K Doğan and S. Tunçer Çağlayan / Hacettepe J. Biol. & Chem., 2024, 52 (1), 11-19

Hacettepe Journal of Biology and Chemistry

Research Article

journal homepage: www.hjbc.hacettepe.edu.tr

Capsaicin Shows Species and Strain-specific Activity: Investigation of the Antibacterial Effects on the Oral Pathogen *Streptococcus mutans* and the Oral Probiotics *Streptococcus salivarius* M18 and K12

Kapsaisinin Oral Patojen *Streptococcus mutans* ve Oral Probiyotikler *Streptococcus salivarius* M18 ve K12 Üzerindeki Antibakteriyel Etkilerin Araştırılması

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ABSTRACT

Extensive research has investigated capsaicin (CAP), the primary bioactive compound in chili peppers, to explore its diverse pharmacological and physiological properties. Recently, the focus has shifted to understanding the potential effects of CAP on gut microbiota due to the strong link between gut bacterial profile and diet. However, there has been no research on the effects of CAP on oral microbiota. Therefore, our study aimed to explore the antibacterial effects of CAP on two oral probiotics, *Streptococcus salivarius* M18, and *S. salivarius* K12, along with the oral pathogen *S. mutans*. Previously, the anti-cancer activity of CAP had been demonstrated, and in accordance with these findings, here, we show its growth inhibitory activity on colorectal cancer cell lines. However, this study is the first to examine the impact of CAP on specific oral micro-organisms while considering the oral consumption of CAP and the interconnectedness of the oral and gut microbiomes. The findings revealed that CAP exhibited antibacterial properties against the M18 strain at concentrations exceeding 100 µg/mL. Surprisingly, it did not show any growth-inhibitory effects on *S. salivarius* K12, even at a concentration of 2 mg/mL. Similarly, CAP did not inhibit the growth of *S. mutans*, a significant factor in dental caries. These results suggest that CAP's effects are species and strain-specific, indicating potential changes in the oral microbiota upon CAP consumption.

Key Words

Capsaicin, *Streptococcus salivarius* M18, *Streptococcus salivarius* K12, *Streptococcus mutans*, antibacterial effect, colorectal cancer.

ÖZ

Acı biberin ana aktif maddesi olan Kapsaisin (CAP), bugüne kadar çoklu farmakolojik ve fizyolojik özellikleri açısından araştırılmıştır. Bağırsak bakteri profilinin beslenme ile güçlü bir ilişkisi olduğundan, CAP'in bağırsak mikrobiyotası üzerinde etkileri giderek daha fazla ilgi çekmektedir. CAP'in anti-kanser etkileri daha once çalışılmış olup, bu makalede de önceki verilerle uyumlu olarak CAP'in kolorektal kanser hücrelerinde büyüme inhibe edici etkisi olduğu gösterilmektedir. Öte yandan, CAP'in ağız mikrobiyotası üzerindeki etkilerini inceleyen mevcut herhangi bir araştırma bulunmamaktadır. CAP'in oral tüketimi ve oral ve bağırsak mikrobiyomları arasındaki karşılıklı etkileşim göz önüne alınarak, burada, literatürde ilk kez olarak CAP'inin, iki oral probiyotik olan *Streptococcus salivarius* M18 ve *S. salivarius* K12 ve oral patojen *S. mutans* üzerindeki antibakteriyel etkisini araştırmayı amaçladık. Sonuçlar, CAP'in M18 suşu üzerinde antibakteriyel etkiye sahip olmasına rağmen (konsantrasyon 100 µg/mL'den yüksek), 2 mg/mL olarak uygulandığında dahi *S. salivarius* K12 üzerinde herhangi bir büyüme inhibe edici etki göstermediğini ortaya koymuştur. Benzer şekilde CAP muamelesi, diş çürüklerinde önemli bir etiyolojik faktör olan *S. mutans*'ın büyümesini de engellemememiştir. Bu veriler, CAP'in tür ve suşa özgü aktivite gösterdiğini ortaya koymakta olup, ayrıca CAP tüketimi sonucu ağız mikrobiyotasında olası değişikliklere dikkat çekmektedir.

Anahtar Kelimeler

Kapsaisin, *Streptococcus salivarius* M18, *Streptococcus salivarius* K12, *Streptococcus mutans*, antibakteriyel etki, kolorektal kanser.

Article History: Received: Aug 5, 2023; Revised: Oct 17, 2023; Accepted: Oct 25, 2023; Available Online: Dec 1, 2023.

