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Original Article

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Antimicrobial Activity of Carvacrol against *Lactobacillus acidophilus* and *Lactobacillus casei*, An In-Vitro Study

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Abstract

***Background & Aims*:** *Lactobacillus acidophilus (L. acidophilus)* and *Lactobacillus casei L. casei)* are the primary bacterial pathogens involved in dental caries and periodontal diseases. In this study, we aimed to investigate the antimicrobial activity of Carvacrol in inhibiting the growth of these two microbial species in-vitro.

***Materials & Methods*:** In this study, we prepared standard colonies of *L. acidophilus* and *L. casei*, then evaluated disk diffusion and well diffusion tests on De Man-Rugose and Sharpe (MRS) agar plates to determine the antimicrobial activity of Carvacrol. We used 30 µg tetracycline disks as control. To evaluate the minimum inhibitory concentration (MIC), Carvacrol was used in the range of 20 to 0.039 µL in MRS broth medium containing bacteria. To determine the Minimum Bactericidal Concentration (MBC), the contents of tubes were subsequently cultured on MRS agar plates.

***Results*:** The MIC and MBC of Carvacrol against *L. casei* were 0.406 ± 0.143 and 0.813 ± 0.287 µg/mL, and against *L. acidophilus* were 0.254 ± 0.072 and 0.406 ± 0.143 µg/mL, respectively. In the disk diffusion test, carvacrol solution (2%) significantly induced inhibitory zones against *L. casei* and *L. acidophilus*. Although In the well diffusion test, 2% carvacrol solution generated inhibitory zones against *L. casei*. and against *L. acidophilus* with detectableinhibitory zones, but they werer not statistically significant.. We noted a significant difference only for the volume of 80 µL of solution (p = 0.03).

***Conclusion*:** The present study indicated that Carvacrol could be used as a natural alternative agent against *L. acidophilus* and *L. casei* generated dental caries.

***Keywords*:** *Lactobacillus acidophilus*, *Lactobacillus casei*, Carvacrol, Antibacterial Agent

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Introduction

Dental caries is one of the most prevalent chronic diseases of people worldwide (1). During dental caries, enamel, dentin, and cement may dissolve by decalcification. The eventual outcome of caries is determined by the dynamic balance between pathological factors and protective factors. Pathological factors include acidogenic bacteria, inhibition of salivary function, and frequently ingestion of fermentable carbohydrates, which leads to demineralization. On the other hand, protective factors include salivary level and quality, antibacterial agents, fluoride from external sources, and the selected dietary component may lead to remineralization (2). It is believed that *Streptococcus mutants* is the main factor that initiates caries and a critical factor of enamel decay. The bacteria of the genus *Lactobacilli* are also essential in further caries development, especially in the dentin (1). *Lactobacilli* are the most important bacteria that contribute to dental caries (3-7). Oral *Lactobacillus* species include: *L. acidophilus, L. casei, L. fermentum, L. delbrueckii, L. Plantarum, L. jensenni, L. Brevis, L. salivarius,* and *L. gasseri* (1, 2). *Lactobacilli* were isolated in 54% of children aged 3 to 4 years with caries and 7% without caries. About 48% of the *lactobacilli* in dental plaques relate to *L. casei*, and 9.6% relate to *L. acidophilus* (7). Dental caries often occur in absence of *lactobacilli* but could not occur without *S. mutants* (8). Chemical plaque control methods, such as chlorhexidine or sodium fluoride, can prevent dental caries and limit the growth and formation of biofilms by cariogenic microorganisms in the oral cavity. Chemical agents are the most common antimicrobial agents, but because of some limitations including microbial resistance, side effects such as vomiting, diarrhea, and postoperative complications, their popularity is low (9-12). The mentioned limitations led to alternative herbal agents because of the their lower cost, fewer side effects, increasing trend in consumers' belief that natural products are harmless and safe (12), and growing demand for natural origin preparations (13-18). Carvacrol is a monoterpene phenol substance found in aromatic plants such as oregano and thyme. Studies showed that Carvacrol has various biological and pharmacological properties such as antioxidant, antibacterial, antifungal, anticancer, anti-inflammatory, immunomodulator, liver protection, and vasorelaxant (19). Carvacrol disintegrates structure and depolarizes the bacterial cell wall, which leads to bacterial cell leakage and death. Carvacrol also interacts with bacterial metabolic enzymes in low doses and lowers their function (20). Carvacrol is generally consumed safely, and is approved by the US Food and Drug Administration (FDA) as food additive. Furthermore, it is used as flavoring agents in alcoholic beverages, chewing gum, and so forth by the Council of Europe (21).

