Biofilm 7 (2024) 100202

Contents lists available at ScienceDirect

Biofilm

journal homepage: www.sciencedirect.com/journal/biofilm

Effect of mutanase and dextranase on biofilms of cariogenic bacteria: A systematic review of *in vitro* studies

Yumi C. Del Rey [a,*], Hian Parize [b], Sahar Assar [a], Gerd Göstemeyer [c], Sebastian Schlafer [a]

[a] Section for Oral Ecology, Cariology, Department of Dentistry and Oral Health, Aarhus University, Vennelyst Boulevard 9, 8000, Aarhus C, Denmark
[b] Department of Prosthodontics, School of Dentistry, University of São Paulo, São Paulo, SP, Brazil
[c] Department of Operative, Preventive and Pediatric Dentistry, Charité – Universitätsmedizin Berlin, Aßmannshauser Straße 4-6, 14197, Berlin, Germany

ARTICLE INFO

Keywords:
Dental caries
Biofilm matrix
Bacterial adhesion
Enzymes
Dextrans
Extracellular polymeric substance

ABSTRACT

Matrix-degrading enzymes are promising non-biocidal adjuncts to dental biofilm control and caries prevention. By disrupting the biofilm matrix structure, enzymes may prevent biofilm formation or disperse established biofilms without compromising the microbial homeostasis in the mouth. This study reviewed whether treatment with mutanase and/or dextranase inhibits cariogenic biofilm growth and/or removes cariogenic biofilms *in vitro*. An electronic search was conducted in PubMed, EMBASE, Scopus, Web of Science, Cochrane, and LIVIVO databases. Manual searches were performed to identify additional records. Studies that quantitatively measured the effect of mutanase and/or dextranase on the inhibition/removal of *in vitro* cariogenic biofilms were considered eligible for inclusion. Out of 809 screened records, 34 articles investigating the effect of dextranase (n = 23), mutanase (n = 10), and/or combined enzyme treatment (n = 7) were included in the review. The overall risk of bias of the included studies was moderate. Most investigations used simple biofilm models based on one or few bacterial species and employed treatment times ≥30 min. The current evidence suggests that mutanase and dextranase, applied as single or combined treatment, are able to both inhibit and remove *in vitro* cariogenic biofilms. The pooled data indicate that enzymes are more effective for biofilm inhibition than removal, and an overall higher effect of mutanase compared to dextranase was observed.

1. Introduction

Bacterial acid production in biofilms that grow attached to dental surfaces is the prime etiological factor in the onset and progression of dental caries [1]. Dental biofilms consist of structured microbial communities embedded in a self-produced matrix of extracellular polymeric substances, predominantly polysaccharides (e.g. glucans), proteins, and extracellular DNA (eDNA). Caries-related bacteria, in particular *Streptococcus mutans*, are recognized as major producers of matrix polysaccharides that play a key role in bacterial adhesion to tooth surfaces, nutrition, and the mechanical stability of dental biofilms [2]. Due to its diffusion-modifying properties, the polymeric matrix also affects the accumulation of acids inside biofilms and acts as a barrier against buffering salivary ions and antimicrobial agents [3].

Mechanical oral hygiene is the mainstay for caries prevention, since it can effectively disrupt and remove dental biofilms; nonetheless, self-performed mechanical biofilm control is not always successful, especially in hard-to-reach areas of the dentition that are particularly prone to the development of oral diseases [4,5]. Antimicrobial agents, such as chlorhexidine and cetylpyridinium chloride, are commonly used adjuncts to dental biofilm control, but their diffusion and efficacy are limited by the insoluble polymeric matrix [6]. Furthermore, antimicrobial adjuncts indiscriminately kill bacteria in the oral cavity, including health-related species that reside on mucosal surfaces, which can affect the homeostasis of the oral microbiome [7,8]. In this context, enzymes that degrade biofilm matrix components, such as proteins (proteases), eDNA (deoxyribonucleases), lipids (lipases) and glucans (glucanohydrolases), represent a promising non-biocidal strategy for dental caries prevention [9–11]. Through the disruption of the matrix structure, matrix-degrading enzymes may prevent biofilm formation or disperse established biofilms without compromising the microbial balance in the mouth [10].

The most thoroughly studied matrix-degrading enzymes are mutanase (α-1,3 glucan 3-glucanohydrolase) and dextranase (α-1,6 glucan 6-

* Corresponding author. Department of Dentistry and Oral Health, Aarhus University Vennelyst Boulevard 9, DK-8000, Aarhus C, Germany.
E-mail addresses: yumi@dent.au.dk (Y.C. Del Rey), hian.parize@gmail.com (H. Parize), sahar@dent.au.dk (S. Assar), gerd.goestemeyer@charite.de (G. Göstemeyer), sebastians@dent.au.dk (S. Schlafer).

https://doi.org/10.1016/j.bioflm.2024.100202
Received 21 February 2024; Received in revised form 20 May 2024; Accepted 21 May 2024
Available online 22 May 2024

glucanohydrolase), which disrupt mutans and dextrans of the biofilm matrix, respectively [11]. Those glucans represent important components of the oral biofilm matrix and are produced by extracellular microbial glucosyltransferases, enzymes that catalyze the transfer of glucosyl residues from dietary sucrose into new or pre-existing glucan chains [2]. Biologically active forms of mutanase and dextranase are usually extracted from fungi or bacteria and, depending on the microbial source and intrinsic structural properties, they differ in optimal pH, optimal temperature, and stability over time [10,11]. All these factors may affect the efficacy of glucan-degrading enzymes against mutans and dextrans of dental biofilms, and their potential use as caries-preventive therapies. To date, a limited number of clinical studies has been conducted to investigate the effect of dextranases and mutanases against oral biofilms [12–19]. Most studies that assessed the effect of enzyme treatment on biofilms have been performed on *in vitro* biofilm models, and the outcome of these studies has not yet been systematically reviewed. Therefore, the aim of this systematic review was to investigate whether treatment of *in vitro* cariogenic biofilms with mutanase and/or dextranase affects biofilm formation.

2. Materials and methods

2.1. Eligibility criteria

The eligibility criteria for inclusion in the review were defined according to the Population-Intervention-Comparison-Outcome-Study Design (PICOS) approach. Population: biofilms of cariogenic bacteria and/or biofilms grown under cariogenic conditions, i.e. supplied with fermentable sugars. Intervention: treatment of biofilms with exogenous mutanase and/or dextranase. Comparison: non-enzymatic treatment or no treatment. Outcome: biofilm formation (i.e. biofilm prevention or biofilm removal). Study design: *in vitro* studies. Outcome measures included biofilm volume, biofilm thickness, biomass, bacterial area coverage, and number of bacteria. Glucan degradation was considered a secondary outcome.

Exclusion criteria were: (1) studies that only evaluated the effect of glucan-degrading enzymes on planktonic bacteria; (2) studies that did not investigate *in vitro* biofilms of cariogenic bacteria or biofilm formation under cariogenic conditions; (3) studies that did not quantitatively measure the effects of the intervention; (4) studies that solely analyzed the degradation of extracellular matrix components; (5) reviews, letters, conference abstracts, personal opinions, case reports, technique articles, hypothesis articles, and studies published in languages other than English.

2.2. Information sources and search strategy

Individual search strategies were developed for the following electronic databases: PubMed, EMBASE, Scopus, Web of Science, Cochrane, and LIVIVO (Table S1). The electronic searches were performed in November 2023 and limited to records in English, but no restriction for the year of publication was applied. Hand searches were also carried out to identify additional eligible studies.

2.3. Selection process

All retrieved records from electronic and hand searches were managed and stored in Endnote 20 software and Rayyan application [20]. After the removal of duplicates, two reviewers (Y.C.D.R. and H.P.) independently screened the title and abstract of the identified records. The full texts of potentially eligible studies were retrieved for a final independent assessment by the two reviewers. Titles and abstracts with insufficient information to assess eligibility were also included in the full-text analysis, to avoid exclusion of potentially relevant studies. The screening process and eligibility assessment were performed using Rayyan application and Endnote 20 software. Disagreements were discussed and resolved by consensus or, if persistent, by the decision of a third independent reviewer (S.S.).

2.4. Data collection and data items

Two independent reviewers (Y.C.D.R and H.P.) collected the data from the included articles using Excel® (Microsoft Office 2017) spreadsheets elaborated specifically for this study. The extracted data included: author names, year of publication, enzyme type and source, enzyme concentration, treatment regimen (time point, frequency and duration), control treatment, biofilm age, model and growth conditions, methodologies for outcome assessment, and main results. Accuracy of the extracted data was checked by a third reviewer (S.A.). When necessary, the authors of the studies were contacted to obtain additional information. Missing or unclear information were extracted as “not reported”. Qualitative data was not extracted and quantitative results that were not reported with numerical values were extracted as “values: not reported”.

2.5. Risk of bias assessment

The risk of bias of the included studies was assessed by two independent reviewers (Y.C.D.R. and S.A.) using an adapted tool elaborated according to previous systematic reviews of *in vitro* studies [21–23]. Disagreements were resolved by consensus or by the decision of a third reviewer (S.S). The following items were included in the assessment: (1) presence of a control group; (2) replication (independent experiments); (3) standardization and description of treatment regimen (enzyme concentration, treatment frequency and duration); (4) standardization and description of biofilm growth conditions (inoculation procedures, atmospheric and nutritional conditions, biofilm age); (5) standardization and description of biofilm quantification (method, sampling procedure); (6) blinding of operator/assessor; (7) description of statistical analysis. Qualitative methods for outcome assessment were not included in the risk of bias judgement. For each study, the items received a “Y” (yes) if the information was described by the authors or by a referred study, or a “N” (no) if the information was unclear, not reported or incomplete. The risk of bias was classified according to the total number of “Y’s” received by each study, as follows: 0 to 3 = high; 4 to 5 = medium; 6 to 7 = low risk of bias.

3. Results

3.1. Study selection

The electronic and manual searches retrieved 1730 records, which were reduced to 809 after the removal of duplicates (n = 921). The screening of titles and abstracts identified 79 studies that potentially fulfilled the eligibility criteria, and 70 were retrieved for full-text analysis. After full-text reading, 36 studies were excluded (Table S2) and 34 were included in the review. The selection process is summarized in Fig. 1.