DOI: https://doi.org/10.15671/hjbc.1337284

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INTRODUCTION

Capsaicin (CAP; 8-methyl-N-vanillyl-6-nonenamide) is an alkaloid present in *Capsicum* plants, imparting the distinctive pungent aroma associated with chili peppers [1,2]. This compound is an off-white solid, crystalline, lipophilic, odorless, and colorless. CAP contains a long hydrophobic chain with a polar amide group and an aromatic ring [3]. CAP possesses a hot and burning taste, which serves as a natural defense mechanism for plants against herbivores. However, this unique taste has also contributed to the popularity of CAP as a spice in various culinary traditions [1]. CAP has been also used in studies where important findings were obtained on how the nervous system perceives heat. David Julius made a significant discovery by identifying the cellular receptor targeted by CAP, which provided valuable insights into pain mechanisms. His research revealed that CAP binds to a specific receptor called transient receptor potential channel vanilloid subtype 1 (TRPV1). This binding event triggers a signaling pathway that leads to the desensitization of afferent nerve fibers, ultimately causing the characteristic hot and burning sensation associated with CAP. Importantly, TRPV1 was shown to be activated by temperatures sensed as painful. After the discovery of TRPV1, David Julius and Ardem Patapoutian independently identified a related cold-sensitive receptor TRPM8. Several additional TRP receptors were subsequently discovered and shown to transduce thermal information in the somatosensory system. In 2021, the Nobel Prize in Physiology or Medicine was awarded to David Julius and Ardem Patapoutian for their groundbreaking discoveries in the field of thermal and mechanical transducers (nobelprize.org).

Due to its diverse pharmacological effects on multiple physiological systems, there is an increasing interest in investigating the potential of CAP as a viable alternative for the treatment of various diseases. CAP has been documented to possess analgesic, anti-obesity, cardioprotective, gastroprotective, anesthetic, neuroprotective, anti-apoptotic, anti-inflammatory, antioxidant, and metabolic modulation effects, along with its potential as an anticarcinogenic agent. It demonstrates high oral bioavailability and is readily absorbed through the skin. The application of CAP topically has demonstrated efficacy in the treatment of musculoskeletal or neuropathic pain conditions, including vasomotor rhinitis, arthritis, vasogenic facial pain, and shingles. Additionally, CAP is used in acupoint therapy for treating chronic kidney disease-associated pruritus, urinary incontinence, and postoperative nausea and vomiting [4,5]. However, it is worth noting that prolonged use of CAP at doses exceeding 100 mg per kg body weight has been associated with the development of peptic ulcers, an increased risk of stomach, prostate, duodenal, and liver cancers, as well as enhanced breast cancer metastasis [6].

The impact of CAP on the abundance, composition, and function of the intestinal microbiota has garnered increasing interest in recent years, primarily due to its close association with dietary factors. These studies aim to uncover the potential applicability of CAP in addressing inflammatory and metabolic diseases, specifically targeting its effects on the gut microbiota. A detailed review of CAP's effects on the gut microbiota was conducted by Rosca *et al*. [7]. Following oral administration, CAP is passively absorbed from the stomach and intestines, with absorption rates ranging from 50% to 90% [1]. Before absorption, CAP reaches concentrations of 500-1000 µM (0.36 mg/mL) within the intestinal lumen. Studies have demonstrated that dietary CAP and its derivatives can enhance the abundance of beneficial gut bacteria, particularly those involved in butyrate production, such as *Faecalibacterium prausnitzii* and *Roseburia*. These bacteria play a critical role in regulating energy metabolism and maintaining a balanced commensal microbiota. However, CAP has been observed to reduce the population of Gram-negative members of the gut microbiota that produce lipopolysaccharide (LPS), while simultaneously enhancing the integrity of the intestinal barrier [7,8]. Additionally, dietary CAP has been demonstrated to restore abnormal changes in the mouse model of LPS-induced depression-like behavior by reshaping the intestinal microbiota [8]. Wang *et al*. conducted a study using high-throughput sequencing to examine the impact of CAP on the mouse intestinal flora and found gender-specific differences in the changes observed in the intestinal flora between male and female mice [9]. In a separate study, researchers examined the effects of cayenne pepper extract containing CAP on 88 strains of intestinal bacteria, including pathogenic and toxigenic species, as well as beneficial *Bifidobacterium* spp. and *Lactobacillus* spp. isolated from the human intestine. The findings demonstrated that the extract promoted the growth of *Bifidobacterium* spp. at concentrations exceeding 2.25 mg/mL, and *Lactobacillus* spp. at concentrations surpassing 1.13 mg/mL [10]. The findings of this study align with a previous investigation that demonstrated the stimulatory effect