In this study, we investigated the antibacterial activity of Carvacrol, a Generally Recognized as Safe (GRAS) compound (22), against common bacteria *L. casei* and *L. acidophilus* as two main bacteria involved in dental caries. Silveria et. al. studied the antibacterial effect and efflux pump reversal of thymol and Carvacrol against the s*taphylococcus*. These compounds had significantly antibacterial effect against the tested strains of *staphylococcus*, however, the efflux pump inhibition was not detected. Previous studies demonstrated that phenolic-rich extracts from edible plants showed antimicrobial effects against different pathogens (23). Chan et. al. conducted a study to investigate the impact of six phenolic-rich extracts on five food-borne bacteria. The results revealed that Carvacrol, the main phenolic compound of the selected herbal extracts, showed an antibacterial effect against the food-borne pathogens involved in this study. Based on the characteristics of Carvacrol mentioned above and because that there were limited studies on the impact of synthetic Carvacrol (not as a combination of herbal extracts) againt *L. casei* and *L. acidophilus*, we aimed to fill in the existing gap by designing the current study (24)..

Materials & Methods

Study design:

In the present study, we purchased standard samples of *L. acidophilus* (DSM 20079) and *L. casei*

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(ATCC 39392) from the Iranian Research Organization for Science and Technology (IROST-Iran) and Carvacrol purified up to 98% from Sigma Aldrich-Germany. Dimethyl Sulfoxide (scharlau-Spain) was used as dilution agent. MRS agar and MRS broth (Qlab-Canada) were used to cultivate both bacterial colonies. We then sequentially dissolved 67.15 and 55.15 gr of them in 1-liter demineralized water at the boiling temperature (100 °C).We then covered the solutions with cotton to prevent vaporization. In the case of *L. acidophilus*, we used HCLO3 to lower the solution pH from 6.5 to 5.5 to trigger *its* growth. All four solutions along with micropipette tips were sterilized in an Autoclave with a degree of 121 °C for 15 minutes. After cooling to 37 °C, the MRS agar solutions were transferred in plastic plates and kept in a 4 °C refrigerator for two hours. MRS broth solutions were then transmitted to experimental tubes.

The pure lyophilized ampoules containing microorganisms were firstly disinfected with alcohol 70%, and 1 mL of distilled water was added to the ampoules and mixed to make microbial suspensions. 1 mL of suspensions was then added to experimental tubes containing MRS broth. the experimental tubes with a Gaspak (Merck-Germany) in an anaerobic jar were incubated at 37 °C temperature. Fresh cultures were grown after 72 h for *L. acidophilus* and 48 h for *L. casei*.

Turbidity appearance in experimental tubes after the incubation period was equal with bacterial growth. Sampling from experimental tubes was conducted with sterile cotton swabs and cultured in MRS agar plates linearly. The plates were incubated in the same method and in the same period to induce bacterial colonies proliferation.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC):

For MIC and MBC test, 3-4 colonies were sampled using sterile inoculation needle and transferred to 1 mL physiologic serum. The solution was then mixed on the vortex with an orbital shaking manner to make a suspension known as 0.5 McFarland. Turbidity was equal to 1.5×10^{8} bacteria which is appropriate for bacterial growth. Firstly 1 mL of MRS broth solution was added to each one of 12 experimental tubes, then 1 mL solution of Carvacrol (4%) was added to the first tube. 1 mL of first tube solution was isolated and added to the second tube, then 1 mL of the second tube was isolated and added to the third tube, and this process continued until the last tube (except the 11th tube). The 11th tube as the positive control was without Carvacrol (containing 100 µL DMSO (Dimethyl sulfoxide) like the first tube), and the 12th tube was negative control without bacteria. Then 10 µL of each microbial suspension were isolated with a micropipette and added to 1 to 11 tubes. After 48 h Incubation at 37 °C under anaerobic conditions, MIC was investigated by macroscopic transparency changes in the tubes. All tubes were cultured in MRS agar plates and incubated for 48-72 h at 37 °C temperature to investigate anaerobic growth for MBC.