3.2. Study characteristics

The main characteristics of the included studies are presented in Table 1. The studies were published from 2004 to 2023, and evaluated the effect of dextranase (23 studies) [25–47], mutanase (ten studies) [25,39,44,48–54], or combined mutanase and dextranase treatment (seven studies) [25,39,44,55–58] on biofilm formation. Mutanase was obtained from fungi in six studies [44,48,53,55,57,58] and from bacteria in six studies [25,49,50–52,56], while fungal and bacterial dextranase were used in 12 [31,32,37,38,40,44,46,47,55–58] and 14 investigations [25–28,30,33–36,38,41–43,45], respectively. Some reports evaluated the effect of enzymes obtained from more than one source organism. Ren et al. (2018) compared a bacterial to a fungal

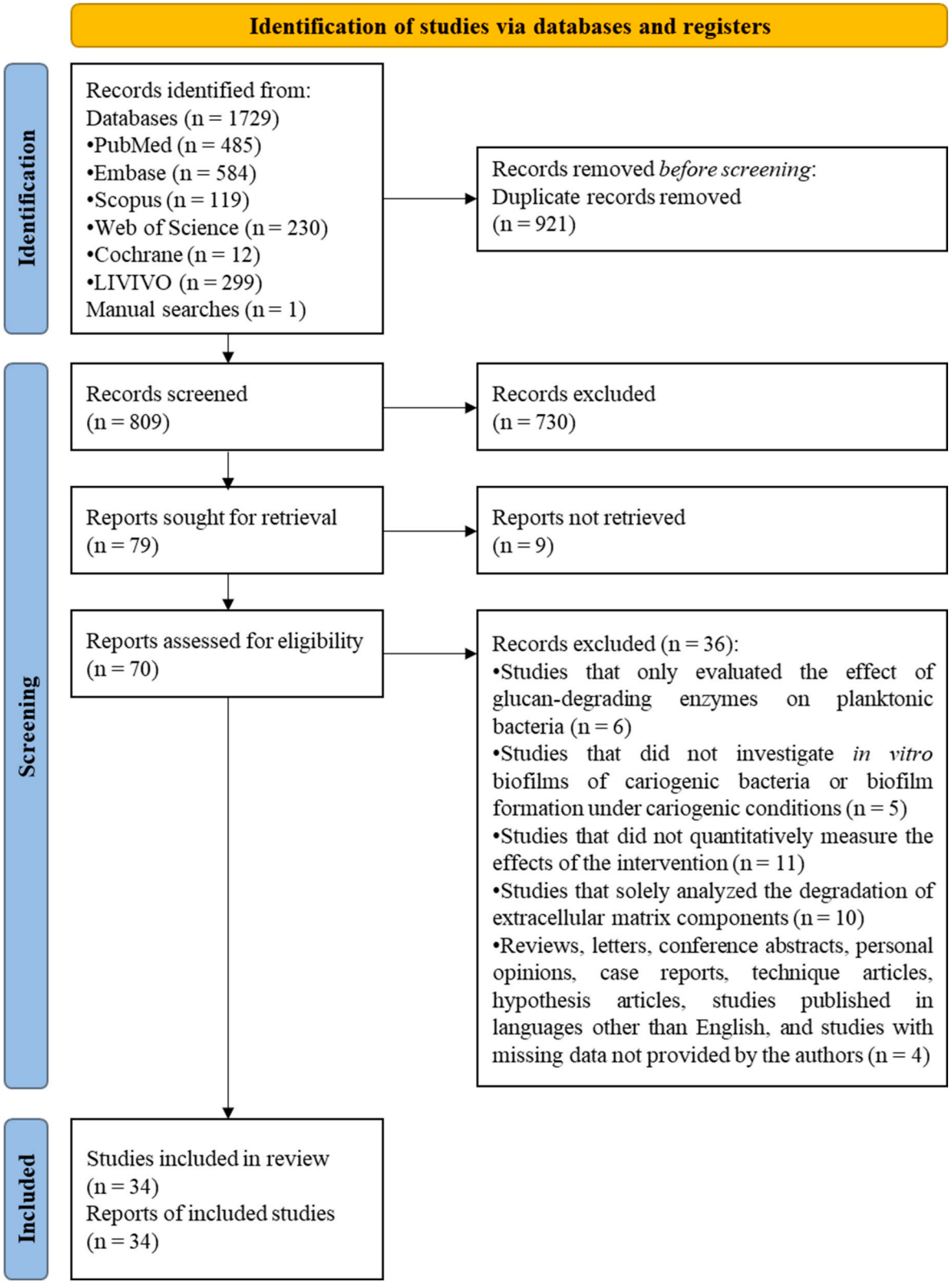

Fig. 1. Flow chart of the screening process (PRISMA 2020) [24].

dextranase [38]. Singh et al. (2021) compared the effect of plant-derived mutanase and dextranase produced via chloroplast genome to commercial fungal and bacterial enzymes [56]. Three studies did not report the enzyme source [29,39,54]. Enzyme concentrations were difficult to compare between studies, as some authors specified the concentration in g/L or mol/L, whereas others provided the enzyme activity in U/g or U/mL. Two reports did not specify the used enzyme concentration [41, 48] and in some studies it was unclear whether the provided concentration referred to the stock solution or the final volume used for biofilm treatment.

Biofilm inhibition (i.e. the prevention of biofilm formation) was the outcome of six studies [31–33,35,38,42], while biofilm removal was

Table 1
Main characteristics of the included studies.

Author, year	Enzyme	Enzyme source	Enzyme concentration	Outcome	Treatment	Control treatment	Biofilm model; growth medium	Surface	Methods for biofilm quantification[e]	Main results for biofilm inhibition (percent reduction)	Main results for biofilm removal (percent reduction)
Bem, 2023 [48]	Mutanase	Fungus *Trichoderma harzianum*	NR	Biofilm removal	Pre-formed 72 h-old biofilms enzyme-treated 2x/day (1 min) for 2 days	Saline; inactivated enzyme	*S. mutans;* TYEB with 0.1 μM glucose	Glass (slide)	CFU	NA	No significant differences between enzyme group and the controls (values: NR).
Boddapati, 2023 [49]	Mutanase	Bacterium *Cellulosimicrobium funkei* SNG1	2.3 U/mL	Biofilm removal	Pre-formed 72 h-old biofilms enzyme-treated for 2 h	No treatment[a]	*S. mutans;* BHI with 5 % sucrose (w/v)	Glass (coverslip)	A_{550}	NA	82.7 %
Cherdvorapong, 2020 [50]	Mutanase	Bacterium *Streptomyces thermodiastaticus* HF3-3	0.01 U/mL	Biofilm inhibition; Biofilm removal	Biofilms grown in the presence of mutanase for 4–16 h; Pre-formed 16 h-old biofilms enzyme-treated for 4–16 h	Enzyme buffer	*S. mutans*; BHI with 1 % sucrose (w/v)	Glass (plate)	Alcian blue staining	12 h: 65–79 %; 16 h: ~50 %	8 h: 60 %; 16 h: 40 %
Cortez, 2023 [25]	Mutanase; Dextranase	Mutanase: Bacterium *Prevotella melaninogenica* PmGH87; Dextranase: Bacterium *Capnocytophaga ochracea* CoGH66	0.125–1 mg/mL	Biofilm inhibition; Biofilm removal	Biofilms grown in the presence of mutanase or dextranase for 24 h; Pre-formed 24 h-old biofilms enzyme-treated for 30 min-24 h	Enzyme buffer (biofilm inhibition); PBS (biofilm removal)	*S. mutans*; TYEB with 0.1 % or 1 % sucrose (w/v)	NR (96-well plate)	Crystal violet staining	Biofilms grown with 0.1 % sucrose: Mutanase or dextranase: ~80 %; Biofilms grown with 1 % sucrose: Mutanase 90 %, dextranase 55 %	Biofilms grown with 0.1 % sucrose: Mutanase (2 h): 97.4 %, dextranase (4 h): 92 %, mutanase + dextranase (30 min): 95.5 %; Biofilms grown with 1 % sucrose: Mutanase (24 h): 92 %, dextranase (24 h) 85.7 %, mutanase + dextranase (2 h): 93.8 %
Deng, 2020 [26]	Dextranase	Bacterium *Catenovolum* sp. DP03	1–8 U/mL	Biofilm inhibition; Biofilm removal	Biofilms grown in the presence of dextranase for 24 h; Pre-formed 24 h-old biofilms enzyme-treated for 24 h	No treatment	*S. mutans*; BHI with 1 % sucrose (w/v)	Glass (coverslip)	Crystal violet staining	$MBIC_{50}$: 4 U/mL; $MBIC_{90}$: 8 U/mL; 4 U/mL: 52.9 %; 8 U/mL: 90.7 %	$MBRC_{50}$: 4 U/mL; $MBRC_{90}$: 8 U/mL; 4 U/mL: 53.8 %; 8 U/mL: 90.8 %
Ding, 2020 [27]	Dextranase	Bacterium *Arthrobacter oxydans* KQ11	359.7 U/g	Biofilm removal	Pre-formed 24-h old biofilms enzyme-treated (free enzyme or immobilized on HA nanoparticles) for 1 h	Pure water	*S. mutans*; BHI with 1 % sucrose (w/v)	NR (96-well plate)	Crystal violet staining	NA	Free enzyme: 14.3 %, Immobilized enzyme: 86.4 %
Dong, 2021 [28]	Dextranase	Bacterium *Bacillus aquimaris* S5	2–10 U/mL	Biofilm removal	Pre-formed 24 h-old biofilms enzyme-treated for 6 h	No treatment	*S. mutans*; BHI	NR (96-well plate)	Crystal violet staining	NA	$MBRC_{80}$: 8 U/mL; 2 U/mL: 41.9 % (3.5 % SD); 4 U/mL: 49.7 % (1.7

(*continued on next page*)

Table 1 (*continued*)

