



Random Amplified Polymorphic DNA for identical *Streptococcus salivarius* strains isolated from tongue of peoples before and after Listerine In vivo

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ABSTRACT

Random amplified polymorphic DNA (RAPD) is one of the most promising methods for distinguishing individual bacterial strains and determining the diversity of nucleotide sequences. Eight isolates (identical in 16S rRNA sequence) of *Streptococcus salivarius* before and after Listerine using In vivo were subjected to RAPD technique showed four strains identical along DNA while other four were not meaning the strains after Listerine using had mutations along DNA strand.

Keywords: *S. salivarius*, RAPD, Listerine

INTRODUCTION

The Random Amplified Polymorphic DNA technique has been widely utilized in recent studies to classify individuals among bacterial species to accomplished phylogenetic relationships and to identify genetic variation among closely related species to describe genetic markers for certain trait and the source of infection (Abd Al-Abbas et al., 2012; Abdul-Ridha and Al-Abbas, 2016; Mahdi et al., 2021). Identification of pathogenic bacteria at the strains level in outbreaks of infection is important, moreover, infection cases may resulted from the transmission of pathogenic strains from one source to humans (Abd Al Wahid and Abd Al-Abbas, 2019), or it may resulted by the transmission of pathogenic strains among humans causing the same disease (Mahdi et al., 2021). Consequently, identifying the source of infection is important to take the necessary measures to avoid outbreak of infection.

Random amplified polymorphic DNA is an analysis has been used for the study of genetic relationships between strains of bacterial species, frequently used in studies of mutagenesis by detecting the presence of genetic differences between strains (Papadopoulos et al., 2002; Arif et al., 2010; To et al., 2021). The aim of this study was to determine if Listerine can cause mutations along the DNA of bacterial strains or not.

MATERIALS AND METHODS

Sample collection

Sample from tongue of peoples were collected before Listerine and after exposure to Listerine for 30 second, eight *S. salivarius* were identified by 16S rRNA sequencing and supplied from the study of Mousa and Abd- Al-Abbas (2023).

Random amplified polymorphic DNA (RAPD)

The sequence of RAPD primer was 5'-AGAGGGCACA-3' (Zhang et al., 2002;

To et al., 2021). Total volume 25 µl contains 12 µl of Go Taq Green master mix (Promega, USA), 7 µl of Nuclease Free water (Bioneer, Korea), 4 µl of DNA template and 2 µl from primer. Thermo cycler (Bioneer, Korea) condition for amplification 95°C for 5min. followed by 35 cycles at 94 °C for 1 min, 42° C for 1 min and 72°C for 2 min, the final extension was done at 72°C for 5 min. Agarose gel electrophoresis was performed (2 % of agarose powder, 100 ml of TBE buffer and 0.5 of Ethidium bromide) with 100bp DNA ladder (Promega, USA) to detect the identical S. salivarius strains bands under UV transilluminator (Wisd, Korea). The distance between RAPD bands of all isolates were calculated according to ladder's bands by

Microsoft word then transferred to the program “ Unweighted pair group method with Arithmetic mean” (UPGMA) to show the result as a dendogram (Garcia-Vallve and Puigbo, 2009).

The result

The bands of RAPD-PCR for eight S. salivarius strains (only identical in 16S rRNA) before and after Listerine were shown in Figure (1). Since, the isolates 176 B (Before) & A (After), 162 B&A, 164 B&A, 131B&A were not identical along DNA strand, while the isolates 76B&A, 140B&A, 128B&A, 66B&A appeared identical along DNA strand (Figure 2) and Table (1).

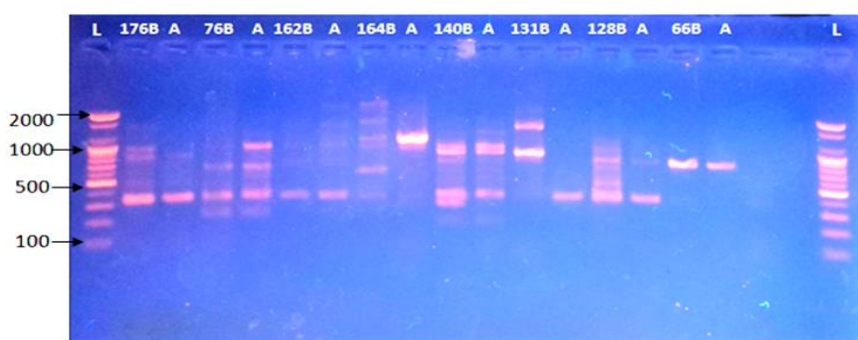


FIGURE 1: Agarose gel electrophoresis (2 %) showing RAPD pattern of Streptococcus salivarius. Lane L: 100 bp Marker, Lane 176 Before and After, 76B and A, 162B and A, 164B and A, 140B and A, 131B and A, 128B and A, 66B and A.