Well diffusion test:

For well diffusion test, 10 µL of each microbial suspension were cultured in MRS agar plates with sterile cotton swabs. We then used warm micropipette tips to create wells with a diameter of 8 mm in MRS agar plates, and added 80, 90, 100, and 110 µL solutions (2% carvacrol) to each well. For the disk diffusion test, 20, 30, 40 and 50 µL solution (2% carvacrol) were added on sterile blank disks (Padtan teb-Iran) and incubated for 30 min. Paper disks were then placed on the "Lawn" of bacteria. In these plates, Antibiotic disks containing 30 µg Tetracycline, (Padtan teb-Iran) was added as a control. Four plates were incubated for 48-72 h, and the growth Diameter of the Inhibition Zone (DIZ) beyond disks and wells was measured. The standard diameter of disks was 6 mm, and DIZ more than 8 mm were categorized as bacterial growth inhibition.

Data Analysis:

Data were obtained and recorded in IBM SPSS 25.0 (IL, Chicago, USA) software. Data were reported as mean ± standard deviation. Intergroup analyzes were conducted using One Way ANOVA and Tukey test. Significance was recorded as $p < 0.05$.

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Results

The results of this study are presented in Tables 1 and 2. In the present study on the two species *L. casei* and *L. acidophilus*, all MIC and MBC experiments with 20 to 0.039 µL volumes of carvacrol, DIZ test with disk, and well diffusion test with 2.2 to 0.4 µL volumes of Carvacrol were done in triplicate.. Also, growth DIZ was measured in both disk and well diffusion, and compared with the control group (30 µg Tetracycline). The results in 3 rounds in the well diffusion test were 20, 24, and 21 mm for *L. casei* and 21, 19, and 22 mm for *L. acidophilus*, compared to the control group. In the disk diffusion test, the growth DIZ for *L. casei* was 20, 25, and 25 mm, and in for *L. acidophilus* was 18, 19, and 20 mm, compared to the control group.

Table 1. Antimicrobial efficacy of different concentrations of carvacrol against *L. casei*

Parameter	*L.casei* (Mean ± SD)	p-value
MIC (µg/mL)	0.406 ± 0.143	N/A[a]
MBC (µg/mL)	0.813 ± 0.287	N/A[a]
DIZ[b] in Well Diffusion Test (mm) 80 µL (2% Carvacrol)	16 ± 2.82	N/S[c]
DIZ in Well Diffusion Test (mm) 90 µL (2% Carvacrol)	18.33 ±1.24	N/S
DIZ in Well Diffusion Test (mm) 100 µL (2% Carvacrol)	20.5 ±1.5	N/S
DIZ in Well Diffusion Test (mm) 110 µL (2% Carvacrol)	20.5 ±1.5	N/S
DIZ in Disk Diffusion Test (mm) 20 µL (2% Carvacrol))	12.66 ± 1.24	< 0.001[d]
DIZ in Disk Diffusion Test (mm) 30 µL (2% Carvacrol))	11.33 ± 0.94	< 0.001
DIZ in Disk Diffusion Test (mm) 40 µL (2% Carvacrol)	13 ± 0.81	< 0.001
DIZ in Disk Diffusion Test (mm) 50 µL (2% Carvacrol)	16 ± 0.81	0.002

a. Not Applicable, b. Diameter of Inhibition Zone (DIZ), c. Not Significant, d. Intergroup comparison with the control group in *L. casei* using Tukey test

Table 2. Antimicrobial efficacy of different concentrations of carvacrol against *L. acidophilus*