Author, year	Enzyme	Enzyme source	Enzyme concentration	Outcome	Treatment	Control treatment	Biofilm model; growth medium	Surface	Methods for biofilm quantification[e]	Main results for biofilm inhibition (percent reduction)	Main results for biofilm removal (percent reduction)
											% SD); 6 U/mL: 52.2 % (2.9 % SD); 8 U/mL: 82.9 % (5.0 % SD); 10 U/mL: 84.2 % (4.2 % SD)
Hwang, 2014 [29]	Dextranase	NR	0.71 U/mL	Biofilm removal	Pre-formed 67 h- and 115 h-old biofilms enzyme-treated for 30 min	Enzyme buffer	*S. mutans*; TYEB with 0.1 % sucrose (w/v)	Saliva-coated HA (disk)	Mechanical stability test; biofilm viscoelasticity test	NA	Shear stress needed to remove 50 % of the biofilm: Dextranase: 0.027 N/m^2, control: 0.184 N/m^2; Storage modulus G': significantly reduced compared to control (values: NR)
Jiao, 2014 [30]	Dextranase	Bacterium *Arthrobacter* sp.	1–10 U/mL	Biofilm inhibition; Biofilm removal	Biofilms grown in the presence of dextranase for 3–24 h; Pre-formed 24 h-old biofilms enzyme-treated for 24 h.	No treatment	*S. mutans*; BHI with 1 % sucrose (w/v)	Glass (coverslip); polystyrene (microtiter plate)	Crystal violet staining; biofilm mass (dry weight); SEM	$MBIC_{50}$: 2 U/mL; $MBIC_{90}$: 6 U/mL; 6 U/mL (24 h): 90 %; Biofilm mass (24 h): Dextranase: 0.6 $\mu g/mm^2$ (1.2 SD), control: 12.3 $\mu g/mm^2$ (1.7 SD); Biofilm coverage (24 h): Dextranase: ~10 %, control: ~100 %	$MBRC_{50}$: 3–7 U/mL; 6 U/mL (24 h): 60 %
Juntarachot, 2020a [31]	Dextranase	Fungus *Penicillium roquefortii* TISTR 351	0.71 U/g	Biofilm inhibition	Biofilms grown in the presence of dextranase for 24 h	No treatment	*S. mutans*; TSB	NR (96-well plate); glass (slide)	Crystal violet staining	OD_{570}: Dextranase: 0.7 (0.0 SD), control 1.0 (0.0 SD)	NA
Juntarachot, 2020b [32]	Dextranase	Fungus *Chaetomium gracile*	3.38 U/g	Biofilm inhibition	Biofilms grown in the presence of dextranase for 24 h	No treatment	*S. mutans*; TSB	NR (96-well plate); glass (slide)	Crystal violet	OD_{570}: Dextranase: 0.5 (0.0 SD), control: 1.0 (0.0 SD)	NA
Lai, 2019 [33]	Dextranase	Bacterium *Catenovolum agarivorans* MNH15	1–7 U/mL	Biofilm inhibition	Biofilms grown in the presence of dextranase for 24 h	No treatment	*S. mutans*; BHI with 1 % sucrose (w/v)	NR (microplate)	Crystal violet staining	$MBIC_{50}$: 3 U/mL; $MBIC_{90}$: 7 U/mL; 3 U/mL: 52.3 %; 7 U/mL: 91.8 %	NA

(*continued on next page*)

Table 1 (*continued*)

Author, year	Enzyme	Enzyme source	Enzyme concentration	Outcome	Treatment	Control treatment	Biofilm model; growth medium	Surface	Methods for biofilm quantification[e]	Main results for biofilm inhibition (percent reduction)	Main results for biofilm removal (percent reduction)
Liu, 2016 [55]	Mutanase; Dextranase	Mutanase: Fungus *Trichoderma harzianum*; Dextranase: Fungus *Penicillium* sp.	Mutanase: 20 U (volume: NR); Dextranase: 100 U (volume: NR)	Biofilm removal	Pre-formed 19 h-old biofilms treated with mutanase + dextranase for up to 1 h	Enzyme buffer	*S. mutans*; TYEB with 1 % sucrose (w/v)	Saliva-coated HA (disk)	CLSM, CFU	NA	No significant differences in cell viability and CFU counts between enzyme group and control (values: NR).
Liu, 2021 [34]	Dextranase	Bacterium *Streptococcus mutans*	1–500 nM	Biofilm inhibition; Biofilm removal	Biofilms grown in the presence of dextranase for 12 h; Pre-formed 12 h-old biofilms enzyme-treated for 30 min	No treatment	*S. mutans*; BHI with 1 % sucrose (w/v)	NR (96-well plate); glass (tube)	Crystal violet staining; biofilm mass (dry weight); CLSM	$MBIC_{50}$: 1 nM; 50 nM: 92.0 %; Biofilm mass: 90.0 % (5.9 % SD); Viable cells: 86.7 % (6.3 % SD); Biofilm thickness: significant reduction (values: NR)	50 nM: 43 % (2 % SD); 500 nM: 77 % (2 % SD)
Mahmoud, 2022 [35]	Dextranase	Bacterium *Bacillus velezensis*; Bacterium *Pseudomonas stutzeri*	28.5 and 30.19 mU/mL	Biofilm inhibition	Biofilms grown in the presence of dextranase for 24 h	No treatment	*S. mutans*; TSB	NR (96-well microtiter plate)	Crystal violet staining	*B. velezensis* dextranase: 82.5 %; *P. stutzeri* dextranase: 84.5 %	NA
Ning, 2021 [36]	Dextranase	Bacterium *Cellulosimicrobium* sp. PX02	3–15 U/mL	Biofilm inhibition; Biofilm removal	Biofilms grown in the presence of dextranase for 24 h. Pre-formed 24 h-old biofilms enzyme-treated for 4 h or 24 h	No treatment	*S. mutans*; BHI with 1 % sucrose (w/v)	NR (microplate); glass (coverslip)	Crystal violet staining; SEM	Dextranase: reduced biofilm growth in a dose-dependent manner (values: NR)	9 U/mL: 64.5 %; 15 U/mL: 93.1 %
Pleszczynska, 2010 [51]	Mutanase	Bacterium *Paenibacillus* sp. MP-1	0.01–2 U/mL	Biofilm removal	Pre-formed biofilms grown for 24 h, enzyme-treated for 3 min, and further grown for 6 h in the presence of enzyme or enzyme buffer	NR	*S. sobrinus/downei*; BHI with 3 % sucrose (w/v)	Glass (tube)	Biofilm mass (dry weight)	NA	2 U/mL (3 min): ~60 %; 2 U/mL (6 h): 64 %
Qiu, 2016 [37]	Dextranase	Fungus *Penicillium* sp.	1, 2 and 4 U/mL	Biofilm removal	Pre-formed 24-h old biofilms enzyme-treated 2x/day (1 min) for 3 days	Saline	*S. mutans*, *Lactobacillus acidophilus*, and *Actinomyces viscosus*; BHI with 1 % sucrose (w/v)	Glass (slide)	Biofilm mass (dry weight); CFU; CLSM	NA	Biofilm mass: 1 U/mL: no reduction (values: NR); 2 and 4 U/mL: significant reduction (values: NR); CFU: 1 and 2 U/mL: no reduction (values: NR); 4 U/mL:

(*continued on next page*)

Table 1 (*continued*)

Author, year	Enzyme	Enzyme source	Enzyme concentration	Outcome	Treatment	Control treatment	Biofilm model; growth medium	Surface	Methods for biofilm quantification[e]	Main results for biofilm inhibition (percent reduction)	Main results for biofilm removal (percent reduction)
											significant reduction (values: NR); Biofilm thickness: 1, 2 and 4 U/mL: significant reduction (values: NR); Cell viability: not affected (values: NR).
Ren, 2018 [38]	Dextranase;	Bacterium *Catenovulum* sp.; Fungus *Penicillium* sp.	5–40 U/mL	Biofilm inhibition	Biofilms grown in the presence of dextranase for 3, 9 and 18 h	No treatment	*S. mutans*; BHI with 1 % sucrose (w/v)	Polypropylene (96-well plate)	Crystal violet staining	*Catenovulum* sp. dextranase: $MBIC_{90}$: 30 U/mL; 30 U/mL: 91.1 %; *Penicillium* sp. dextranase: $MBIC_{90}$: 40 U/mL 40 U/mL: 89.3 %	NA
Ren, 2019 [39]	Mutanase; Dextranase	NR	Mutanase: 1.75 U/mL; Dextranase: 8.75 U/mL	Biofilm removal	Pre-formed 19 h-old biofilms enzyme-treated for 30 min, 1 h, or 2 h	Enzyme buffer	*S. mutans*; TYEB with 1 % sucrose (w/v).	Saliva-coated HA (disk)	CFU; biofilm mass (dry weight)	NA	CFU: Mutanase + dextranase (2 h): No reduction (values: NR); Biofilm mass: Mutanase or dextranase (2 h): No reduction (values: NR), Mutanase + dextranase (2 h): Significant reduction (values: NR)
Rikvold, 2023 [54]	Mutanase	NR	62 μM/mL	Biofilm inhibition; Biofilm removal	Biofilms grown in the presence of mutanase for 24 h Pre-formed 24 h-old biofilms enzyme-treated for 30 min under agitation (150 rpm).	Enzyme buffer	Salivary inoculum; BHI with 5 % sucrose (w/v) and sterile saliva	Polystyrene (96-well microplate, RTCA electronic plates)	Crystal violet staining, biofilm adhesion and stability (RTCA)	96.8 % (95 % CI: 70.2–123.4 %). RTCA: reduced mechanical stability of enzyme-treated biofilms compared to control (values: NR)	60.3 % (95 % CI: 43.3–75.2 %)
Shimotsuura, 2008 [52]	Mutanase	Bacterium *Paenibacillus* sp. RM1	NR-1.4 U/mL	Biofilm removal	Pre-formed 18 h-old biofilms enzyme-treated for 3 min and further incubated with buffer for 6 h	NR	*S. mutans*; BHI with 1 % sucrose (w/v)	Glass (tube)	A_{550}	NA	1.4 U/mL: 98 %
Singh, 2021 [56]	Mutanase; Dextranase	Mutanase, Dextranase: Plant-derived via	Mutanase: 0.84 or 105 U/mL;	Biofilm removal	Pre-formed 6 h-old biofilms treated with	Enzyme buffer	*S. mutans* and *Candida albicans*;	Saliva-coated and enzyme-	CFU; biofilm mass (dry weight); CLSM	NA	Biovolume: Commercial mutanase/dextranase

(*continued on next page*)

Table 1 (*continued*)

Author, year	Enzyme	Enzyme source	Enzyme concentration	Outcome	Treatment	Control treatment	Biofilm model; growth medium	Surface	Methods for biofilm quantification[e]	Main results for biofilm inhibition (percent reduction)	Main results for biofilm removal (percent reduction)
		chloroplast genome; Dextranase: Fungus *Penicillium* sp.; Mutanase: Bacterium *Bacillus* sp.	Dextranase: 7.08 or 525 U/mL		mutanase + dextranase for 1 h and allowed to continue growing until 19 h		TYEB with 1 % sucrose (w/v)	treated (1 h) HA (disk)			(0.84/7.08 U/mL): Significant reduction in total and bacterial, but not in fungal biovolume (values: NR), Plant-derived mutanase/dextranase (0.84/7.08 U/mL): Significant reduction in total and bacterial, but not in fungal biovolume (values: NR), Plant-derived mutanase/dextranase (105/525 U/mL): No reduction in total, bacterial or fungal biovolume (values: NR); Total Biofilm Inhibition index[b]: No significant reduction, Plant-derived mutanase/dextranase (105/525): 0.67, control: 1.0.
Tsutsumi, 2018 [40]	Dextranase	Fungus *Chaetomium erraticum*	10, 20 and 40 U (volume: NR)	Biofilm removal	Pre-formed 24 h-old biofilms enzyme-treated for 3 min	No treatment	*S. gordonii, S. mutans, Actinomyces naeslundii, Fusobacterium nucleatum, Veillonella parvula*; artificial saliva medium with 0 % or 1 % sucrose (w/v)	Polystyrene (24-well plate)	Crystal violet staining	NA	Removal was significantly higher for biofilms grown with 1 % sucrose compared to biofilms grown without sucrose at all enzyme concentrations (values: NR)
Wang D, 2014 [41]	Dextranase	Bacterium *Arthrobacter oxydans* KQ11	NR	Biofilm removal	Pre-formed 4 h-old biofilms enzyme-treated (duration: NR) and further incubated for 5 days	No treatment	*S. mutans* or biofilms of *S. mutans*, *S. salivarius*, *S. sanguinis*, *Lactobacillus*[c], *Actinomyces viscosus*; BHI with 1 % sugar[d]	NR (coverslip)	CLSM	NA	Thickness: *S. mutans* biofilms: Dextranase: 36670 nm, control 54340 nm, Multispecies biofilms: Dextranase: 43320 nm, control: 64260 nm
Wang X, 2014 [42]	Dextranase	Bacterium *Arthrobacter oxydans* KQ11	1–8 U/mL	Biofilm inhibition	Biofilms grown in the presence of dextranase for 24 h.	No treatment	*S. mutans;* BHI with 1 % sucrose (w/v)	NR (coverslip)	SEM	5 U/mL: ~90 %	NA

