Original Article

Antagonistic activity of a Multi-Functional Gold Standard Chlorhexidine against Lactobacillus acidophilus Isolated from Childhood Caries

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Abstract

Keywords:
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Lactobacillus acidophilus and Antimicrobial activity.

Dental caries, second to common cold, remains one of the most prevalent chronic diseases that affect children and young adults globally. The world is now aiming to prevent this disease at an early stage and provide children the best quality of life. The background of this study chlorhexidine gluconate is an effective bactericidal agent and broad-spectrum antimicrobial drug. It has been extensively researched and is the “gold standard” antimicrobial in oral hygiene. Among different kind of microorganisms the cariogenic Lactobacillus acidophilus is recognized as a predominant pathogen in childhood caries. So plaque samples were collected from different dental clinics in and around Tirupur district. All the isolates were identified by biochemical characterization and 16SrDNA gene sequencing. Further, the potentially of Lactobacillus acidophilus in antimicrobial production was assessed. Lactic acid production by Lactobacillus acidophilus is partially growth associated and about 180.16 mg/ml of lactic acid/ml was synthesized in the oral cavity of childhood caries followed by quantification of organic acid and determination of pH value indicates that organic acid production was increased with the highest acidity (1.8%) was observed after 72 h of incubation and determination of H2O2 production by the isolates MTVG02, MTVG20, MTVG25, MTVG38 and MTVG46 are generated H2O2. The highest proportion was found among all cariogenic isolates, 5 out of 5 strain (100%) being positive. Finally antimicrobial efficacy of CHX was used at 2% concentration (50µl, 100µl and 150µl) against L. acidophilus. The gold standard CHX was used and it has proven efficacy as a broad-spectrum antimicrobial for reducing childhood plaque.

Introduction

Childhood caries is a chronic and transmissible disease characterized by demineralization of tooth owing to production of acids by bacteria in biofilms formed on its surface. The elimination of cariogenic microorganisms is one of the vital factors for the primary caries prevention (Bjorndal and Larsen, 2000).

Modern studies demonstrate that the microbes include Streptococcus mutans, Lactobacillus acidophilus, Actinomyces viscosus, and L. rhamnosus are the most common cariogenic microorganisms involved in caries progression (Oda et al, 2015). The progression of caries associated with the microbe and is also isolated both from enamel carious lesions and hidden dentin caries (Simon-Soro and Mira, 2015).

Mainly causative agent of Lactobacillus is a set of bacteria that occur at elevated levels in deep childhood caries lesions (Byun et al, 2004). The analysis shows that among the genus Lactobacillus, L. acidophilus is most prevalent, and L. paracasei, L. rhamnosus, and L. fermentum are also present in deep caries lesions and promote caries progression (Callaway, 2013). If these caries lesions progress, they may eventually result in pain and pulp exposure in young children. For this reason to diminish the possible of chief caries and enhance the postoperative sensitivity, antibacterial agents may be chosen according to their ability to reduce the possibility of existing bacteria. The use of antimicrobial solutions as an oral rinse disinfectant for reducing cariogenic microorganisms according to the target of the application has been recommended (Esra Uzer Celik et al, 2016).

Chlorhexidine gluconate (chlorhexidine) is a gold standard broad-spectrum antimicrobial drug. Acting as an antiseptic, it is an effective bactericidal agent against all categories of microbes, including bacteria, yeast, and viruses and also commonly used...
was regarded as end point. A decolourization of the sample was equivalent to 1.070 mg of H₂O₂. Titration was carried out with 0.1 N acidophilus broth culture of test organisms. Phenolphthalein was added to the 20 ml of supernatant as an indicator for titrimetric estimation. One ml of 0.1M NaOH is collected by centrifuging at 10,000 rpm for 15 m at 4°C. When chlorhexidine binds to microbial cell walls it induces changes, damaging the surface structure, leading to an osmotic imbalance with consequent precipitation of cytoplasm causing cell death. The substantively of chlorhexidine enhances this bactericidal effect, which allows for the retention of chlorhexidine in the oral cavity and a prolonged residual antimicrobial effect for up to 12 hours or longer depending on the dosage and form. As a result chlorhexidine can be used repeatedly and over long periods of time to eliminate the oral bacteria. Furthermore, it destroys all categories of microbes, not just bacteria, and there is little risk for the development of opportunistic infections. So hence the present study has planned to evaluate the antimicrobial activity of multifunctional gold standard chlorhexidine against the caries infections.

Materials and methods

Sample Collection

Dental samples were obtained from different dental clinics around Tirupur district (Fig.1). It was kept in glass vial with saline solution and was brought to the laboratory of PG and Research Dept. of Zoology, Division of Microbial Technology, Chikkanna Govt. Arts College, Tirupur. Then, the samples were incubated at 37°C for 24 hours and the samples were used for isolation of cariogenic bacteria.

Identification of Lactobacillus acidophilus

The organism isolated by Man Rogosa Sharpe agar was the selective media for Lactobacillus acidophilus (Fig.2). After, the identification was done by Gram staining, IMViC test, Nitrate reduction test, Sugar fermentation test, Catalase test, and antimicrobial production (H2O₂, Lactic acid and Organic acid), and genotypic characterization.

Production of antimicrobial compound by oral Lactobacillus acidophilus

Determination of lactic acid production by Lactobacillus acidophilus

The strains were grown in MRS broth for 48 h and supernatant was collected by centrifuging at 10,000 rpm for 15 m at 4°C. Phenolphthalein was added in to the 20 ml of supernatant as an indicator for titrimetric estimation. One ml of 0.1M NaOH is equivalent to 9.08 mg of lactic acid.