Parameter	*L.acidophilus* (Mean ± SD)	p-value
MIC (µg/mL)	0.254 ± 0.072	N/A[a]
MBC (µg/mL)	0.406 ± 0.143	N/A
DIZ[b] in Well Diffusion Test (mm) 80 µL (2% Carvacrol)	17 ± 0.81	0.03
DIZ in Well Diffusion Test (mm) 90 µL (2% Carvacrol)	19.33 ± 0.47	N/S[c]
DIZ in Well Diffusion Test (mm)	20 ± 0.81	N/S

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100 µL (2% Carvacrol)		
DIZ in Well Diffusion Test (mm)	19 ± 1.41	N/S
110 µL (2% Carvacrol)		
DIZ in Disk Diffusion Test (mm)	12 ± 0.81	< 0.001[d]
20 µL (2% Carvacrol)		
DIZ in Disk Diffusion Test (mm)	12.66 ± 0.47	< 0.001
30 µL (2% Carvacrol)		
DIZ in Disk Diffusion Test (mm)	13.33 ± 0.47	< 0.001
40 µL (2% Carvacrol)		
DIZ in Disk Diffusion Test (mm)	14 ± 0.81	< 0.001
50 µL (2% Carvacrol)		

a. Not Applicable, b. Diameter of Inhibition Zone (DIZ), c. Not Significant d. intergroup Comparison with the control group in L. acidophilus using Tukey test

MIC of Carvacrol against *L. acidophilus* was 0.254 ± 0.072 µg/mL and for *L. casei* was 0.406 ± 0.143 µg/mL Also, MBC against *L. acidophilus* was 0.406 ± 0.143 µg/mL and for *L. casei* was 0.813 ± 0.287 µg/mL. These findings are illustrated in Tables 1 and 2. Results for Growth DIZ in disk diffusion test showed a significant difference between the control groups in all concentrations ($p < 0.05$). Results for growth DIZ in well diffusion test showed a significant difference only between the control group and the volume of 80 µL carvacrol solution (2%). In other concentrations, DIZwas determined but was not significant.

Discussion

As the primary food and water consumption access, the oral cavity is constantly exposed to irritants, pollutants, and disease factors (25). Decayed teeth are one of the most prevalent chronic disorders affecting people worldwide (1). Dental biofilm initiates dental caries, and it is associated with specific types of bacteria, mainly *streptococcus mutants* and *Lactobacilli* (4). According to Badet et. al. (26), *lactobacillus acidophilus* and *Lactobacillus casei* are the most common *Lactobacilli* species on bacterial plaque. A significant number of aromatic plants such as thyme and oregano produce a monoterpene phenol called Carvacrol. Carvacrol (C10H14O) had various biological and pharmacological characteristics, including antibacterial and antifungal effects (19). Our results indicated that carvacrol showed ≥ 98% more antibacterial effect against *L. acidophilus* than *L. casei.* Two studies by Chandra S. Mathela et. al. (27) in India and Soon-Nang Park et. al. (32) in South Korea evaluated the effect of Carvacrol against *S. mutants* as the most important bacteria that triggers dental caries. We aimed to study the impact of Carvacrol against *Lactobacilli* as a critical contributory bacterium in tooth decay. A study by Machado et. al. indicated that Thymbra capitata essential oil thatconstituted by Carvacrol 75%, showed an MIC level of 2.5 µg/mL against *L. casei* (ATCC393) and *L. acidophilus* (KS400). The results are probably due to the differences in strains, Carvacrol concentration, and the test method.

Disk diffusion test for Carvacrol (19.52 mg/mL) showed significant growth of DIZ against *L. acidophilus* and *L. casei* (Table 1, 2). In a study done by Manconi et al., 15 µL of each thymus essential oil extraction formulation mainly consisted of 817 mg/mL Carvacrol was used for the disk diffusion test. The mean diameter of the inhibition halo of thymus essential oil extraction formulations against *L. acidophilus* (ATCC4365) was 9.6 mm (28). The difference in the diameter of inhibition halo between this study and our experiment was due to the difference in the volume of the Carvacrol (29). Chan et al. dissolved 20 µL/cup of six phenolic-rich extracts and indicated that the inhibition halo was not established