(*continued on next page*)

Table 1 (*continued*)

Author, year	Enzyme	Enzyme source	Enzyme concentration	Outcome	Treatment	Control treatment	Biofilm model; growth medium	Surface	Methods for biofilm quantification[e]	Main results for biofilm inhibition (percent reduction)	Main results for biofilm removal (percent reduction)
Wang X, 2016 [43]	Dextranase	Bacterium *Arthrobacter oxydans* KQ11	1–9 U/mL	Biofilm inhibition; Biofilm removal	Biofilms grown in the presence of dextranase for 24 h. Pre-formed 24 h-old biofilms enzyme-treated for 24 h or 2x/day (5 min) for 5 days.	No treatment (biofilm inhibition); sterile saline (biofilm removal)	*S. mutans;* BHI with 1 % sucrose (w/v)	NR (coverslip); glass (tube)	Crystal violet staining; cell adherence (OD_{550}); CLSM	Adherence (2 U/mL): 50 % $MBIC_{50}$: 2 U/mL; 2 U/mL: 53.8 % 6 U/mL: 90 %	$MBRC_{50}$: 5 U/mL; 5 U/mL: 53.5 % Thickness (5 U/mL): 54.5 μm (control), 36.67 μm (dextranase).
Wiater, 2004 [44]	Mutanase; Dextranase	Mutanase: Fungus *Trichoderma harzianum* CCM F-340; Dextranase: Fungus *Penicillium* sp.	Mutanase: 0.3 U/mL; Dextranase: 5 U/mL	Biofilm inhibition; Biofilm removal	Biofilms grown in the presence of mutanase, dextranase, or a mixture of both enzymes for 24 h. Pre-formed 24 h-old biofilms enzyme-treated for 24 h	No treatment	*S. mutans, S. sobrinus,* and oral sample from a volunteer; sucrose-containing BHI	Glass (plate)	Erythrosine staining	Mutanase: 96 %; Dextranase: 61 %; Mutanase + dextranase: 99.5 %	Mutanase: 85 %; Dextranase: 71 %; Mutanase + dextranase: 95 %
Wiater, 2008 [57]	Mutanase; Dextranase	Mutanase: Fungus *Trichoderma harzianum* CCM F-340; Dextranase: Fungus *Penicillium* sp.	Mutanase: 0.25 U/mL; Dextranase: 1 U/mL	Biofilm removal	Pre-formed 24 h-old treated with mutanase + dextranase for 3 and 6 h	NR	*S. mutans, S. sobrinus, S. sobrinus/downei, Candida albicans*; sucrose-containing BHI	Saliva-coated glass (plate)	Erythrosine staining	NA	Mutanase + dextranase (3 h): 86.6 %, (6 h): ~100 %
Wiater, 2013a [58]	Mutanase; Dextranase	Mutanase: Fungus *Trichoderma harzianum CCM F-340*; Dextranase: Fungus *Penicillium* sp.	Mutanase: 0.25 U/mL; Dextranase: 1 U/mL	Biofilm removal	Pre-formed 24 h-old biofilms treated with mutanase + dextranase for 1 and 3 h	No treatment	*S. mutans, S. sobrinus, S. sobrinus/downei, Candida albicans*; BHI	Saliva-coated glass (coupon)	Erythrosine staining	NA	Mutanase + dextranase (1 h): 63.4 %; (3 h): 81.7 %
Wiater, 2013b [53]	Mutanase	Fungus *Trichoderma harzianum* CCM F-340	1 U/mL	Biofilm removal	Pre-formed biofilms (age: NR) enzyme-treated 3x/day (3 min) for 3 days	PBS	*S. mutans, S. sobrinus, S. sobrinus/downei, Candida albicans;* BHI with 3 % sucrose (w/v)	Saliva-coated glass (coupon)	Erythrosine staining	NA	~30 %
Xu, 2022 [45]	Dextranase	Bacterium *Cellulosimicrobium* sp. THN1	2–10 U/mL	Biofilm inhibition; Biofilm removal	Biofilms grown in the presence of dextranase for 24 h; Pre-formed 24 h-old biofilms enzyme-treated (treatment duration: NR)	Deionized water	*S. mutans*; BHI with 1 % sucrose (w/v)	NR (microplate)	Crystal violet staining	$MBIC_{50}$: 6 U/mL; $MBIC_{90}$: 10 U/mL; 6 U/mL: 52.3 %; 10 U/mL: 91.6 %	$MBRC_{50}$: 4 U/mL; $MBRC_{90}$: 10 U/mL; 4 U/mL: 53.4 %; 8 U/mL: 90.6 %
Yang, 2019 [46]	Dextranase	Fungus *Chaetomium globosum*	10–70 U/mL	Biofilm inhibition; Biofilm removal	Biofilms grown in the presence of dextranase for 24 h; Pre-formed 24 h-	No treatment	*S. mutans*; BHI with 1 % sucrose (w/v)	NR (96-well plate)	Crystal violet staining	50 U/mL: 71.6 %	50 U/mL: 49.1 %

(*continued on next page*)

Table 1 (*continued*)

Author, year	Enzyme	Enzyme source	Enzyme concentration	Outcome	Treatment	Control treatment	Biofilm model; growth medium	Surface	Methods for biofilm quantification[e]	Main results for biofilm inhibition (percent reduction)	Main results for biofilm removal (percent reduction)
					old biofilms enzyme-treated for 24 h						
Yano, 2010 [47]	Dextranase	Fungus *Chaetomium globosum*	120,000 U/mL	Biofilm inhibition; Biofilm removal	Biofilms grown in the presence of dextranase (0.125 % v/v) for 16 h. Pre-formed 16 h-old biofilms treated with dextranase (0.25 % v/v) for 12 h	No treatment	*S. mutans* or *S. sobrinus*; BHI with 0.5 % sucrose (w/v)	Polystyrene (culture plate)	Crystal violet staining	Dextranase + BSA: *S. mutans*: 37 % (14 % SD), *S. sobrinus*: 42 % (16 % SD)	Dextranase + BSA: *S. mutans* or *S. sobrinus*: 1 % (0 % SD)

A550 = absorbance read at 550 nm; BHI = brain heart infusion; BSA = bovine serum albumin; CHX = chlorhexidine; CLSM = confocal laser scanning microscopy; HA = hydroxyapatite; MBIC50 = minimal enzyme concentration to inhibit more than 50 % of biofilm formation; MBIC90 = minimal enzyme concentration to inhibit more than 90 % of biofilm formation; MBRC50 = minimal enzyme concentration to remove more than 50 % of pre-formed biofilms; MBRC80 = minimal enzyme concentration to remove more than 80 % of pre-formed biofilms; MBRC90 = minimal enzyme concentration to remove more than 90 % of pre-formed biofilms; NA = not applicable; NR = not reported; OD570 = optical density read at 570 nm; OD550 = optical density read at 550 nm; PBS = phosphate buffered saline; rpm = rotations per minute; RTCA = SD = standard deviation; *S. gordonii* = *Streptococcus gordonii*; *S. mutans* = *Streptococcus mutans*; *S. oralis* = *Streptococcus oralis*; *S. salivarius* = *Streptococcus salivarius*; *S. sanguinis* = *Streptococus sanguinis*; *S. sobrinus/downei* = *Streptococcus sobrinus/downei*; TSB = tryptic soy broth; TYEB = tryptone yeast extract broth.

[a] No treatment: no enzyme, buffer or water was added to the control samples.

[b] Calculated as inhibition rate of fungal CFU x inhibition rate of bacterial CFU x inhibition rate of dry weight.

[c] Unspecified species of Lactobacillus.

[d] Unspecified type of sugar.

[e] Methods for biofilm quantification: methods used for qualitative analyses are not reported.

investigated by 16 studies [27–29,37,39–41,48,49,51–53,55–58]. Twelve reports compared the effect of enzymes on both outcomes [25,26,30,34,36,43–47,50,54]. Biofilm age ranged from 3 to 24 h in inhibition studies, with the majority of biofilms being grown for 24 h with the enzymes present in the growth medium. Most frequently, the control group was not exposed to any treatment in inhibition studies, which means that no water, buffer or heat-inactivated enzyme was added to the control samples. One study used deionized water as the control [45], and three studies used enzyme buffer [25,50,54].

Biofilm removal studies exhibited a greater degree of variation regarding the study design and treatment regimen. Pre-formed biofilms were grown from 4 to 115 h prior to enzyme treatment, with most studies growing biofilms for 24 h. Ding et al. (2020) was the only study to treat pre-formed biofilms with enzymes immobilized on hydroxyapatite (HA) nanoparticles [27]; all other investigations applied enzymes in solution as a single treatment for periods varying from 3 min to 24 h [25,26,28–30,34,36,39–41,44,46,47,49–52,54–58], or as pulsed treatments with durations between 1 and 5 min [37,43,48,53]. After enzyme treatment, the biofilms were immediately prepared for analysis, or further incubated [41,51,52,56] for periods ranging from 6 h to five days. Rikvold et al. (2023) was the only study to apply agitation during enzyme treatment [54]. Four articles did not fully report the treatment protocol [41,45] or specify the control treatment [52,57]. Most often, the control samples received no treatment [26,34,36,40,41,44,58,28,30,46,47,49], followed by treatment with enzyme buffer [29,39,50,55,56,54], saline [37,43,48], water [27,45], or PBS [25,53]. Bem et al. (2023) was the only removal study to use heat-inactivated enzymes as one of the controls [48], while Plesczynska et al. (2010) [51] did not describe a control group that was not exposed to mutanase.