Determination of hydrogen peroxide by Lactobacillus acidophilus

Take 25 ml of dilute sulphuric acid were added to 25ml of MRS broth culture of test organisms. Titration was carried out with 0.1 N of Potassium permanganate. Each ml of Potassium permanganate is equivalent to 1.070 mg of H₂O₂. A decolourization of the sample was regarded as end point.

Quantification of organic acid and determination of pH value by Lactobacillus acidophilus

In this study 1% (V/V) of 24 h active culture of Lactobacillus was used to inoculate 10% sterilized skim milk and initial pH (6.6) was determined by digital electrode pH meter. The inoculated skim milk was incubated at 37°C for 72 h and samples were collected in every 24h; 48 h, 72 h and liquid of coagulated milk were separated by filtration. The pH of the separated liquid was recorded using a digital electrode pH meter. The quantification of organic acid was performed through titration with 0.1 N NaOH using phenolphthalein as pH indicator.

Preparation of gold standard CHX stock solution

In this study the 2% CHX powder was weighed and it was thoroughly dissolved in 100ml of sterile distilled water. Further, it was used against dental pathogen Lactobacillus acidophilus.

Antimicrobial efficacy of gold standard CHX against Lactobacillus acidophilus

The antimicrobial activity of CHX was done by well diffusion method. The solid medium was prepared with using Muller Hinton agar. After solidification of medium the 0.1ml overnight culture was spread over the surface of agar. After puncture was made by using different range of Cork borer (50µl, 100 µl and 150µl). Finally the hole was filled with 2% con. of CHX at different µl (50, 100 and 150). After it was kept in incubator at 37°C, 24h for its antimicrobial activity. Measure the zone of inhibition around the well.

Result

Isolation and identification of Lactobacillus acidophilus

Totally ten isolates were used in this study. The Lactobacillus acidophilus was identified by grams reaction (Fig.3) and biochemical characterization, genotypic characterization.

Production of antimicrobial compound by oral lactobacillus acidophilus

Lactic acid estimation

Lactic acid production of EPS producing cariogenic plasmid strains were studied by titrimetric estimation which include MTVG08, MTVG13, MTVG14, MTVG15, MTVG22, MTVG47 and MTVG48 produce equal amount of lactic acid 180.16 mg/ml in the oral cavity of rural caries patients (Fig. 4).

Quantification of organic acid and determination of pH value

The present experiment indicates that organic acid production was increased with the incubation time. On the other hand, pH of the media decreased with the increasing acid production. In this study highest acidity (1.8%) was observed after 72 h of incubation at 37°C for Lactobacillus acidophilus isolated from childhood caries (Fig.4).

Determination of H₂O₂ production by oral Lactobacillus acidophilus

The finding from the present studies of H₂O₂ production by oral Lactobacillus acidophilus. A substantial proportion of the isolates MTVG02, MTVG20, MTVG25, MTVG38 and MTVG46 are...
generated H2O2 (Table: 08), as revealed by decolourization of pink color that appeared during titration. The highest proportion was found among all cariogenic isolates, 5 out of 5 strain (100%) being positive. Among five strains including only one strain namely MTVG02 (340.20 mg / ml) strongly to produce maximum amount of H2O2 compare than other cariogenic isolates namely (MTVG20 - 255.15 mg / ml), (MTVG25 - 212.625 mg / ml), (MTVG46 - 212.625 mg / ml), and (MTVG38 – 127.575 mg / ml) (Fig. 4).

**Antimicrobial activity of Gold standard Chlorhexidine**

Antimicrobial activity of 2% concentration of CHX was performed by Agar Well Diffusion assay against cariogenic Lactobacillus acidophilus. Three different concentrations (50µl, 100µl and 150µl) were used in this assay against oral Lactobacillus acidophilus. The maximum zone of inhibition 22.5mm, 35mm and 31mm was observed in strain no. MTVG75 followed by the minimum zone of inhibition 25mm, 27mm and 30mm was observed in strain no. MTVG06 at different µl concentration of (50, 100 and 150) CHX (Fig. 5, Table. 1).

**Table No. 1: Antibacterial activity of Chlorhexidine Diacetate**

<table>
<thead>
<tr>
<th>Strain No.</th>
<th>2% Concentration of CHX</th>
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<tbody>
<tr>
<td></td>
<td>50µl</td>
</tr>
<tr>
<td>MTVG02</td>
<td>25</td>
</tr>
<tr>
<td>MTVG06</td>
<td>25</td>
</tr>
<tr>
<td>MTVG75</td>
<td>22.5</td>
</tr>
<tr>
<td>MTVG85</td>
<td>25</td>
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</tbody>
</table>

C: Control; P: Positive

**Lactic acid production**

C: Control; P: Positive

**Hydrogen peroxide production**

C: Control; P: Positive

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Fig. 1: Tooth Decay Sample

Fig. 2: Isolation of Lactobacillus acidophilus from tooth decay sample using Man Rogosa Sharpe Agar

Fig. 3: Gram staining of L. acidophilus

Fig. 4: Antimicrobial production from Lactobacillus acidophilus

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Organic acid production

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Fig. 5: Antimicrobial activity of gold standard CHX against Lactobacillus acidophilus

Conclusion

The findings of the present study suggest that CHX is significantly effective against cariogenic microorganisms at 2% concentrations of different range of µl (50, 100 and 150). The gold standard multi functional chlorhexidine can be considered as a broad spectrum activity to kill the cariogenic pathogen around the oral environment of school children's. The varied and effective applications of chlorhexidine make it a viable option for use in all dental settings and within a variety of dental procedures and pre-procedures with very few undesirable side effects.

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References