of an aqueous extract of red chili on the growth of *L. reuteri* and *L. rhamnosus* [11]. The researchers tested different concentrations of the extract, up to 9 mg/mL, and did not observe any antibacterial activity. Additionally, the extract (>9 mg/mL) did not exhibit bactericidal effects against the tested *Escherichia coli* and *Salmonella typhi* strains [10]. However, in another study, it was found that incubating with 2 mM (0.6 mg/mL) CAP for 24 hours (but not at lower concentrations) resulted in a reduction in the abundance of *L. rhamnosus* GG (LGG) *in vitro*, while *L. rhamnosus* L34 (L34) was unaffected. Moreover, the administration of a chili extract at a dose of 0.5 mL (approximately 50 mg capsaicin/dose) to mice led to negative effects on gut permeability and induced gut dysbiosis [12]. In a separate study conducted *in vivo*, the administration of 40 mg/kg CAP did not result in significant adverse effects on the GI tract in mice. However, higher doses of CAP, specifically 60 and 80 mg/kg, were associated with GI injury. These higher doses were characterized by reduced levels of IL-10, inflammation, and histopathological changes observed in the ileum, jejunum, colon, and stomach. These effects may be linked to the impact of CAP on the gut microbiota, particularly affecting the abundance of *Lactobacillus*, *Faecalibacterium*, *Bifidobacterium*, and *Butyricimonas* [13].

As can be seen, these studies were mainly intended to explore the effects of CAP on gut microbiota. Considering the oral consumption of CAP and the oral-gut microbial axis [14], here, first time in the literature, we investigated the effect of CAP on two oral probiotics, namely *S. salivarius* M18 and *S. salivarius* K12 [15], and the oral pathogen *S. mutans* as an important etiologic agent in dental caries [16]. In consideration of the potential impact of the compound on the outcomes [1], we initially evaluated the activity of the CAP solution employed in this study on HCT-116 and HT-29 cell lines. These cell lines are commonly utilized in colorectal cancer (CRC) research, and CAP is well-documented for its recognized anticancer properties against these cells.

MATERIALS and METHODS

Colorectal cancer cell cultures, treatments, and MTT assay

HT-29 (ŞAP Enstitüsü, Ankara, Turkey) and HCT-116 (DSMZ, Braunschweig, Germany) CRC lines were cultured in RPMI-1640 without phenol red containing 10% Fetal Bovine Serum (FBS), 2 mM L-glutamine and 1% penicillin/streptomycin as previously described [17]. CAP with ≥98% purity was obtained from Enzo Life Sciences, Inc. (New York, USA; Cat no: BML-EI125-0200) and dissolved in cell culture grade DMSO (Santa Cruz Biotechnology, Dallas, Texas, USA; Cat no: sc-358801) as a stock concentration of 61 mg/mL (~200 mM). The day before the treatment with CAP, the cells were seeded as $1x10^4$ cells/well of a 96-well plate. The cells were treated with 15 µg/mL (50 µM)-120 µg/mL (400 µM) or the corresponding volume of DMSO (ie., 0.2% v/v DMSO for 120 µg/mL CAP) for 24 h. After the completion of the incubation period, the cellular metabolic activity was assessed using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, which serves as an indicator of cell cytotoxicity, viability, and proliferation. For this, the medium was discarded and the cells were incubated for 4 h at 37ºC in 100 µL of complete RPMI-1640 medium containing 1.2 mM of MTT reagent (BioVision, Pennsylvania, USA). In each well, 100 µL of an SDS solution (1 g of SDS in 10 mL of 0.01 M HCl) was added to dissolve the MTT formazan crystals. Subsequently, the plates were further incubated at 37ºC for 16 h. The absorbances were measured at 570 nm using a microplate reader from Thermo Fisher Scientific (MA, USA). The reduction of MTT was expressed as a percentage relative to the untreated (UT) control cells. Absorbance values which were read from the wells without cells but containing complete medium with MTT and SDS solutions were used as blanks. Before conducting MTT assays, cell observations were made using a Nikon Eclipse TS100 inverted light microscope (4X objective), and images were captured using a Toupcam HD camera from China [18]. The experiments were replicated at least twice, with technical replicates.

