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Antifungal Activity Of Purified Dextrnase From Dental Caries Staphylococcus Salivarius*against*oral Candidiasis Caused Bycandida Albicans

Karrar N.Shareef[*], Ghassan N Talib[*], Jawaher M Taher[**], Ali Abdul Hussein[***]

*College of Dentistry, University of Alkafeel, Najaf, Iraq

**College of |Dentistry, University of Mustansiriyah, Najaf, Iraq

***Faculty of Pharmacy, University of Alkafeel, Najaf, Iraq

Abstract

Dental caries from the most populardental illnesses, and it is caused by chronic infections of the normal flora in the mouth. Cavities are created by acidic metabolites produced by surviving bacteria found in the mouth that feed on sugar.When the dextran used as a substrate seven*Staphylococcus aureus and Staphylococcus salivarius* isolatesisolated from dental caries can secret extracellular dextranase with potential index for ranger between 0.78-1.72. An ammonium sulfate, DEAE-cellulose column and then sephadexG-150 column were used to purify of dextranase with a final recovery 32.3%. Ten *Candida albicans* isolates were isolated from patients infected with oral candidiasis and these fungal isolates revealed high level of antifungal activity with MIC values ranged from 25 to 50 µg/ ml. In contrast, the fungicidal effect of dextranase appeared against *Candida albicans* isolates with concentrations values from 50-100 µg/ ml, thus the dextranase may be an alternative promising treatment for fungal infections caused by *Candida albicans* such as oral candidiasis.

Key words: Dental caries, dextranase , oral candidiasis

Introduction

Dental caries considered from the widely distributed chronic infections around the world(1).The common causative factor for dental caries is bacterial plaque, which is composed of native oral flora and and accumulates on teeth surfaces(2). Dental caries is a bacterial infection that destroys the enamel, dentin, and cementum of teeth. Dental caries is a disease thatdepended on the biofilm forming organisms that can ferment different carbohydrates which play a crucial role in their growth and progression(3). It is caused by the interaction of certain bacteria with dietary ingredients in a biofilm known as "dental plaque". Because the sucrose that fermented to form the extra and intracellular polysaccharides in dental plaque, it is concedered as the most cariogenic dietary carbohydrate(4).

Dextranase, α-1,6-d- glucan-6-glucanohydrolase that degredate of dextran polymer (polysaccharide of glucose) by cleaving the α-1,6-glycosidic bond for dextraninto different fractions with low molecular weights(5).Some bacterial species, filamentous fungi and yeast had an ability to produced the dextranase (6). The dextranases have great importance in pharmaceutical, analytical,industrial and agricultural fields(7).

Most of oral candidiasis that also known as oral thrush caused by Candida albican(8).Candida albicans is a commensalismyeast and opportunistic pathogens on the human body. Oral infection that caused by Candida albicans such as oral candidosis (9).Most Candida spp. infections frequently have been associated with biofilm formation on different biological surfaces and non viable roofs like the catheters(10,22-39).The widely used fungal antibiotics made the infections that associated with the biofilm formation more difficult to treat with common used drags so that There is a need to discover newer and more effective therapies and the goal of the present research to screen of dextranase bacterial producers from dental caries, purification of dextranase and using it as antifungal agent for treatment of oral candidiasis.

Materials and methods

Collection of specimens screening for dextranase producers

Forty-five dental plaques from patients suffering from dental caries were takenbysterile forceps and floodedin amount of normal saline solution then homogenized by vortex. Each homogenized sample was inoculated to modified cultivation medium that consist per 100 ml of1g dextran,0.05g yeast extract, 3g glucose, 0.5g peptone, 0.1g $KH_2PO_4$and 0.05g $MgSO_4$:$7H_2O$, and incubated at 37ºC for 24 hours. The qualitative screening included measurement of the potential index of hydrolytic zone that surroundedthe colonies by dividing the diameter of clear area on the colony diameter(11). The pure isolated colonies were sub cultured for pure culture preservation.

Identification of bacterial isolates

The morphological properties of colonies such as shape, size, margins and pigmentation with Gram stain were determined for bacterial isolates(12). Biochemical properties withVitek-2 system used to ensure these isolated.