The majority of studies used *S. mutans* biofilms grown in the presence of sucrose as a cariogenic biofilm model [25–27,29,30,33,34,36,38,39,42,43,45–47,49,50,52,55]. Single-species biofilms of *Streptococcus sobrinus* were employed by Pleszczynska et al. (2010) [51] and Yano et al. (2010) [47]. Multispecies biofilms grown in the presence of sucrose were used as biofilm models in eight studies [37,40,44,53,54,56,57]; these included a variety of different acidogenic bacteria (e.g. *S. mutans*, *S. sobrinus*, *Lactobacillus acidophilus*, *Actinomyces viscosus*, *Streptococcus salivarius*) and fungi (e.g. *Candida albicans*), as well as other typical colonizers of the oral cavity (e.g. *Fusobacterium nucleatum*, *Veillonella parvula*). Eight studies did not supplement the biofilm growth media with sucrose [28,31,32,35,40,48,58] or did not specify the type of sugar added [41]. Many reports did not specify the surface the biofilms were grown on, only the type of device used for biofilm formation (e.g. microtiter plates). The most commonly employed surface was uncoated glass [26,30–32,34,36,37,43,44,48–52], followed by polymers (e.g. polystyrene or polypropylene) [30,38,40,47,54]. Some studies used saliva-coated glass [53,57,58] or saliva-coated HA [29,39,55,56].

Different biofilm quantification methods were reported in the included articles. Crystal violet staining was the most common method and was used in 18 studies [25–28,30–36,38,40,43,45–47,54]. Some studies employed other colorimetric analyses like alcian blue [50] or erythrosine staining [44,53,57,58]. Absorbance [49,52] or optical density [43] measurements performed without the aid of colorimetric agents were employed in three investigations. Biofilm mass (dry weight) was quantified in six studies [30,34,37,39,51,56], while colony-forming unit (CFU) counts were used by five investigations [37,55,39,48,56]. One study investigated the biofilm adhesion and stability using impedance-based real-time cell analysis (RTCA) [54]. Some reports performed quantitative analyses of images obtained by scanning electron microscopy (SEM) [30,36,42] or confocal laser scanning microscopy (CLSM) [34,37,41,43,55,56], but in most reports, microscopy images were only used for qualitative analyses (data not extracted). Hwang et al. (2014) was the only study to use mechanical stability and viscoelasticity tests as a measure of the effect of enzymes on biofilm removal [29].

3.3. Risk of bias

The risk of bias assessment resulted in six studies judged to be at high risk [36,41,42,45,51,57], twenty three at medium risk [26,27,30–35,37–40,43,44,46,48–50,52,53,55,56,58], and five at low risk [25,28,29,47,54] of bias (Table 2). The most frequent items of the risk of bias tool that contributed to the judgement were the blinding of operator/-assessor (item 6), which was not reported by any study, and the description of statistical analysis (item 7; judged as "N" in 22 articles), as illustrated in Fig. 2.

3.4. Results of individual studies

A summary of the results of the included studies is presented in Fig. 3.

3.4.1. Inhibition of biofilm formation

The inhibition of biofilm formation by mutanase was investigated by four studies, two of them with medium [44,50] and two with low risk [25,54] of bias. Of the four studies, only two analyzed the treatment effect statistically [25,54]. Cortez et al. (2023) [25] found mutanase to significantly reduce *S. mutans* biofilm formation by 80–90 % after 24 h, while Rikvold et al. (2023) [54] reported 97 % inhibition of a multispecies biofilm grown for 24 h in the presence of mutanase compared to buffer control. Similarly, Wiater et al. (2004) [44] observed 96 % reduction of biofilm formation in a multispecies model after 24 h. Cherdvorapong et al. (2020) [50] reported an inhibition of *S. mutans* biofilms grown for 4–16 h in the presence of one of two types of recombinant mutanases, rAglST1 and rAglST2, which were added to the growth medium. The authors reported that both mutanases had a similar biofilm inhibition effect, which was highest after 12 h of incubation (65–79 %) [50].

Sixteen studies evaluated the effect of dextranase on biofilm inhibition [25,26,30–36,38,42–47], and all of them reported reductions (30%–92 % for the highest applied concentration) in biofilm formation. Two studies received a low risk of bias assessment [25,47]. Yano et al. (2010) [47] reported dextranase to significantly inhibit biofilm formation of *S. mutans* or *S. sobrinus* by 37–42 % after 16 h. Cortez et al., 2023 [25] found 80 % inhibition of *S. mutans* biofilm formation after 24 h when the medium was supplemented with 0.1 % sucrose. Interestingly, the effect decreased to 55 % when the sucrose concentration was increased to 1 %. The majority of studies was assessed to have a medium risk of bias [26,30–35,38,43,44,46], whereas three reports received a high risk of bias judgement [36,42,45]. Out of those, three investigations did not include sucrose in the biofilm growth medium [31,32,35]. The highest observed treatment effects ranged from 61 to 92 % and 30–84 % for studies that did and did not provide sucrose during biofilm growth, respectively.

Two studies compared the effect of mutanase and dextranase, and reported a higher inhibitory effect of mutanase compared to dextranase (96 % vs. 61 % [44]; 90 % vs. 55 % [25]). Only one investigation [44] evaluated the effect of combined treatment with mutanase and dextranase on biofilm inhibition. The effect considerably exceeded the inhibitory effect of dextranase, but not of mutanase (mutanase: 96 %; dextranase: 61 %; combined treatment: 99 %) [44].

3.4.2. Removal of established biofilms

The removal of pre-formed biofilms by mutanase was investigated by ten studies [25,39,44,48,49,50–54]. The studies by Rikvold et al. (2023) [54] and Cortez et al. (2023) [25] were classified as having a low risk of bias and reported significant removal of 60% and 92%–97%, respectively, of 24 h-old biofilms. The studies classified as having medium [39,44,48–50,52,53] or high [51] risk of bias also reported biofilm removal by mutanase (30%–98 %), but only two of them [39,48] performed a statistical analysis of the results. Bem et al. (2023) [48] found no significant difference between treatment with active and heat-inactivated

Table 2
Risk of bias assessment of the included studies.

Author, year	Item 1	Item 2	Item 3	Item 4	Item 5	Item 6	Item 7	Risk of bias
Bem, 2023	Y	Y	N	Y	Y	N	Y	Medium
Boddapati, 2023	Y	Y	Y	Y	Y	N	N	Medium
Cherdvorapong, 2020	Y	Y	Y	Y	Y	N	N	Medium
Cortez, 2023	Y	Y	Y	Y	Y	N	Y	Low
Deng, 2020	Y	Y	Y	Y	Y	N	N	Medium
Ding, 2020	Y	N	Y	Y	Y	N	N	Medium
Dong, 2021	Y	Y	Y	Y	Y	N	Y	Low
Hwang, 2014	Y	Y	Y	Y	Y	N	Y	Low
Jiao, 2014	Y	Y	Y	Y	Y	N	N	Medium
Juntarachot, 2020a	Y	Y	Y	Y	Y	N	N	Medium
Juntarachot, 2020b	Y	Y	Y	Y	Y	N	N	Medium
Lai, 2019	Y	Y	Y	Y	Y	N	N	Medium
Liu, 2016	Y	Y	N	Y	N	N	Y	Medium
Liu, 2021	Y	Y	N	Y	Y	N	Y	Medium
Mahmoud, 2022	Y	Y	Y	Y	Y	N	N	Medium
Ning, 2021	Y	N	Y	Y	N	N	N	High
Pleszczynska, 2010	N	Y	Y	N	Y	N	N	High
Qiu, 2016	Y	Y	Y	Y	N	N	Y	Medium
Ren, 2018	Y	N	Y	Y	Y	N	N	Medium
Ren, 2019	Y	N	Y	Y	Y	N	Y	Medium
Rikvold, 2023	Y	Y	Y	Y	Y	N	Y	Low
Shimotsuura, 2008	Y	Y	N	Y	Y	N	N	Medium
Singh, 2021	Y	N	Y	Y	Y	N	Y	Medium
Tsutsumi, 2018	Y	Y	N	Y	Y	N	Y	Medium
Wang D, 2014	Y	N	N	Y	N	N	N	High
Wang X, 2014	Y	N	Y	Y	N	N	N	High
Wang X, 2016	Y	Y	Y	Y	Y	N	N	Medium
Wiater, 2004	Y	Y	Y	Y	Y	N	N	Medium
Wiater, 2008	N	Y	N	Y	Y	N	N	High
Wiater, 2013a	Y	Y	Y	Y	Y	N	N	Medium
Wiater, 2013b	Y	Y	Y	Y	N	N	N	Medium
Xu, 2022	Y	N	N	Y	Y	N	N	High
Yang, 2019	Y	N	Y	Y	Y	N	N	Medium
Yano, 2010	Y	Y	Y	Y	Y	N	Y	Low

"Y" (yes) = information was described by the authors; **"N" (no)** = information was unclear, not reported or incomplete.
Item 1 = Presence of a control group.
Item 2 = Replication (independent experiments).
Item 3 = Standardization and description of treatment regimen (enzyme concentration, treatment frequency and duration).
Item 4 = Standardization and description of biofilm growth conditions (inoculation procedures, atmospheric and nutritional conditions, biofilm age).
Item 5 = Standardization and description of biofilm quantification (method, sampling procedure).
Item 6 = Blinding of operator/assessor.
Item 7 = Description of statistical analysis.

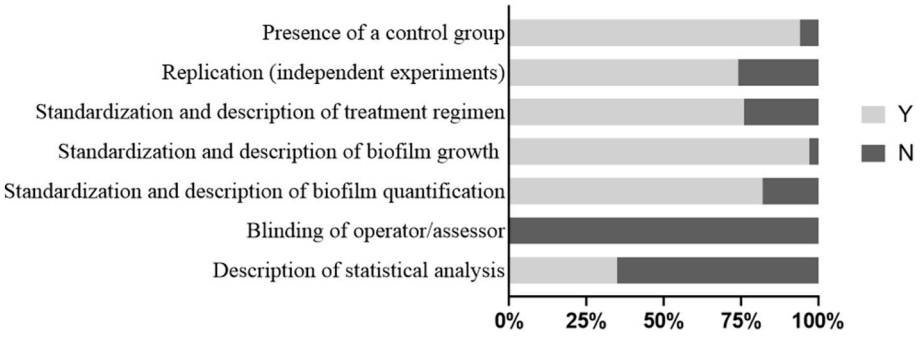

Fig. 2. Distribution of scores (Y: information was described, N: information was unclear, not reported, or incomplete) for each item of the risk of bias tool for the included studies.

enzymes against *S. mutans* biofilms grown with glucose as carbohydrate source. Similarly, Ren et al. (2019) [39] reported no significant reduction in biomass of mutanase-treated *S. mutans* biofilms grown in the presence of sucrose compared to the control.