Bacterial culture conditions, treatments, and growth inhibition analysis

S. salivarius K12 (BLIS Technologies, New Zealand), *S. salivarius* M18 (BLIS Technologies, New Zealand) [15], and the oral origin strain *S. mutans* Clarke (ATCC 35668) [19] were grown at 37ºC in TSB (Tryptic Soy Broth) medium (Merck Millipore, Burlington, MA, USA; Cat. no: 1.05459). After overnight incubation, the bacteria were diluted as 103 colony forming units (CFU)/mL in TSB and treated with the indicated concentrations of CAP (Enzo Life Sciences, Inc. (Cat. no: BML-EI12;) which was dissolved in DMSO (Santa Cruz Biotechnology, Cat no: sc-358801) as a stock solution of 61 mg/mL (~200 mM). In all treatments, DMSO was used as "vehicle control" in a volume (0.08-3.25% v/v) that the cells were treated with CAP (0.05-2.0 mg/mL). The bacteria were cultured

in screw cap tubes filled with 95% growth medium and sealed with paraffin film to create low oxygen conditions. The tubes were placed in a shaking incubator set at 160 rpm and incubated at 37°C for 24 h [18]. After the incubation, OD_{600} of the cultures was measured and the viability was calculated with respect to untreated (UT) control. TSB medium without bacteria or medium containing CAP (or DMSO) were used as blanks. The experiments were repeated four times with two technical replicates each.

After the CAP treatments, the bacterial growth was assessed using a spot plate assay. Following a 24 h incubation period, the bacterial cultures were diluted in TSB using the serial dilution method. Subsequently, 3.0 µL of the diluted or undiluted bacteria were dispensed onto agar plates (TSA) containing 1.5% w/v agar in TSB. The plates were then incubated overnight at 37ºC [20]. To document the results, photographs of the plates were captured using the Gel Logic-212 Pro imaging system (Carestream, USA).

Statistical analysis

GraphPad Prism 8.0.1 (GraphPad, CA, USA) was utilized for graph preparation and statistical analysis. The findings were presented as mean ± standard error of the mean (mean ± SEM), and the t-test was employed for comparisons. Significance was determined at $p \leq 0.05$ level (*p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001; **** p ≤ 0.0001), while ns (nonsignificant) denoted lack of significance. The statistical analysis was performed in comparison to the control group treated with the vehicle (DMSO).

RESULTS and DISCUSSION

Capsaicin shows anticancer effect on colon cancer cell lines

To test the activity of the CAP solution, we first treated the HCT-116 and HT-29 CRC cell lines with CAP or corresponding volume of DMSO for 24 h. As shown in Figure 1, CAP treatment inhibited the growth of the CRC cell lines for all the concentrations analyzed, in agreement with the literature [21, 22]. Of note, HT-29 cells (Figure 1B, left panel) were found slightly more sensitive to the treatment with respect to HCT-116 cells (Figure 1A, left panel). Representative microscope images of the cells treated with CAP or DMSO or left untreated are presented in the right panels of Figure 1.