Extraction and purification of dextranase

The selected bacterial isolate was grown in dextranase production broth at 37C for 24h. and after fermentation process the centrifugation with 8000 rpm for 20min was performed. The supernatant was used as crude extract and the purification performed by some modification for(6) . This crude solution presented to serialconcentrations of saturated ammonium sulfate solutions from 30 to 90%. After cooling incaution period, the precipitated products were collected by centrifugation and suspended with 0.1Macetate buffer at pH 6.2.The dextranase activity was assayed for each percentage of saturation. After that the solution was prepared to DEAE- cellulose and the elution performed by gradient concentrations of NaCl in 0.1Macetate buffer at pH 6.2. The active parts that contained higher dextranase activity were

collected and supported to sephadex G-150 column with retention by 0.1Macetate buffer at pH 6.2. The concentrated fraction with higher activity were pooled for the next application.

Dextranase and protein assays

For dextranase assay(6), 25µl of the prepared enzyme was incubated with 25µl of 2.5 mM dexan in 0.1M citrate buffer with pH 6.2 for 20 min at 37ºC. The reaction was stopped and the DNS reagent was added and the reducing sugars released was estimated at 500 nm(5).Dextranase activity(U) was expressed with the amount of enzymethat librates 1µ mole of maltose in one min. The estimation of protein content with method of (13) depending on bovine serum albumin as standard solution for preparation the standard curve.

Isolation of identification of Candida SPP.

Twenty-two oral specimens were taken by cotton swabs from patients infected with oral candidiasis. The specimens were streaked on sabouraud dextrose agar as well as chromogenic Candida agar then incubated aerobically for 48h at 37C. The diagnosis included the cultural and microscopical examination for the colonies properties(14)besides to the growth on chromogenic agar and using of Vitek 2 system.

Detection of dextranase antifungal activity

The antifungal activity was determined by two ways:

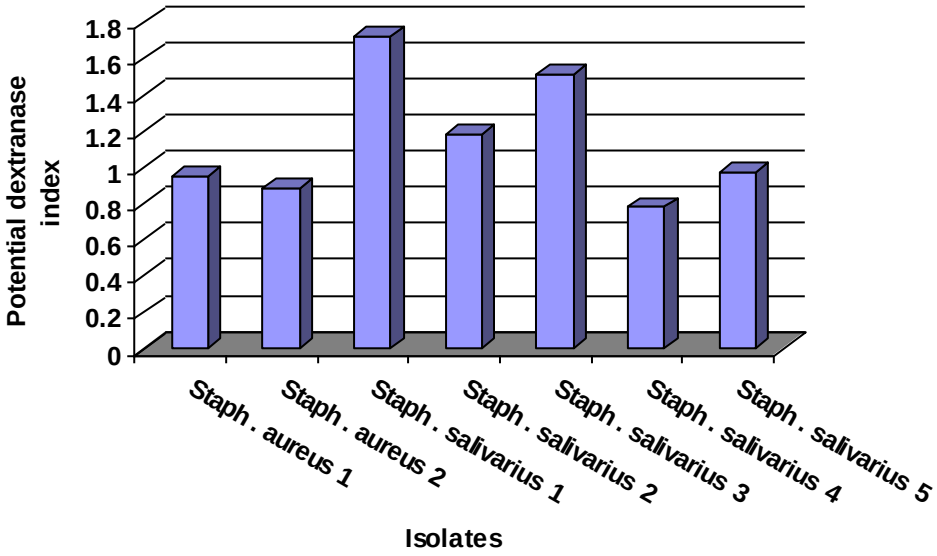
A- The antifungal activity of purified dextranase against Candida isolates were done by microdilution method for determination the minimum inhibitory(MIC) concentration and fungicidal (MFC)concentration. Different concentrations with 100 µl were prepared from the purified dextranaseranged 400-12.5 µ g/ml with sabouraud dextrose broth on microplates, then 2.5µl of overnight culture of Candida isolates were inoculated tomicroplate wells and incubated at 37Cfor 48 h. The MIC can express as the concentration in which no visible growth was appeared with naked eye , while MFC can express as the concentration at which no growth on sabouraud dextrose agaraccording to (8) with some modifications .

B-The antifungal activity of purified dextranaseat MICs concentrationsagainst different Candida isolates were screened using agar well diffusion method. The fungal isolates at concentration 0.5 x 10^8 cell/ ml were spread on sabouraud dextrose agarplatesusing a sterile swab.The wells were made with the help of a sterile cork borer with the diameter of 5 mm then50 µl of purified dextranase was places in each well. After incubation period at 37◦C for 48h, the formed inhibition zone was recorded(9).