Sixteen studies evaluated the effect of dextranase on the removal of established biofilms [25–30,34,36,37,39–41,43–45,47], and the reported results ranged from 1 % to 93 % biofilm removal. Four studies were classified as having a low risk of bias [25,28,29,47]. Hwang et al. (2014) [29] found that the mechanical stability of *S. mutans* biofilms was significantly reduced after 30 min of dextranase treatment, whereas Yano et al. (2010) [47] reported no significant removal of *S. mutans* or *S. sobrinus* biofilms by dextranase treatment (1 % reduction). Dong et al. (2021) [28] reported 84 % removal of *S. mutans* biofilms after 6 h of treatment, but the biofilm model was grown without added sucrose to the media. One study [25] compared the removal effect of dextranase against *S. mutans* biofilms grown with 0.1% or 1% sucrose, and reported a higher susceptibility of biofilms grown in lower sucrose concentrations (92% vs. 86% removal, respectively). The remaining studies were

A) Biofilm inhibition effect of mutanase

Author, year	0-50%	51-70%	71-89%	≥ 90%
Cherdvorapong, 2020				
Cortez, 2023				
Rikvold, 2023				
Wiater, 2004				

B) Biofilm inhibition effect of dextranase

Author, year	0-50%	51-70%	71-89%	≥ 90%
Cortez, 2023				
Deng, 2020				
Jiao, 2014				
Wang X, 2014				
Wang X, 2016				
Wiater, 2004				
Ren, 2018				
Juntarachot, 2020a				
Juntarachot, 2020b				
Lai, 2019				
Liu, 2021				
Mahmoud, 2022				
Xu, 2022				
Yang, 2019				
Yano, 2010				

C) Biofilm inhibition effect of mutanase + dextranase

Author, year	0-50%	51-70%	71-89%	≥ 90%
Wiater, 2004				

D) Biofilm removal effect of mutanase

Author, year	0-50%	51-70%	71-89%	≥ 90%
Boddapati, 2023				
Cortez, 2023				
Cherdvorapong, 2020				
Rikvold, 2023				
Shimotsuura, 2008				
Pleszcynska, 2010				
Wiater, 2004				
Wiater, 2013b				

E) Biofilm removal effect of dextranase

Author, year	0-50%	51-70%	71-89%	≥ 90%
Cortez, 2023				
Deng, 2020				
Ding, 2020				
Dong, 2021				
Hwang, 2014				
Jiao, 2014				
Liu, 2021				
Ning, 2021				
Wang D, 2014				
Wang X, 2016				
Wiater, 2004				
Xu, 2022				
Yang, 2019				
Yano, 2010				

F) Biofilm removal effect of mutanase + dextranase

Author, year	0-50%	51-70%	71-89%	≥ 90%
Cortez, 2023				
Singh, 2021				
Wiater, 2004				
Wiater, 2008				
Wiater, 2013a				

Low risk of bias Medium risk of bias High risk of bias

Fig. 3. Highest biofilm inhibition (A–C) and removal (D–F) effects of mutanase and/or dextranase observed by the included studies and their respective risk of bias. Studies where the quantitative results were not reported with numerical values and could not be extracted are not listed.

classified as having a medium [26,27,30,43,44,46,37,39,40] or a high [36,41,45] risk of bias. Qiu et al. (2016) [37] observed a significant biofilm mass removal after short pulsed treatment (1 min, 2x/day) of multispecies biofilms grown in the presence of 1 % sucrose. Tsutsumi et al. (2019) [40] applied a single 3-min treatment to 24 h-old multispecies biofilms, and found a limited effect of dextranase against biofilms grown in salivary medium without sucrose. Interestingly, biofilm removal was significantly higher when sucrose was present during growth [40]. Wang D. et al. (2014) [41] reported a reduction in the thickness of both *S. mutans* and multispecies biofilms after dextranase treatment, but the treatment regimen was not specified. Ren et al. (2019) [39] observed no significant reduction in the biomass of *S. mutans* biofilms after 2 h of dextranase treatment, whereas several other studies reported moderate (49–60%) [30,43,46] to high (71–93%) [26,34,36,44,45] removal effects of dextranase using similar biofilm models. One study [27] reported an increased effect of dextranase immobilized on hydroxyapatite particles compared to free enzyme in solution (86 % vs. 14 %), but did not analyze the results statistically.

The biofilm removal effect of combined mutanase and dextranase treatment was evaluated by seven studies [25,39,44,55–58], one of them with a low risk of bias [25]. Cortez et al. (2023) [25] reported that combined treatment removed 94–95 % of pre-formed biofilms compared to 92–97 % for mutanase and 86–92 % for dextranase alone. The remaining studies were classified was having a medium [39,44,55,56,58] or a high [57] risk of bias. Ren et al. (2019) [39] observed a significant reduction in biofilm mass after combined treatment with mutanase and dextranase, but not when the enzymes were applied individually. Wiater et al. (2004) [44] also compared the effect of combined and individual enzyme treatment, and reported a higher removal rate when both enzymes were applied simultaneously (combination: 95 %, mutanase: 85 %, dextranase: 71 %). Wiater et al. (2008) [57] and Wiater et al. (2013a) [58] both reported a time-dependent effect of combined mutanase and dextranase treatment, which ranged from 63 % (1 h) to almost 100 % (6 h), but in the latter study the media was not supplemented with sucrose, and in both studies no statistical analysis was performed. Three studies found no differences in CFU counts [39,55] or total biofilm inhibition index [56] between enzymatic and control treatment.

3.4.3. *Inhibition vs. removal of biofilms*

Twelve studies compared the effect of enzymes on biofilm inhibition and removal [25,26,30,34,36,43–47,50,54]. In most studies, both

mutanase [44,50,54] and dextranase [30,34,43,45,46,47] were more effective in preventing biofilm formation than in removing established biofilms, whereas Deng et al. (2020) [26] found a similar effect of dextranase for *S. mutans* biofilm inhibition and removal (91 %). Interestingly, Wiater et al. (2004) [44] reported a higher effect of dextranase for the removal of pre-formed biofilms (71 %) than for the inhibition of biofilm formation (61 %), whereas the opposite trend was observed for mutanase (inhibition: 96 %, removal: 85 %). Yano et al. (2010) [47] found that dextranase had no significant effect in removing pre-formed biofilms (1 %), but that it significantly inhibited biofilm formation (37–42 %). Cortez et al. (2023) [25] was the only study that reported a higher effect of both mutanase and dextranase on the dispersal of pre-formed 24 h biofilms than on the prevention of biofilm formation.

4. Discussion

Enzymatic therapy for the removal of microbial biofilms has recently gained increased attention in a variety of fields, such as the medical and food industries [59]. In the context of oral health, matrix-degrading enzymes, in particular mutanase and dextranase, have been considered promising non-biocidal adjuncts to biofilm control and dental caries prevention. This systematic review evaluated the current evidence regarding the effect of mutanase and/or dextranase on the inhibition and/or removal of *in vitro*-grown cariogenic biofilms. A total of 34 articles were included in this review, and the pooled data indicate that mutanase and dextranase, applied as single or combined treatment, are able to both inhibit biofilm formation and remove established biofilms *in vitro*. Most studies found a higher effect of the enzymes on biofilm inhibition than on removal [30,34,43–47,50,54], which can be explained by a longer exposure and an easier access of mutanase and/or dextranase to their targets, as the enzymes are present from the beginning of biofilm formation and matrix production in inhibition experiments. Overall, mutanase exhibited a stronger biofilm inhibition and removal effect than dextranase, which suggests an important role of mutans (α-1,3 glucans) in biofilm stability. However, most studies were conducted using *S. mutans* biofilm models, which have a particularly high abundance of water-insoluble glucans rich in α-1,3 glucan, the specific target for mutanase [60]. Only one study [44] included in this review compared the effect of mutanase and dextranase in a multispecies biofilm model, but there, too, a higher inhibition and removal effect was found for mutanase. The few studies that compared single and combined enzyme treatment reported an increased effect of the latter on biofilm formation [25,39,44]. This apparent synergistic effect shows that both matrix components (mutans and dextrans) contribute to adhesion and/or cohesion and thereby biofilm stability.

Several studies found that the enzyme effect increased in a time- and dose-dependent manner, until it reached a plateau. Most inhibition studies applied the enzymes for longer periods, typically 24 h, while common treatment times in removal studies spanned from 30 min to 24 h. Such extended treatment times may be difficult to achieve in a clinical setting, but a few studies tested short treatment periods of 1–3 min and still reported significant biofilm removal [37,40]. All studies that compared different enzyme concentrations found improved effects for higher concentrations, but comparisons between individual studies were not possible, due to a considerable heterogeneity in the reporting of enzyme concentrations/activities. In general, the reports failed to provide all the details required by the Standards for Reporting Enzyme Data (STRENDA) commission, such as the assay conditions and enzyme activity calculations [61].

Due to the high heterogeneity among the included studies, it was not possible to perform a meta-analysis. The main differences in study design included the employed biofilm model, treatment regimen, and outcome measures. Furthermore, enzymes are a very heterogeneous class of therapeutics compared to other oral adjuncts, such as fluoride or arginine. Many different source organisms and production methods can be used to obtain biologically active enzymes, and those significantly affect enzyme specificity, activity and stability, as well as the optimal pH and temperature range [10,11].

Most studies included in this review were conducted on simple biofilm models, composed of one or a very limited number of bacterial species. The microbial composition of biofilms grown intraorally is significantly more diverse, and so is their biofilm matrix, which has been shown to comprise of many distinct carbohydrate components [62,63], eDNA [64], lipids and proteins [65]. It therefore remains unclear if treatment with mutanase and/or dextranase can achieve significant effects in a clinical context. Furthermore, few investigations mimicked a clinically relevant treatment regimen, and in some studies, no sucrose was present during biofilm growth [28,31,32,35,40,48,58], or the type of sugar added to the growth medium was not specified [41]. In the absence of sucrose, alternative substrates can potentially act as donor molecules for streptococcal glucosyltransferases [66,67]; nonetheless, sucrose remains the primary and optimal substrate for glucan synthesis [2]. Consequently, studies that did not include sucrose in the growth medium may not accurately reflect the typical glucan production of oral biofilms and hence the effect of glucan-degrading enzymes.

The majority of studies in this review were classified as having a medium risk of bias. Strikingly, the lack of a statistical analysis or the incomplete reporting of important elements of the statistical analysis, such as the employed statistical test and the adopted level of significance, were among the most frequent shortcomings of the reports (Fig. 2). Moreover, none of the investigations reported blinding of the investigators, which may introduce significant bias in the assessment of treatment effects. Operator/assessor blinding is routinely performed in most clinical trials [68] and also advocated by the Checklist for Reporting *In-vitro* Studies (CRIS) guidelines [69], but rarely implemented in laboratory studies. The findings from this review are in line with other systematic reviews of *in vitro* work, which also reported the complete absence of investigator blinding in all included studies [21–23]. Another concern that is especially relevant in the context of *in vitro* studies is a publication bias towards positive outcomes. Studies with non-significant or inconclusive results are less likely to be published, which may lead to an overrepresentation of positive findings in the current literature. It is, however, virtually impossible to assess the extent of such bias for laboratory studies.