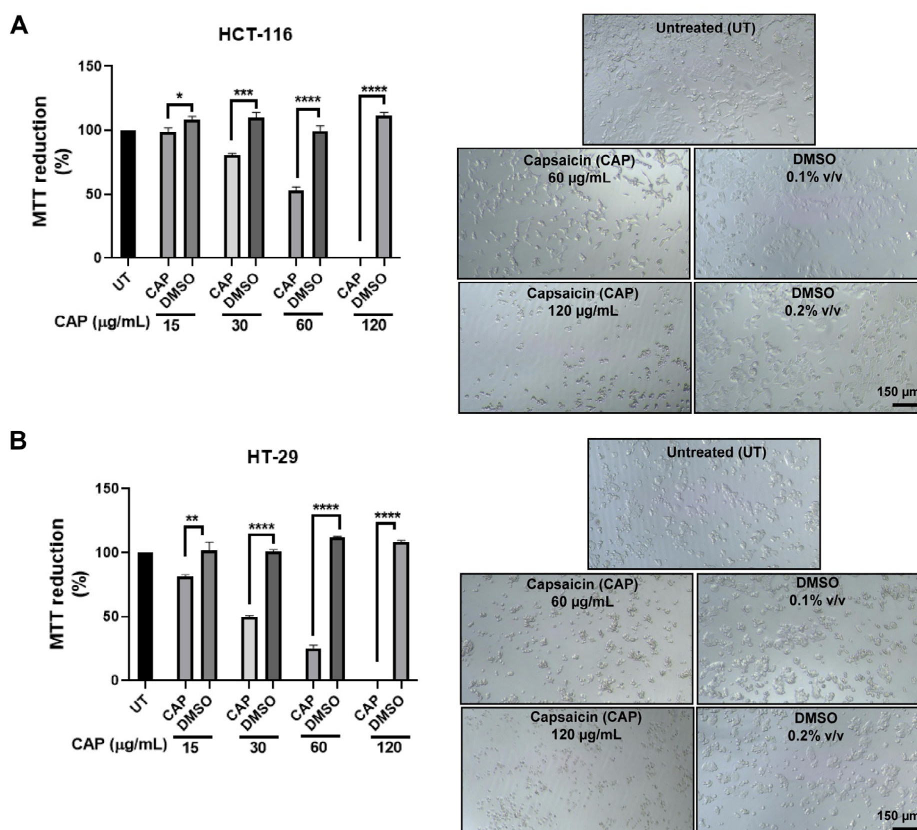

Figure 1. Cytotoxicity of CAP in colorectal cancer cell lines (A) HCT-116 and (B) HT-29.

CAP is known for its anticancer activities on CRC by inhibiting the growth of CRC cells and tumor formation. CAP exhibits its anti-carcinogenesis effects in CRC through inhibition of proliferation, induction of apoptosis, and cell cycle arrest [21,22]. Based on these well-known anticancer properties of CAP, here we first tested the activity of the pure CAP solution in our hands, on HCT-116 and HT-29 CRC cell lines. The obtained data were consistent with the literature presenting that CAP concentrations above 15 µg/mL (~50 µM) show strong cytotoxic effects on the cell lines [23]. The respective CAP compound (such as employing various plant extracts obtained through different isolation methods, pure CAP or CAP derivatives, or combining CAP with other capsaicinoids) can affect the results on the anti-bacterial activity of CAP [1]. Here, we used the same CAP reagent (with ≥98% purity) which was used for anti-cancer activity experiments, for further experiments carried out with the bacteria.

CAP shows antibacterial activity against the M18 strain of *Streptococcus salivarius*

Responses of *S. salivarius* strain M18, K12, and *S. mutans* to CAP treatment are shown in Figure 2. Between the analyzed bacteria, only the growth of the probiotic *S. salivarius* M18 was inhibited with the CAP treatment (Figure 2A). Growth inhibition, calculated with respect to the OD_{600} of the untreated (UT) control culture is shown on the left panel and the spot plate assay is presented on the right panel of Figure 2A. However, treatment of *S. salivarius* K12 (Figure 2B) and *S. mutans* (Figure 2C) with CAP did not result in growth inhibition.

