The effect of mouthwash on the DNA yield and quality of oral bacteria

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Received 14 November 2017; Received in revised form 27 July 2018 ; Accepted 27 July 2018

ABSTRACT

Aims: The application of mouthwash is one of the oral hygiene treatments that commonly use after tooth brushing to control the bacterial colonization from overgrowth. This research is focused on investigating the effect of mouthwash on oral microbiome by analyzing the quality and yield of DNA obtained before and after using mouthwash and also to compare the bacterial abundance via 16S rRNA PCR detection.

Methodology and results: The DNA was extracted from the saliva samples before and after using mouthwash using Phenol-Chloroform extraction method. The DNA extract was then evaluated using Nano Drop ND-1000 UV/VIS Spectrophotometer to determine the DNA quality and DNA yield. After that, the 16S rRNA gene was amplified via PCR for bacterial detection in the saliva using 27 F and 1492 R primers set, and the PCR products were observed on 1.5% gel electrophoresis. Statistical analysis was performed by using Graphpad Prism 7.03 software. For DNA yield, there was significantly higher yield observed after mouthwash usage with 80% of the samples was found to yield more DNA. To assess DNA quality, absorbance ratio of A260/A280 and A260/A230 absorbance ratio even after the usage of mouthwash. The DNA quality was seen to be similar for both A260/A280 and A260/A230 absorbance ratio even after the usage of mouthwash. The amplification of 16S rRNA gene was successful and 1500 bp expected band size was observed.

Conclusion, significance and impact of study: This study demonstrated the usage of mouthwash is useful to increase the DNA yield as compared to without using mouthwash. However in terms of quality, no difference is seen. This result can be used to provide insight on mouthwash usage for saliva sampling in a non-invasive manner.

Keywords: Saliva, mouthwash, DNA yield, DNA quality, 16S rRNA gene

INTRODUCTION

Every part of the human body consists of bacteria that colonize to be symbiont to the host subject. The mouth is the second part of the body that comes after gut that has the highest bacterial communities (Killian et al., 2016). About 500 to 700 bacterial species were detected for the oral microbiome and most of them interact with each other to complement the host. The bacteria from the saliva are from intraoral surface of the mouth shaded and also from the environment or anything that being consume or in contact with mouth (Takeshita et al., 2016). The application of mouthwash is one of the oral hygiene treatments that is commonly used after tooth brushing to control the bacterial colonization from overgrowth.

Its ability to reduce bacterial colonization in mouth had been approved in many studies including the studies done by Wade (2013) and Killian et al. (2016) based on targeted ingredient. The mouthwashes in the market today have added features such as antimicrobial effects for the purpose of improvement of oral health maintenance (Mat Ludin and Md Radzi, 2014). The identification of oral bacteria may be useful as a prognostic tool for early treatment of oral disease and any related disease. Not all the bacteria present in the oral cavity are harmful to the host since some are co-exist without causing health risk. This includes Porphyromonas gingivalis and Tannerella forsythensis from Bacteroidetes phylum whereas Treponema denticola from Spirochaete phylum have been associated with periodontal disease and considered as pathogenic (Tamura et al., 2006; Kang et al., 2009). The bacteria is not the only component of the saliva (Chartier and Birnboim, 2005), there is need of specific identification to distinguish the bacterial DNA from another. The use of PCR based on 16S rRNA gene commonly use in study of bacteria because this gene are commonly found in bacteria and it is reliable for phylogenetic analysis due to its ultra-conserves regions (Zayats et al., 2009; Tanner et al., 2011). This study aims to compare the quality and yield of DNA obtained before and after using mouthwash. Another objective is to identify the difference in bacterial...