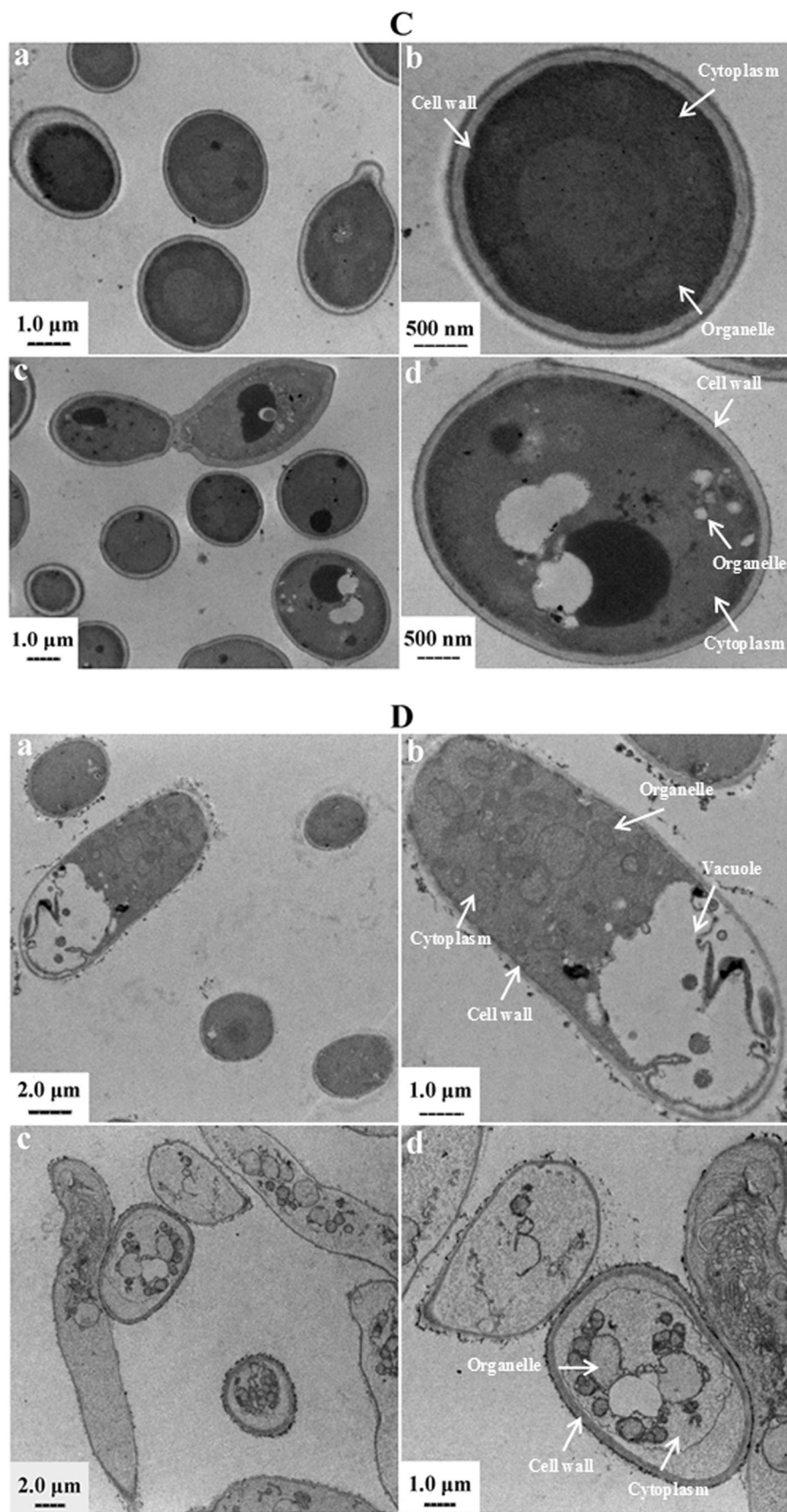


Fig. 4 (continued)

cytoplasm, and organelles that were intact, clear, and showed a regular arrangement. After treatment with 2.17 mg/mL TTO, the internal structures of the *A. niger* hyphae were also altered (Fig. 4 (D—c, d)). Cytoplasmic loss appeared to have occurred, and the organelles were deformed, damaged, and arranged irregularly.

Discussion

The results of the present study indicated that TTO had antimicrobial activity against four microbe strains, with MIC values of 1.08 mg/mL against both *E. coli* and *S. aureus* bacteria and 2.17 mg/mL against both *C. albicans* and *A. niger* fungi. We previously reported that the MIC of citronella oil against *A. niger* was 1.08 mg/mL (Li et al. 2013), the MICs of garlic oil against *P. funiculosus* and *C. albicans* were 0.69 mg/mL (Li et al. 2014a) and 0.35 mg/mL (Li et al., 2016a, b), respectively, and the MIC of *Litsea cubeba* oil against *E. coli* was 1.08 mg/mL (Li et al. 2014b). Compared to these essential oil antimicrobial activities, TTO showed moderate antibacterial and antifungal activity. Furthermore, the bacterial strains employed in the present study were more sensitive to TTO than the fungal strains. Some essential oils such as citronella oil (Delespaul et al. 2000) and garlic oil (Avato et al. 2000) have strong antifungal activities but weak antibacterial activities. This reflects the varied chemical compositions of essential oils, which produce different antibacterial and antifungal activities. The present GC-MS analysis showed that the main components of TTO were alkenes and alcohols. It also identified terpinen-4-ol as the major individual component, which accounted for approximately 23 % of the TTO. Terpinen-4-ol has previously shown strong antimicrobial and anti-inflammatory properties (Kwieciński et al. 2009). Mondello et al. (Mondello et al. 2006) found that terpinen-4-ol was as effective as TTO for accelerating vaginal clearance of all the examined *Candida* strains. The present study also showed that terpinen-4-ol had MICs of 1.17, 1.17, 2.34, and 2.34 mg/mL against *E. coli*, *S. aureus*, *C. albicans*, and *A. niger*, respectively; these values were equivalent to those of TTO against these four strains. These data strengthened the conclusion that terpinen-4-ol was a major component and a contributor to the antimicrobial activity of TTO.