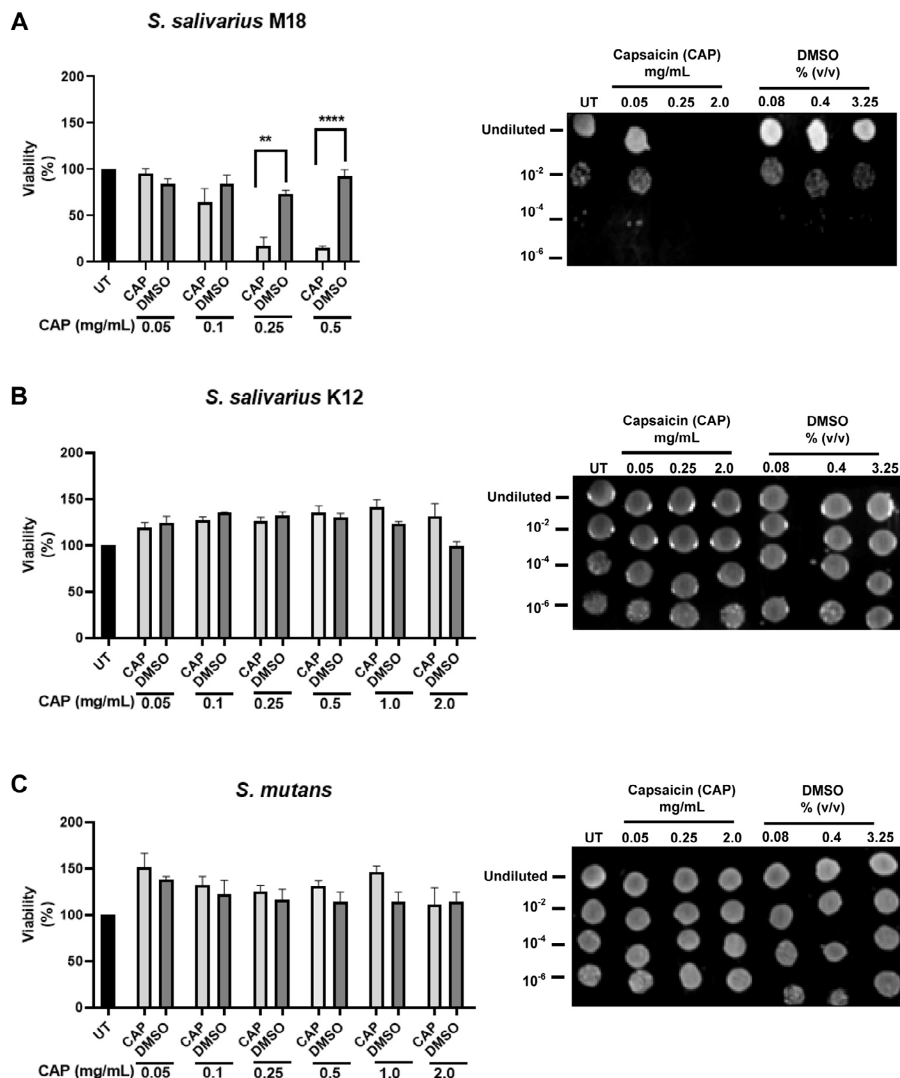

Figure 2. Antibacterial activity of CAP in (A) *S. salivarius* M18, (B) *S. salivarius* K12, and (C) *S. mutans*.

Green and red peppers, which are used as food additives worldwide, have CAP contents ranging between 0.1%-1.0% [22]. Studies with isolated CAP showed that oral supplementation of 0.375-33 mg CAP per day found no thermogenic effect on humans [24]. On the other hand, due to the side effects including intestinal cramping and stomach discomfort, it is not recommended either 10 mg or a higher dose of CAP for daily human consumption [25, 26]. Therefore, considering the concentrations of the CAP used in the human trials and the volume of saliva in the oral cavity before swallowing (0.5-2.1 mL depending on the individual) [27], CAP concentrations ranging from 0.05 mg/mL-2.0 mg/mL were used to treat the oral bacteria in this study (corresponding to approximately 0.1-4.0 mg oral consumption). Additionally, the vehicle effect of DMSO was also considered during the determination of CAP concentrations for treatments, since 6.5% v/v DMSO (solvent for 4.0 mg/mL CAP) was shown growth inhibitory effect on *S. salivarius* strains, CAP concentrations higher than 2 mg/mL was not used in the study (Figure 3).

CAP, as a natural molecule, previously has been investigated mainly as a complementary or replacement molecule for antibiotics for its antibacterial potential on different Gram-negative and Gram-positive bacteria to combat bacterial infections. However, the studies are mostly incomparable because of the differences in experimental setups [1]. The antibacterial activity of a crude methanol extract obtained from *Capsicum annuum* was observed against several Gram-negative bacteria including *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Proteus* spp. The Minimum Inhibitory Concentration (MIC) for these strains was determined to be 512 µg/mL. Additionally, the extract showed slightly lower MIC values (256 µg/mL) against methicillin-resistant strains of *Staphylococcus aureus*. The lowest MIC value (128 µg/mL) was observed against *Bacillus subtilis* [28]. Pure CAP on the other hand, only retarded the growth of *E. coli* (DH5α Difco ATCC 25922) at concentrations up to 300 mg/mL, and the growth of *P. solanacearum* was only decreased by about 20% with a concentration of 300 µg/mL. In the *B. subtilis* (Kodiak) strain, it was observed that pure CAP exhibited a more pronounced inhibitory effect, which became evident at concentrations of 25 µg/mL and higher. Within the same study, the impact of CAP on *Saccharomyces cerevisiae* (wild strain 288C) yielded uncertain results. During the early 6 h incubation period, cellular growth appeared to be stimulated even at concentrations as high as 300 µg/mL. However, when the incubation time was prolonged to 24 h with the same concentration of CAP, no discernible effect on growth was observed [29]. In another study, the concentration of 100 µg/mL of CAP inhibited the growth of the two strains of *Chromobacterium violaceum*, *Serratia marcescens* MG1 and *P. aeruginosa* PAO1 partially. However, CAP at 25 and 50 µg/mL enhanced the growth of the opportunistic Gram-negative *S. marcescens* MG1 by 46.0% and 38.1% compared to the control and authors suggested for metabolization of CAP by this bacteria [30]. CAP demonstrated inhibitory effects on the growth of *Porphyromonas gingivalis*, a key agent in periodontitis, with a MIC of 16 µg/mL and a minimum bactericidal concentration (MBC) of 64 µg/mL [31]. Santos *et al*. discovered that the ethyl acetate crude extract obtained from *C. annuum* inhibited the