The scope of this review was limited to investigating the sole effect of mutanase and/or dextranase treatment on biofilm inhibition and removal. As enzymes seek to disrupt the biofilm matrix and thereby both increase the permeability and reduce the mechanical stability of biofilms [6,29], their action could be employed to enhance the effect of other therapeutic approaches, like antimicrobial treatment, mechanical oral hygiene, or fluoride supplementation. Some of the studies included in this review combined enzyme treatment with other methods of biofilm control and reported favorable results for combination treatments [34,37,39,47,55], but the results of supplementary treatments were not extracted in this review.

5. Conclusions

The pooled data suggest that mutanase and dextranase, applied as single enzymes or combined treatment, are able to both inhibit and remove *in vitro* cariogenic biofilms; however, most included studies were classified as having a medium risk of bias, and no meta-analysis could be performed due to a considerable heterogeneity between studies. A higher reporting standard and practices to minimize the introduction of bias, such as blinding of the operators, the conduction and proper description of independent experiments (biological replicates), as well as the use of biofilm models that better mimic natural biofilms are needed in future *in vitro* studies on enzymes.

6. Other information

6.1. Protocol and registration

This systematic review was conducted in accordance with the Preferred Reporting Items for systematic Reviews and Meta-analyses guidelines (PRISMA 2020) [24]. The review protocol was registered at the Open Science Framework (OSF) database and is publicly available at https://osf.io/tq8ah.

CRediT authorship contribution statement

Yumi C. Del Rey: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Hian Parize:** Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis. **Sahar Assar:** Writing – review & editing, Investigation, Formal analysis. **Gerd Göstemeyer:** Writing – review & editing, Visualization, Methodology, Conceptualization. **Sebastian Schlafer:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

This study was supported by the Faculty of Healthy, Aarhus University, Denmark.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioflm.2024.100202.

References

[1] Nyvad B, Takahashi N. Integrated hypothesis of dental caries and periodontal diseases. J Oral Microbiol 2020;12:1710953. https://doi.org/10.1080/20002297.2019.1710953.
[2] Bowen WH, Koo H. Biology of Streptococcus mutans-derived glucosyltransferases: role in extracellular matrix formation of cariogenic biofilms. Caries Res 2011;45:69–86. https://doi.org/10.1159/000324598.
[3] Koo H, Falsetta ML, Klein MI. The exopolysaccharide matrix: a virulence determinant of cariogenic biofilm. J Dent Res 2013;92:1065–73. https://doi.org/10.1177/0022034513504218.
[4] Hujoel PP, Hujoel MLA, Kotsakis GA. Personal oral hygiene and dental caries: a systematic review of randomised controlled trials. Gerodontology 2018;35:282–9. https://doi.org/10.1111/ger.12331.
[5] Stein C, Santos NML, Hilgert JB, Hugo FN. Effectiveness of oral health education on oral hygiene and dental caries in schoolchildren: systematic review and meta-analysis, Community Dent. Oral Epidemiol 2018;46:30–7. https://doi.org/10.1111/cdoe.12325.
[6] Takenaka S, Trivedi HM, Corbin A, Pitts B, Stewart PS. Direct visualization of spatial and temporal patterns of antimicrobial action within model oral biofilms. Appl Environ Microbiol 2008;74:1869–75. https://doi.org/10.1128/AEM.02218-07.
[7] Bescos R, Ashworth A, Cutler C, Brookes ZL, Belfield L, Rodiles A, Casas-Agustench P, Farnham G, Liddle L, Burleigh M, White D, Easton C, Hickson M. Effects of Chlorhexidine mouthwash on the oral microbiome. Sci Rep 2020;10:5254. https://doi.org/10.1038/s41598-020-61912-4.
[8] Al-Kamel A, Baraniya D, Al-Hajj WA, Halboub E, Abdulrab S, Chen T, Al-Hebshi NN. Subgingival microbiome of experimental gingivitis: shifts associated with the use of chlorhexidine and N-acetyl cysteine mouthwashes. J Oral Microbiol 2019;11:1608141. https://doi.org/10.1080/20002297.2019.1608141.
[9] Okshevsky M, Regina VR, Meyer RL. Extracellular DNA as a target for biofilm control. Curr Opin Biotechnol 2015;33:73–80. https://doi.org/10.1016/j.copbio.2014.12.002.
[10] Pleszczyńska M, Wiater A, Bachanek T, Szczodrak J. Enzymes in therapy of biofilm-related oral diseases. Biotechnol Appl Biochem 2017;64:337–46. https://doi.org/10.1002/bab.1490.
[11] Pleszczyńska M, Wiater A, Janczarek M, Szczodrak J. (1→3)-α-D-Glucan hydrolases in dental biofilm prevention and control: a review. Int J Biol Macromol 2015;79:761–78. https://doi.org/10.1016/j.ijbiomac.2015.05.052.
[12] Caldwell RC, Sandham HJ, Mann WV, Finn SB, Formicola AJ. 1. The effect of a dextranase mouthwash on dental plaque in young adults and children. J Am Dent Assoc 1971;82:124–31. https://doi.org/10.14219/jada.archive.1971.0023.
[13] Lobene RR. 2. A clinical study of the effect of dextranase on human dental plaque. J Am Dent Assoc 1971;82:132–5. https://doi.org/10.14219/jada.archive.1971.0014.
[14] Keyes PH, Hicks MA, Goldman M, McCabe RM, Fitzgerald RJ. 3. Dispersion of dextranous bacterial plaques on human teeth with dextranase. J Am Dent Assoc 1971;82:136–41. https://doi.org/10.14219/jada.archive.1971.0016.
[15] Murayama Y, Wada H, Hayashi H, Uchida T, Yokomizo E. Effects of dextranase from Spicaria violaceae (IFO 6120) on the polysaccharides produced by oral streptococci and on human dental plaque. J Dent Res 1973;52:658–67. https://doi.org/10.1177/00220345730520040401.
[16] Inoue M, Yakushiji T, Mizuno J, Yamamoto Y, Tanii S. Inhibition of dental plaque formation by mouthwash containing an endo-alpha-1, 3 glucanase. Clin Prev Dent 1990;12:10–4.
[17] Juntarachot N, Sivamaruthi BS, Sirilun S, Tongpong P, Sittiprapaporn P, Kantachote D, Chaiyasut C. An introductory report on the effect of use of dextranase-containing mouthwash on oral health status of human volunteers. Asian J Med Sci 2019;11:22–5. https://doi.org/10.3126/ajms.v11i1.26499.
[18] Kelstrup J, Holm-Pedersen P, Poulsen S. Reduction of the formation of dental plaque and gingivitis in humans by crude mutanase, Scand. J Dent Res 1978;86:93–102. https://doi.org/10.1111/j.1600-0722.1978.tb00613.x.
[19] Nyman S, Lindhe J, Janson JC. The effect of a bacterial dextranase on human dental plaque formation and gingivitis development. Odontol Revy 1972;23:243–52.
[20] Ouzzani M, Hammady H, Fedorowicz Z, Elmagarmid A. Rayyan-a web and mobile app for systematic reviews. Syst Rev 2016;5:210. https://doi.org/10.1186/s13643-016-0384-4.
[21] Wang C, Shi Y-F, Xie P-J, Wu J-H. Accuracy of digital complete dentures: a systematic review of in vitro studies. J Prosthet Dent 2021;125:249–56. https://doi.org/10.1016/j.prosdent.2020.01.004.
[22] Maske TT, van de Sande FH, Arthur RA, Huysmans MCDNJM, Cenci MS. In vitro biofilm models to study dental caries: a systematic review. Biofouling 2017;33:661–75. https://doi.org/10.1080/08927014.2017.1354248.
[23] Bohrer TC, Fontana PE, Lenzi TL, Soares FZM, Rocha RdO. Can endodontic irrigating solutions influence the bond strength of adhesives to coronal dental substrates? A systematic review and meta-analysis of in vitro studies. J Adhesive Dent 2018;20:481–94. https://doi.org/10.3290/j.jad.a41633.
[24] Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, Shamseer L, Tetzlaff JM, Akl EA, Brennan SE, Chou R, Glanville J, Grimshaw JM, Hróbjartsson A, Lalu MM, Li T, Loder EW, Mayo-Wilson E, McDonald S, McGuinness LA, Stewart LA, Thomas J, Tricco AC, Welch VA, Whiting P, Moher D. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. Syst Rev 2021;10:89. https://doi.org/10.1186/s13643-021-01626-4.
[25] Cortez AA, de Queiroz MX, de Oliveira Arnoldi Pellegrini V, Pellegrini VOA, de Mello Capetti CC, Dabul ANG, Liberato MV, Pratavieira S, Ricomini Filho AP, Polikarpov I. Recombinant Prevotella melaninogenica α-1,3 glucanase and Capnocytophaga ochracea α-1,6 glucanase as enzymatic tools for in vitro degradation of S. mutans biofilms. World J Microbiol Biotechnol 2023;39:357. https://doi.org/10.1007/s11274-023-03804-z.
[26] Deng T, Feng Y, Xu L, Tian X, Lai X, Lyu M, Wang S. Expression, purification and characterization of a cold-adapted dextranase from marine bacteria and its ability to remove dental plaque. Protein Expr Purif 2020;174:105678. https://doi.org/10.1016/j.pep.2020.105678.
[27] Ding Y, Zhang H, Wang X, Zu H, Wang C, Dong D, Lyu M, Wang S. Immobilization of dextranase on nano-hydroxyapatite as a recyclable catalyst. Materials 2020;14:130.
[28] Dong D, Wang X, Deng T, Ning Z, Tian X, Zu H, Ding Y, Wang C, Wang S, Lyu M. A novel dextranase gene from the marine bacterium Bacillus aquimaris S5 and its expression and characteristics. FEMS (Fed Eur Microbiol Soc) Microbiol Lett 2021;368. https://doi.org/10.1093/femsle/fnab007.
[29] Hwang G, Klein MI, Koo H. Analysis of the mechanical stability and surface detachment of mature Streptococcus mutans biofilms by applying a range of external shear forces. Biofouling 2014;30:1079–91. https://doi.org/10.1080/08927014.2014.969249.
[30] Jiao YL, Wang SJ, Lv MS, Jiao BH, Li WJ, Fang YW, Liu S. Characterization of a marine-derived dextranase and its application to the prevention of dental caries. J Ind Microbiol Biotechnol 2014;41:17–26. https://doi.org/10.1007/s10295-013-1369-0.
[31] Juntarachot N, Kantachote D, Peerajan S, Sirilun S, Chaiyasut C. Optimization of fungal dextranase production and its antibiofilm activity, encapsulation and stability in toothpaste. Molecules 2020;25. https://doi.org/10.3390/molecules25204784.
[32] Juntarachot N, Sirilun S, Kantachote D, Sittiprapaporn P, Tongpong P, Peerajan S, Chaiyasut C. Anti-Streptococcus mutans and anti-biofilm activities of dextranase