The dynamic curves showed that the minimum bactericidal concentrations of TTO against *E. coli* and *S. aureus* were 2.17 and 4.34 mg/mL, respectively, and the minimum fungicidal concentration against *C. albicans* was 4.34 mg/mL; however, the *A. niger* conidia were not completely eradicated by exposure to 17.34 mg/mL TTO for 3 days. *E. coli* was thus the most sensitive to TTO, while the *A. niger* conidia were the most resistant to TTO. The numbers of surviving *A. niger* conidia only have minor decreases after 72-h incubation with TTO. The survival conidia in 2.17 mg/mL TTO germinated and

grew mycelia after incubation for 48 h, but the germination of conidia was inhibited after the 72-h incubation with 4.34 mg/mL and beyond TTO. Furthermore, a trend was observed that with increasing concentrations of TTO, the rate of cell killing and the duration of growth lag phase increased correspondingly. These data indicated that TTO had time- and concentration-dependent antibacterial and antifungal effects; this finding was consistent with previous studies of citronella oil (Li et al. 2013), garlic oil (Li et al. 2016a, b; Li et al. 2014a), and *Litsea cubeba* oil (Li et al. 2014b).

TEM analysis of *E. coli*, *S. aureus*, *C. albicans*, and *A. niger* showed irreversible TTO-induced changes, as compared with the control experimental groups. Gaps were observed between the bacterial cell wall and cytoplasmic membrane. Carson et al. (Carson et al. 2002) also found that TTO altered the cellular morphology of *S. aureus* and compromised its cytoplasmic membrane. In contrast, no significant changes were observed in the fungal cell wall. The cytoplasmic electron densities showed heterogeneity in all the TTO-treated bacterial and fungal strains, with some cytoplasmic loss from the cells. The organelles of fungal cells were damaged, deformed, and irregularly arranged. This was consistent with the reported antifungal effects of garlic oil (Li et al., 2016a, b) and citronella oil (Li et al. 2013), which destroyed some organelles such as mitochondria in *C. albicans* and *A. niger* hyphae. However, it differed from our previous report of the antibacterial effects of silver nanoparticles (Li et al. 2011), which cause DNA a tense state in *S. aureus*. The present results indicated that TTO shared some antimicrobial effects observed with other essential oils, but differed from other types of antimicrobial materials. Taken together, these studies suggest that TTO penetrates through the cell wall and cytoplasmic membrane of the tested bacterial and fungal strains, causing damage to these structures and the subsequent loss of cytoplasmic material. Essential oils can penetrate the cytoplasmic membrane due to their lipophilicity (Nogueira et al. 2010; Rassoli and Owlia 2005). In addition, TTO may penetrate fungal organelle membranes, and induce organelle damage. Finally, these irreversible TTO-mediated changes lead to cell death.

In summary, the main components of TTO were alkenes and alcohols, with the major individual component (terpinen-4-ol) accounting for approximately 23 % of the TTO as a whole. TTO showed moderate antibacterial and antifungal activities, with MICs of 1.08, 1.08, 2.17, and 2.17 mg/mL against *E. coli*, *S. aureus*, *C. albicans*, and *A. niger*, respectively. This activity was mainly attributed to the presence of terpinen-4-ol, which showed equivalent antimicrobial activity against these bacterial and fungal strains. Bacterial strains were more sensitive to TTO than fungal strains. TTO showed good antimicrobial dynamics, with a concentration- and time-dependent effect. The minimum bactericidal and fungicidal concentrations of TTO against *E. coli*, *S. aureus*, and *C. albicans* were 2.17, 4.34, and 4.34 mg/mL, respectively,

while *A. niger* conidia were not completely eradicated by 17.34 mg/mL TTO. TTO may penetrate and damage the cell wall and cytoplasmic membrane of all four tested bacterial and fungal strains, leading to cytoplasmic loss. Additionally, TTO could penetrate fungal organelle membranes, inducing deformation and damage. Ultimately, these irreversible damages result in microbe cell death.

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Compliance with ethical standards

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Conflict of interest The authors declare that they have no conflict of interests.

Ethical statements This paper is our original work. It has not been submitted elsewhere, and it is not under consideration in any other journal. This article does not contain any studies with human participants or animals performed by any of the authors. All the authors have seen the manuscript and approved its submission to applied microbiology and biotechnology.

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