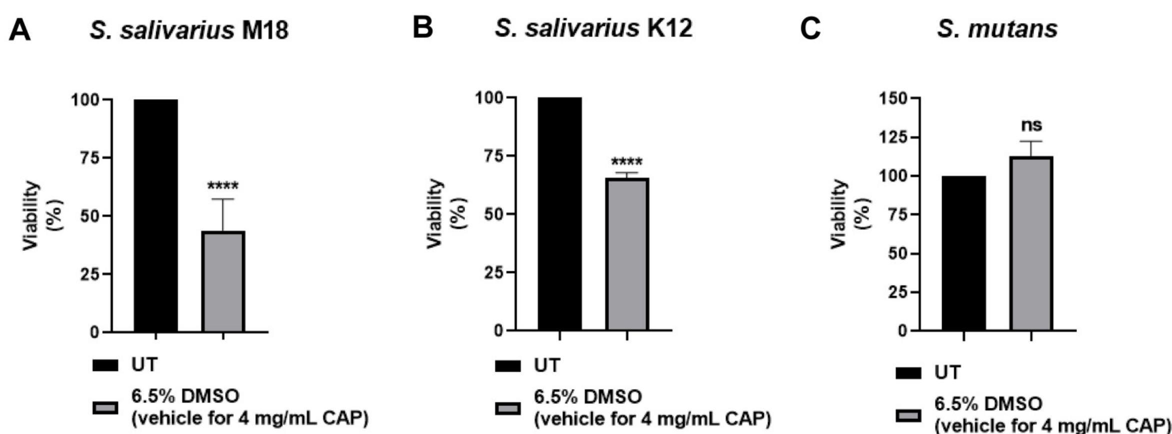

Figure 3. DMSO (vehicle) toxicity in (A) *S. salivarius* M18 (B) *S. salivarius* K12 and (C) *S. mutans*.

growth of *S. mutans* ATCC 25175 strain at a concentration of 2.5 mg/mL. Furthermore, CAP and dihydrocapsaicin, isolated from the ethyl acetate extract of *C. annuum*, showed growth-inhibitory effects on the bacteria at a concentration of 1.25 µg/mL [32]. In another study, a natural extract derived from plants containing CAP (65%) and dihydrocapsaicin (35%) demonstrated inhibition of *Paenibacillus larvae*, a Gram-positive bacillus that forms endospores and causes infectious diseases in honey bees, with a MIC of 32 µg/mL [33]. The antibacterial effect of the CAP was also tested on Gram-positive and Gram-negative rumen bacteria to evaluate its potential as an alternative to antibiotic treatment. While not causing significant growth inhibition, CAP demonstrated potential antibacterial activity against *Ruminococcus flavefaciens* and *Methanobacterium formicicum*. Additionally, CAP exhibited a growth-stimulating effect on *R. albus* at concentrations ranging from 0.5 to 128 µg/mL, but at 256 µg/mL, it demonstrated antibacterial activity. In the case of other Gram-positive rumen bacteria, CAP stimulated the growth of *Butyrivibrio fibrisolvens* at concentrations of 0.5 to 64 µg/mL and *Eubacterium ruminantium* at concentrations of 8 to 128 µg/mL, though these effects diminished at higher concentrations. Furthermore, CAP also stimulated the growth of Gram-positive bacteria *S. bovis* and the Gram-negative species *Megasphaera elsdenii* and *Fibrobacter succinogenes* [34]. Taken together, these findings indicate that the antibacterial properties of CAP are influenced by several factors, including the specific bacterial species and strains, experimental conditions, administered concentrations, and the nature of the compounds used. It is important to note that when CAP is extracted, other plant-derived compounds with antibacterial properties may be present in the mixture, potentially influencing the outcomes of the experiments. To avoid this drawback, a commercial CAP reagent with ≥98% purity was used in this study. The findings of the manuscript reveal distinct responses of two closely related oral probiotics, namely *S. salivarius* M18 and K12, to CAP treatment: *S. salivarius* M18 exhibited sensitivity to CAP, while no growth inhibition was observed for *S. salivarius* K12. Additionally, CAP did not inhibit the growth of *S. mutans*, the major microbial etiological agent of dental caries [35]. *S. salivarius* is a group of streptococci that plays a vital role in maintaining microbial balance in the oral microbiome and GI system. It establishes colonization in the mouth and nasopharynx of newborns shortly after birth and is a dominant species found in breast milk, contributing significantly to the development of the infant's gut microbiome. *S. salivarius* can colonize various parts of the body, including the GI system, genitourinary tracts, and paranasal sinuses [18,20]. Among the *S. salivarius* probiotics, *S. salivarius* M18 and K12 are considered highly promising oral strains. *S. salivarius* M18 was originally isolated from the oral cavity of a healthy adult female, while *S. salivarius* K12 was isolated from the saliva of a healthy child. Both strains have demonstrated inhibitory effects against numerous pathogens in the mouth, throat, and nasal regions [15]. However, these two strains, *S. salivarius* M18 and K12, exhibit distinct bacteriocin profiles. *S. salivarius* K12 produces A2 and B, whereas *S. salivarius* M18 synthesizes salivaricin A2, 9, MPS, and M. Notably, salivaricin A2, MPS, and 9 are encoded by megaplasmids and demonstrate inhibitory effects against *S. pyogenes*. A2 and 9 possess broader inhibitory actions against additional respiratory tract pathogens, while MPS specifically targets *S. pyogenes*. Salivaricin M on the other hand, plays a role in inhibiting *S. mutans* and its expression is regulated chromosomally [36]. *S. mutans* is capable of forming dental plaque, a biofilm on tooth surfaces [37]. *S. salivarius* M18 has been proposed for use in cavity prevention and disruption of dental plaque formation. It accomplishes this by producing bacteriocins that target cariogenic species, as well as dextranase and urease enzymes that aid in reducing dental plaque accumulation and acidification, respectively [38]. Clinical trials have demonstrated that individuals colonized with *S. salivarius* M18 exhibit a significant decrease in plaque formation compared to those who were not colonized or only exposed to the bacterial probiotic. Furthermore, M18-colonized patients showed a marked reduction in *S. mutans* levels and an overall decrease in the development of dental caries [36]. Accordingly, the commercial form of the strain M18 (BLIS M18®) is indicated to target gum disease and plaque build-up while strain K12 (BLIS K12®) is claimed to target the pathogens that cause infections such as tonsillitis, pharyngitis, and ear infections (blis.co.nz/). Based on the findings of this study, it is possible to suggest that CAP could potentially reduce the oral population of M18 and also decrease the bioavailability of the probiotic when administered in the form of probiotic lozenges. Furthermore, since *S. mutans* is not affected by CAP treatment, a decrease in the oral M18 count may facilitate the colonization of *S. mutans*.