and its encapsulation in alginate beads for application in toothpaste. PeerJ 2020;8. https://doi.org/10.7717/peerj.10165.
[33] Lai X, Liu X, Deng T, Feng Y, Tian X, Lyu M, Wang S. The marine catenovulum agarivorans MNH15 and dextranase: removing dental plaque. Mar Drugs 2019;17. https://doi.org/10.3390/md17100592.
[34] Liu N, Li X, Wang M, Zhang F, Wang C, Zhang K, Wang H, Xu S, Hu W, Gu L. DexA70, the truncated form of a self-produced dextranase, effectively disrupts Streptococcus mutans biofilm. Front Microbiol 2021;12. https://doi.org/10.3389/fmicb.2021.737458.
[35] Mahmoud S, Gaber Y, Khattab RA, Bakeer W, Dishisha T, Ramadan MA. The inhibitory effect of dextranases from Bacillus velezensis and Pseudomonas stutzeri on Streptococcus mutans biofilm, Iran. J Microbiol 2022;14:850–62. https://doi.org/10.18502/ijm.v14i6.11260.
[36] Ning Z, Dong D, Tian X, Zu H, Lyu M, Wang S. Alkalic dextranase produced by marine bacterium Cellulosimicrobium sp. PX02 and its application. J Basic Microbiol 2021;61:1002–15. https://doi.org/10.1002/jobm.202100310.
[37] Qiu YX, Mao MY, Jiang D, Hong X, Yang YM, Hu T. Co-operative effect of exogenous dextranase and sodium fluoride on multispecies biofilms. J Dent Sci 2016:41–7.
[38] Ren W, Cai R, Yan W, Lyu M, Fang Y, Wang S. Purification and characterization of a biofilm-degradable dextranase from a marine bacterium. Mar Drugs 2018;16. https://doi.org/10.3390/md16020051.
[39] Ren Z, Kim D, Paula AJ, Hwang G, Liu Y, Li J, Daniell H, Koo H. Dual-targeting approach degrades biofilm matrix and enhances bacterial killing. J Dent Res 2019;98:322–30. https://doi.org/10.1177/0022034518818480.
[40] Tsutsumi K, Maruyama M, Uchiyama A, Shibasaki K. Characterisation of a sucrose-independent in vitro biofilm model of supragingival plaque. Oral Dis 2018;24:465–75. https://doi.org/10.1111/odi.12779.
[41] Wang DL, Lu MS, Wang SJ, Jiao YL, Li WJ, Zhu Q, Liu ZP. Purification and characterization of a novel marine Arthrobacter oxydans KQ11 dextranase. Carbohydr Polym 2014;106:71–6.
[42] Wang XB, Lu MS, Wang SJ, Fang YW, Wang DL, Ren W, Zhao GM. The atmospheric and room-temperature plasma (ARTP) method on the dextranase activity and structure. Int J Biol Macromol 2014;70:284–91.
[43] Wang XB, Cheng HX, Lu MS, Fang YW, Jiao YL, Li WJ, Zhao GM, Wang SJ. Dextranase from Arthrobacter oxydans KQ11-1 inhibits biofilm formation by polysaccharide hydrolysis. Biofouling 2016;32:1223–33.
[44] Wiater A, Szczodrak J, Rogalski J. Hydrolysis of mutan and prevention of its formation in streptococcal films by fungal alpha-D-glucanases. Process Biochem 2004;39:1481–9.
[45] Xu L, Zhang Y, Liu N, Wei Z, Wang Z, Wang Y, Wang S. Purification and characterization of cold-adapted and salt-tolerant dextranase from Cellulosimicrobium sp. THN1 and its potential application for treatment of dental plaque. Front Microbiol 2022;13:1012957. https://doi.org/10.3389/fmicb.2022.1012957.
[46] Yang L, Zhou ND, Tian YP. Characterization and application of dextranase produced by Chaetomium globosum mutant through combined application of atmospheric and room temperature plasma and ethyl methyl sulfone. Process Biochem 2019;85:116–24.
[47] Yano A, Kikuchi S, Yamashita Y, Sakamoto Y, Nakagawa Y, Yoshida Y. The inhibitory effects of mushroom extracts on sucrose-dependent oral biofilm formation. Appl Microbiol Biotechnol 2010;86:615–23. https://doi.org/10.1007/s00253-009-2323-y.
[48] Bem JSP, Lacerda NGS, Polizello ACM, Cabral H, da Rosa-Garzon NG, Aires CP. Mutanase from Trichoderma harzianum inductively produced by mutan: short-term treatment to degrade mature Streptococcus mutans biofilm. Curr Microbiol 2023;80:312. https://doi.org/10.1007/s00284-023-03417-7.
[49] Boddapati S, Gummadi SN. Production and application of purified mutanase from novel Cellulosimicrobium funkei SNG1 in the in vitro biofilm degradation. Biotechnol Appl Biochem 2023;70:1371–83. https://doi.org/10.1002/bab.2446.
[50] Cherdvorapong V, Panti N, Suyotha W, Tsuchiya Y, Toyotake Y, Yano S, Wakayama M. Prevention of oral biofilm formation and degradation of biofilm by recombinant α-1,3-glucanases from streptomyces thermodiastaticus hf3-3. J Gen Appl Microbiol 2020;66:256–64. https://doi.org/10.2323/jgam.2019.11.003.
[51] Pleszczyńska M, Wiater A, Szczodrak J. Mutanase from Paenibacillus sp. MP-1 produced inductively by fungal α-1,3-glucan and its potential for the degradation of mutan and Streptococcus mutans biofilm. Biotechnol Lett 2010;32:1699–704. https://doi.org/10.1007/s10529-010-0346-1.
[52] Shimotsuura I, Kigawa H, Ohdera M, Kuramitsu HK, Nakashima S. Biochemical and molecular characterization of a novel type of mutanase from Paenibacillus sp. strain RM1: identification of its mutan-binding domain, essential for degradation of Streptococcus mutans biofilms. Appl Environ Microbiol 2008;74:2759–65. https://doi.org/10.1128/AEM.02332-07.
[53] Wiater A, Pleszczynska M, Rogalski J, Szajnecka L, Szczodrak J. Purification and properties of an alpha-(1 -> 3)-glucanase (EC 3.2.1.84) from Trichoderma harzianum and its use for reduction of artificial dental plaque accumulation. Acta Biochim Pol 2013;60:123–8.
[54] Rikvold PD, Hansen Skov LB, Louise Meyer R, Rose Jorgensen M, Tiwari MK, Schlafer S. The effect of enzymatic treatment with mutanase, beta-glucanase and DNase on a saliva-derived biofilm model. Caries Res 2023. https://doi.org/10.1159/000535980.
[55] Liu Y, Kamesh AC, Xiao Y, Sun V, Hayes M, Daniell H, Koo H. Topical delivery of low-cost protein drug candidates made in chloroplasts for biofilm disruption and uptake by oral epithelial cells. Biomaterials 2016;105:156–66. https://doi.org/10.1016/j.biomaterials.2016.07.042.
[56] Singh R, Ren Z, Shi Y, Lin S, Kwon KC, Balamurugan S, Rai V, Mante F, Koo H, Daniell H. Affordable oral health care: dental biofilm disruption using chloroplast made enzymes with chewing gum delivery. Plant Biotechnol J 2021;19:2113–25. https://doi.org/10.1111/pbi.13643.
[57] Wiater A, Szczodrak J, Pleszczyńska M. Mutanase induction in Trichoderma harzianum by cell wall of Laetiporus sulphureus and its application for mutan removal from oral biofilms. J Microbiol Biotechnol 2008;18:1335–41.
[58] Wiater A, Janczarek M, Choma A, Prochniak K, Komaniecka I, Szczodrak J. Water-soluble (1 -> 3),(1 -> 4)-alpha-D-glucan from mango as a novel inducer of cariogenic biofilm-degrading enzyme. Int J Biol Macromol 2013;58:199–205.
[59] Khalikova E, Susi P, Korpela T. Microbial dextran-hydrolyzing enzymes: fundamentals and applications. Microbiol Mol Biol Rev 2005;69:306–25. https://doi.org/10.1128/mmbr.69.2.306-325.2005.
[60] Klein MI, Hwang G, Santos PHS, Campanella OH, Koo H. Streptococcus mutans-derived extracellular matrix in cariogenic oral biofilms. Front Cell Infect Microbiol 2015;5.
[61] Tipton KF, Armstrong RN, Bakker BM, Bairoch A, Cornish-Bowden A, Halling PJ, Hofmeyr J-H, Leyh TS, Kettner C, Raushel FM, Rohwer J, Schomburg D, Steinbeck C. Standards for Reporting Enzyme Data: the STRENDA Consortium: what it aims to do and why it should be helpful. Perspectives Sci 2014;1:131–7. https://doi.org/10.1016/j.pisc.2014.02.012.
[62] Dige I, Paqué PN, Del Rey YC, Lund MB, Schramm A, Schlafer S. Fluorescence lectin binding analysis of carbohydrate components in dental biofilms grown in situ in the presence or absence of sucrose. Molecular Oral Microbiol 2022;37:196–205. https://doi.org/10.1111/omi.12384.
[63] Tawakoli PN, Neu TR, Busck MM, Kuhlicke U, Schramm A, Attin T, Wiedemeier DB, Schlafer S. Visualizing the dental biofilm matrix by means of fluorescence lectin-binding analysis. J Oral Microbiol 2017;9:1345581. https://doi.org/10.1080/20002297.2017.1345581.
[64] Schlafer S, Meyer RL, Dige I, Regina VR. Extracellular DNA contributes to dental biofilm stability. Caries Res 2017;51:436–42. https://doi.org/10.1159/000477447.
[65] Jakubovics NS, Goodman SD, Mashburn-Warren L, Stafford GP, Cieplik F. The dental plaque biofilm matrix. Periodontol 2000 2021;86:32–56. https://doi.org/10.1111/prd.12361.
[66] Binder TP, Robyt JF. p-Nitrophenyl alpha-D-glucopyranoside, a new substrate for glucansucrases. Carbohydr Res 1983;124:287–99. https://doi.org/10.1016/0008-6215(83)88464-X.
[67] McCabe MM, Hamelik RM. An enzyme from Streptococcus mutans forms branches on dextran in the absence of sucrose. Biochem Biophys Res Commun 1983;115:287–94. https://doi.org/10.1016/0006-291X(83)91002-1.
[68] Karanicolas PJ, Farrokhyar F, Bhandari M. Practical tips for surgical research: blinding: who, what, when, why, how? Can J Surg 2010;53:345–8.
[69] Krithikadatta J, Gopikrishna V, Datta M. CRIS Guidelines (Checklist for Reporting In-vitro Studies): a concept note on the need for standardized guidelines for improving quality and transparency in reporting in-vitro studies in experimental dental research. J Conserv Dent 2014;17:301–4. https://doi.org/10.4103/0972-0707.136338.