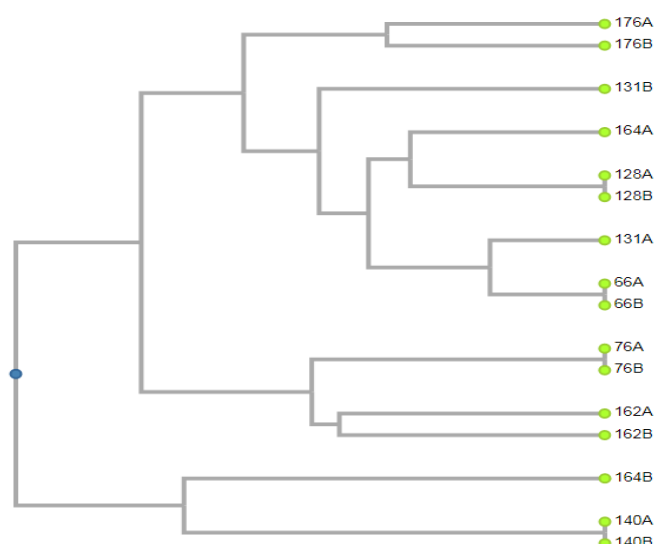


FIGURE 2: Dendogram of Streptococcus salivarius strains (176Before and After, 76B and A, 162B and A, 164B and A, 140B and A, 131B and A, 128B and A, 66B and A) performed by variables

related to RAPD band using the Unweighted Pair Group Method with Arithmetic mean (UPGMA) algorithm. Strains No. 176B, 176A, No. 131B, 131A, No.164 B, 164A and No. 162B, 162A demonstrate mutation, while strains No. 128B identical to 128A, No. 66B identical to 66A, No. 76B identical to 76A and No. 140B identical to 140A.

TABLE 1: The distance matrix of Streptococcus salivarius isolates

	17 6B	17 6A	76B	76A	162 B	16 2A	164 B	164 A	140 B	140 A	131 B	13 1A	12 8B	128 A	66 B	66A
17 6B	0	1.3 21	2.10 0	2.10 0	2.13 2	2.3 55	2.03 3	1.59 7	2.51 6	2.51 6	2.03 6	1.8 51	1.8 71	1.87 1	2.1 95	2.19 5
17 6A		0	1.63 2	1.63 2	1.85 8	2.2 00	2.42 5	1.35 7	2.65 4	2.65 4	1.85 4	1.2 96	1.6 71	1.67 1	1.7 53	1.75 3
76 B			0	0.00 0	1.67 0	1.4 75	2.28 4	2.12 3	2.09 2	2.09 2	2.47 0	2.0 84	2.3 36	2.33 6	2.3 95	2.39 5
76 A				0	1.67 0	1.4 75	2.28 4	2.12 3	2.09 2	2.09 2	2.47 0	2.0 84	2.3 36	2.33 6	2.3 95	2.39 5
16 2B					0	1.4 27	1.55 8	1.75 7	2.07 6	2.07 6	1.99 0	1.7 11	2.0 09	2.00 9	2.0 78	2.07 8
16 2A						0	1.74 4	1.85 4	2.08 5	2.08 5	2.07 7	2.0 77	2.0 95	2.09 5	2.3 89	2.38 9
16 4B							0	2.00 9	1.81 8	1.81 8	2.21 6	2.3 14	2.0 60	2.06 0	2.5 98	2.59 8
16 4A								0	2.49 7	2.49 7	1.26 2	1.1 48	1.1 80	1.18 0	1.6 46	1.64 6
14 0B									0	0.00 0	2.31 6	2.3 15	2.2 99	2.29 9	2.5 99	2.59 9
14 0A										0	2.31 6	2.3 15	2.2 99	2.29 9	2.5 99	2.59 9
13 1B											0	1.3 25	1.3 53	1.35 3	1.7 74	1.77 4
13 1A												0	1.0 54	1.05 4	1.1 80	1.18 0
12 8B													0	0.00 0	1.5 82	1.58 2
12 8A														0	1.5 82	1.58 2
66 B															0	0.00 0
66 A																0

DISCUSSION

The antibacterial effect of Listerine mostly comes from its alcohol content. Since, alcohol like any chemical substances has the ability to cause mutations in DNA or RNA. DNA mutations can affect on the synthesis of amino acids and thus affect on the functional proteins altering their action (Aminetzach et al., 2005; Anstee et al., 2013; Haerian-Ardakani et al., 2015). Previous studies demonstrated the ability of Listerine to denature protein as the mechanism

action of Listerine involves bacterial cell wall destruction with denaturation of protein leading to release the cellular material such as ion, ATP and nucleic acid, inhibition the enzymatic activity of bacteria, extraction of bacterial lipopolysaccharides (endotoxin) from gram negative bacteria and increases the time takes for germs to regrowth (Walker, 1988; Mandel, 1994). While the present study proved its ability to cause mutations, the mutagenic effect of Listerine was proved by comparing 16S rRNA

gene sequence of bacterial isolates before and after Listerine recording 10 out of 42(23.8%) mutations in 16S rRNA bacterial isolates (Mousa and Abd- Al-Abbas, 2023). On the other hand, RAPD-PCR technique demonstrated mutations on the length of DNA strand. The present study showed the following strains No. 176B, 176A, No. 131B, 131A, No. 164B, 164A and No. 162B, 162A (Figure 2 and Table 1) were appeared genetic differences along DNA strand by replacement, insertion or deletion of nitrogenous bases. These mutations caused by Listerine can cause DNA damage leading to effect on the genetic stability of the cell. Nevertheless, there is no evidence of a relationship between alcohol-containing mouthrinse, the risk of oral and pharyngeal cancer (Depaola and Spolarich., 2007; Milić et al., 2019).

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