Results and discussion

Screening for dextranase producers

After culturing the dental plaques from forty two patients suffering from dental caries in screening dextranase agar medium ,the results revealed that only 7 isolates had the ability to produce the dextranase by hydrolysis the dextran that found in the medium. The dextranse producer isolates included 2 Staphylococcus aureus isolates and 5 Staphylococcus salivarius isolates and the potential index for dextranase production ranger between 0.78-1.72(figure-1) with higher level of productivity forStaphylococcus salivarius1.Dextranase had obtained from the genera Pseudomonas, Streptococcus and Brevebacterium which isolated from different sources(7). Members of genera could colonize the tooth surface included Niesseria, Hemophilus and Streptococcusas well as Micrococcus and Staphylococcus as mentioned by(15) and Streptococcus sp. was the most frequeningindental plaque followed byPseudomonas sp that were isolated from the patients with dentalplaque from hospital.



Figure(1):th

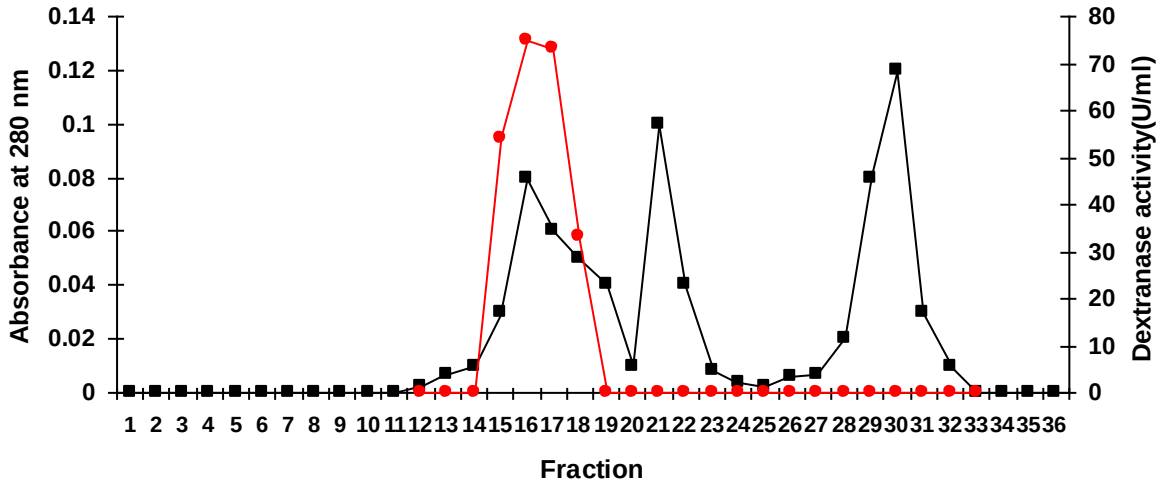
Extraction and purification of dextranase

Dextranase was extracted from Staphylococcus salivarius1that showed higher productivity for dextranase using dextranase production broth that contained dextran as a substrate with 52U/ml. In the first step of purification, the dextranase was precipitated with higher activity 68U/ml at 70% saturation of ammonium sulfate. In the second step the precipitate was applied to chromatography columns which included DEAE- cellulose with elution by gradient concentrations of NaCl in 0.1M acetate buffer at pH 6.2 that showed three peaks of proteins and the dextranase activity located in the first peak as located in figure(2) with a yield of 35.7 % then sephadex G-150 column with recovering the active fractions that eluted from the second protein peak with final specific activity 62.6U/ mg and a yield of 32.3%(figure-3) and table(1).

Table-1: Sequenced steps for Purification of dextranase from Staphylococcus salivarius1

Purification step	Size(ml)	Dextranase activity (U/ml)	Protein conc. (mg/ml)	Specific activity (U/ mg)	Total activity	Purification fold	Yield (%)
Crude extract	55	52	3.66	14.20	2860	1	100
$(NH_4)_2SO_4$ precipitation	21	68	2.11	32.22	1428	2.26	49.9
DEAE-Cellulose	14	73	1.99	36.68	1022	2.58	35.7
Sephadex G-150	12	77	1.23	62.60	924	4.4	32.3

The dextranase was purified from by 70% saturation of $NH_4)_2SO_4$ precipitation and chromatographically on sephadex G-100 as reported by(16).Also ammonium sulphate precipitation with 70% and urogel-AcA-34 gel filtration used for purification of dextranase produced by Streptococcus sobrinus(17).