Various health conditions, encompassing a wide range of ailments such as hepatic or brain abscesses, cardiovascular diseases, pneumonia, dementia, cystic fibrosis,

and GI cancers, have been linked to the composition of the oral microbiota, both directly and indirectly. Pointing out the oral-colon interaction, the dysbiosis of colonic microbiota is proposed to be affected by oral microbiota. Given the colonization pattern of *S. salivarius*, it is reasonable to propose that this bacterium plays a crucial role not only in the oropharyngeal region but also in the ecological balance of the digestive tract [20]. Consequently, when assessing the growth inhibitory impact of CAP on *S. salivarius* M18, it is essential to consider not only its effect on the oral microbiota but also its potential influence on the equilibrium of the gut microbiota, within the context of the oral-colon axis.

CONCLUSION

This is the first study in the literature to investigate the effect of CAP on oral probiotics and an oral pathogen. The obtained results indicate that CAP has the potential to reshape oral microbiota, as shown for the gut microbiota previously. To comprehend the alterations in the abundance of particular bacteria induced by CAP, it is essential to unravel the fundamental molecular mechanisms responsible for its antibacterial effects, including investigating its impact on efflux pumps and the expression of bacterial virulence factors.

Finally, it is important to note that many studies in the literature employ plant extracts that contain various compounds, along with CAP, to assess CAP's antibacterial activity as discussed above. These extracts often encompass a spectrum of ingredients such as a range of radical scavengers, flavonoids, and additional plant-derived elements with potential antibacterial properties. It is essential to recognize that the diversity of these ingredients within the extracts, as well as varying concentrations of CAP among different extracts, create challenges in comparing results across studies. Consequently, inconsistencies in outcomes frequently arise. In our study, we deliberately chose to work with pure CAP to focus exclusively on CAP's antibacterial activity, resulting in outcomes that are not only more consistent but also readily comparable with other investigations.

Acknowledgments - This project is partially supported by the TÜBİTAK 2209-A University Students Research Projects Support Program (Project number: 1919B012114445) to undergraduate student KD under the supervision of ST. The authors thank the Molecular Biology and Genetics Department and Biotechnology Application and Research Center of Bilecik Şeyh Edebali University, for the laboratory facilities and Dr. Sreeparna Banerjee (Middle East Technical University) for the CRC cell lines and Dr. Rafig Gurbanov (Bilecik Şeyh Edebali University) for sharing the bacterial strains.

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