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for *L. acidophilus* (CSCC2400) and *L. casei* (ASCC290) (30). However, in the control groups of Gentamicin (20 µg) and Ampicillin (20 µg), the diameter of inhibition halo of them was measured as 21.3 ± 1.15 and 15 ± 1 mm for *L. acidophilus* and 19.7 ± 0.58 and 15.3 ± 1.15 mm for *L. casei*, respectively. In our study, prefabricated Tetracycline control disks (30 µg) were used, and the measured inhibition halo diameter against *L. acidophilus* and *L. casei* 19 ± were 0.81 and 23.3 ± 2.35 mm, respectively. MIC levels for phenolic-rich extracts for five *lactobacillus* bacteria species were more than 2500 µg/mL. In summary, the extracts used in this study did not have any antibacterial effect on the five groups of lactic acids. Silveria et. al. evaluated the antimicrobial effect of Carvacrol and thymol against *staphylococcus aureus* (MIC of Carvacrol against *staphylococcus aureus* was measured 256 µg/mL). They used tetracycline for the control group (MIC = 128 µg/mL). A combination of Carvacrol and tetracycline reported to have an antagonistic effect (23). Aimmo et. al. studied six *L. acidophilus* strains and six *L. casei* isolated from dairy and pharmaceutical products. The MIC of tetracycline against the *L. casei* and *L. acidophilus* strains were 16 and 4 µg/mL, respectively. Therefore, our antimicrobial material in a smaller volume affected the two strains of bacteria and was more effective than tetracycline (30).

Conclusion

According to the present in-vitro study, Carvacrol can be used as an alternative agent for chemical antimicrobials to encounter *L. acidophilus* and *L. casei*. Further investigations are suggested for evaluating the antimicrobial activity of Carvacrol in the in-vivo studies.

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Conflict of interest

The authors have no conflict of interest in this study.

References

1. Karpiński TM, Szkaradkiewicz AK. Microbiology of dental caries. J Biol Earth Sci 2013;3(1):M21-4.
2. Featherstone J. The continuum of dental caries—evidence for a dynamic disease process. J. Dent. Res 2004;83(1_suppl):39-42.
3. Carminative T. A review on antimicrobial activity of vegetables, herbs and spices against cariogenic bacteria. Res J of Bio 2016;4(4):12-20.
4. Tanzer JM, Livingston J, Thompson AM. The microbiology of primary dental caries in humans. J. Dent. Educ 2001;65(10):1028-37.
5. Staat RH, Gawronski TH, Cressey DE, Harris RS, Folke LE. Effects of dietary sucrose levels on the quantity and microbial composition of human dental plaque. J. Dent. Res 1975;54(4):872-80.
6. Tanzer J, Freedman M, Fitzgerald R, Larson R. Diminished virulence of glucan synthesis-defective mutants of Streptococcus mutans. Infect. Immun 1974;10(1):197-203.
7. Holbrook W, De Soet J, De Graaff J. Prediction of dental caries in pre-school children. Caries Res 1993;27(5):424-30.
8. Ikeda T, Sandham H, Bradley Jr E. Changes in Streptococcus mutans and lactobacilli in plaque in relation to the initiation of dental caries in Negro children. Arch. Oral Biol 1973;18(4):555-66.
9. Brookes ZL, Bescos R, Belfield LA, Ali K, Roberts A. Current uses of chlorhexidine for management of oral disease: a narrative review. J. Dent 2020:103497.
10. Shekar BRC, Nagarajappa R, Suma S, Thakur R. Herbal extracts in oral health care-A review of the current scenario and its future needs. Pharmacogn Rev 2015;9(18):87.
11. Palombo EA. Traditional medicinal plant extracts and natural products with activity against oral bacteria: potential application in the prevention and treatment of oral diseases. Evid. -based Complement. Altern Med 2011;2011.