Figure(2): The purification Staphylococcus salivarius1dextranase using ion exchange chromatography

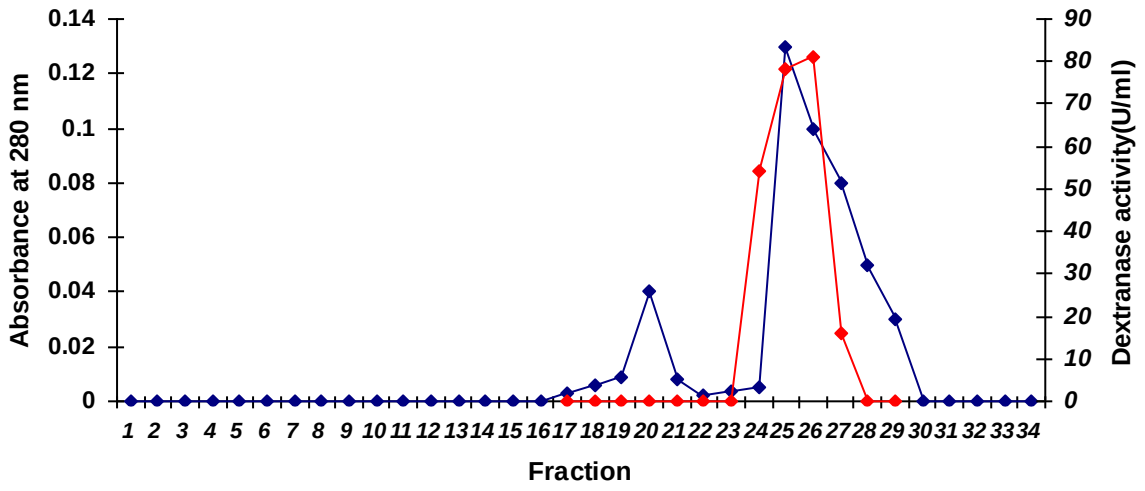


Figure (3): The purification Staphylococcus salivarius1dextranase using gel filtration chromatography

Isolation of identification of Candida SPP.

Out of 22 oral specimens were taken by cotton swabs from patients infected with oral candidiasis, there were 10 isolates belonged to Candida albicans. In microscopic examination appeared as gram positive with oval to spherical shape besides their growth on chromogenic agar with light greenish colonies.

Candida albicans are found in the mouth as a microbiota without causing a disease, but when converted from nonpathogenic to pathogenic and invasive the tissues of the host lead to oral candidiasis(18,19).The majority of isolated The dental caries fromthe oral cavity belonged to Candida albicans with larger percentage then Candida tropicalis and Candida krusaei with lower percentage(20).

Detection of dextranase antifungal activity

In microdilution method, the antifungal activity of purified dxtranase against Candida albicans was performed. The results revealed that the purified dextranase had an inhibitory effect on growth of Candida albicans isolates with MIC values ranged from 25 to 50 µg/ ml. In contrast, the fungicidal effect of dextranase appeared against Candida albicansisolates with concentrations values from 50-100 µg/ ml as recorded in table(2).

On the other hand, the antifungal activity of purified dxtranasewas determined with agar well diffusion method using the MIC and MFC values.In the current study found that dextranase recorded maximum antifungal activityagainst Candida albicansO5 isolate with18 and 20mm at 25 and 50µg/ml for MIC and MFC, respectively. The hydrolytic enzymes can break down the cell wall of Candida and biofilm thus lead to appear the β- glucan layer that protect the biofilm and enhance the immunoresponse against Candida infections(10).The formation of biofilm and hypha, secretion of some

enzymes and adhesion to the tissues of the host have an important role in Candida pathogenesis(21).Therefore the research suggested that dextranase may be used to prevent oral disease such as dental caries.

Table(2): Detection of antifungal activity of purified dextranase against Candida albicans isolates

Candidal isolate	Purified dextranase	
	MIC(µg/ml)	MFC(µg/ml)
Candida albicansO1	50	100
Candida albicansO2	50	50
Candida albicansO3	25	50
Candida albicansO4	25	100
Candida albicansO5	25	50
Candida albicansO6	50	50
Candida albicansO7	50	100
Candida albicansO8	25	50
Candida albicansO9	25	100
Candida albicansO10	50	100

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