[DOI: 10.52547/rabms.7.4.172]

12. Bidhendi N, Ahmadi Ashtiani H, Ayatollahi A, Yadangi S, Ghorban Dadras O, Firooz A. Comparison of the efficacy and safety of a herbal extract lotion with clotrimazole 1% lotion in the treatment of seborrheic dermatitis: A randomized clinical trial. J. Cosmet. Dermatol 2017;7(4):189-99.
13. Dias DA, Urban S, Roessner U. A historical overview of natural products in drug discovery. Metabolites 2012;2(2):303-36.
14. Paczesny J, Bielec K. Application of bacteriophages in nanotechnology. Nanomaterials 2020;10(10):1944.
15. Raza S, Matuła K, Karoń S, Paczesny J. Resistance and Adaptation of Bacteria to Non-Antibiotic Antibacterial Agents: Physical Stressors, Nanoparticles, and Bacteriophages. Antibiotics 2021;10(4):435.
16. Onsea J, Post V, Buchholz T, Schwegler H, Zeiter S, Wagemans J, et al. Bacteriophage Therapy for the Prevention and Treatment of Fracture-Related Infection Caused by Staphylococcus aureus: a Preclinical Study. Microbiol. Spectr 2021;9(3):e01736-21.
17. Sun Y, Oliver J. Antimicrobial action of some GRAS compounds against Vibrio vulnificus. Food Addit Contam 1994;11(5):549-58.
18. Gonelimali FD, Lin J, Miao W, Xuan J, Charles F, Chen M, et al. Antimicrobial properties and mechanism of action of some plant extracts against food pathogens and spoilage microorganisms. Front. Microbiol 2018;9:1639.
19. Suntres ZE, Coccimiglio J, Alipour M. The bioactivity and toxicological actions of carvacrol. Crit Rev Food Sci Nutr 2015;55(3):304-18.
20. Tiwari BK, Valdramidis VP, O'Donnell CP, Muthukumarappan K, Bourke P, Cullen P. Application of natural antimicrobials for food preservation. J. Agric. Food Chem 2009;57(14):5987-6000.
21. Kachur K, Suntres Z. The antibacterial properties of phenolic isomers, carvacrol and thymol. . Crit Rev Food Sci Nutr 2020;60(18):3042-53.
22. Magi G, Marini E, Facinelli B. Antimicrobial activity of essential oils and carvacrol, and synergy of carvacrol and erythromycin, against clinical, erythromycin-resistant Group A Streptococci. Front. Microbiol 2015;6:165.
23. Sousa Silveira Zd, Macêdo NS, Sampaio dos Santos JF, Sampaio de Freitas T, Rodrigues dos Santos Barbosa C, Júnior DLdS, et al. Evaluation of the antibacterial activity and efflux pump reversal of thymol and carvacrol against Staphylococcus aureus and their toxicity in drosophila melanogaster. Molecules 2020;25(9):2103.
24. Chan C-L, Gan R-Y, Shah NP, Corke H. Polyphenols from selected dietary spices and medicinal herbs differentially affect common food-borne pathogenic bacteria and lactic acid bacteria. Food Control 2018;92:437-43.
25. Le Bars P, Matamoros S, Montassier E, Le Vacon F, Potel G, Soueidan A, et al. The oral cavity microbiota: between health, oral disease, and cancers of the aerodigestive tractCan. J. Microbiol 2017;63(6):475-92.
26. Badet C, Thebaud N. Ecology of lactobacilli in the oral cavity: a review of literature. Open Microbiol. J 2008;2:38.
27. Mathela CS, Singh KK, Gupta VK. Synthesis and in vitro antibacterial activity of thymol and carvacrol derivatives. Acta Pol Pharm 2010;67(4):375-80.
28. Manconi M, Petretto G, D'hallewin G, Escribano E, Milia E, Pinna R, et al. Thymus essential oil extraction, characterization and incorporation in phospholipid vesicles for the antioxidant/antibacterial treatment of oral cavity diseases. Colloids Surf. B 2018;171:115-22.
29. Machado D, Gaspar C, Palmeira-de-Oliveira A, Cavaleiro C, Salgueiro L, Martinez-de-Oliveira J, et al. Thymbra capitata essential oil as potential therapeutic agent against Gardnerella vaginalis biofilm-related infections. Future Microbiol 2017;12(5):407-16.
30. D'Aimmo MR, Modesto M, Biavati B. Antibiotic resistance of lactic acid bacteria and Bifidobacterium spp. isolated from dairy and pharmaceutical productsInt. J. Food Microbiol 2007;115(1):35-42.

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