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Analysis of Transcriptome in *Enterococcus faecalis* Treated with Silver Nanoparticles

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Silver nanoparticles can be used to effectively disinfect *Enterococcus faecalis* (*E. faecalis*) in dental root canal therapy, of which the mechanism remains unclear. In the current study, we explored the effect of silver nanoparticles on bacterial growth and changes in the transcriptome of *E. faecalis* using RNA-sequencing technology. Results showed that silver nanoparticles significantly inhibited growth of *E. faecalis* by prolonging the lag phase in a dose dependent manner. A total of 100 differentially expressed genes (DEGs) including 3 upregulated and 97 downregulated DEGs were obtained in *E. faecalis* after treatment with silver nanoparticles. We performed Gene Ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses on these DEGs. Functional annotation of DEGs showed that transport and metabolism, such as carbohydrate metabolic process, peptide transport, and amide transport were most significantly enriched. Dysregulated genes were significantly enriched in pathways related to carbohydrate and energy metabolism, and the TCA cycle may be a major mechanism for *E. faecalis* with silver nanoparticle treatment. These data indicated changes in the transcriptome of *E. faecalis* after silver nanoparticle treatment, thus providing a mechanism for supporting application of silver nanoparticles for disinfection of *E. faecalis* during and after root canal therapy.

Keywords: *Enterococcus faecalis*, Silver Nanoparticles, Transcriptome.

1. INTRODUCTION

Enterococcus faecalis (*E. faecalis*) is not the main bacterial species present in the root canal of a tooth before treatment. However, *E. faecalis* is significantly increased and becomes one of the most common bacteria species after treatment [1, 2]. Indeed, *E. faecalis* is one of the main bacteria species responsible for persistent infection and reinfection, as well as root canal treatment failure [2, 3]. Disinfection of *E. faecalis* in the root canal is therefore essential for successful dental treatment.

E. faecalis grows in the biofilms of root canal and can survive harsh environments, such as those with nutrient deficiencies, high alkalinity, and presence of antimicrobial agents [4, 5]. Multiple strains of *E. faecalis* have been isolated from root canal and systemic infections [6], and they differ in their abilities to coexist in biofilms with other root canal bacteria [2].

Many approaches have been used in clinical root canal infection control, including root canal irrigation, root canal drug treatments (using sodium hypochlorite, chlorhexidine, MTAD, iodine or calcium hydroxide), disinfection of root canal sealers, and physical disinfection [7–12]. However, these agents can only reduce *E. faecalis* infection, but can't remove all *E. faecalis* colonized in the root canal [13, 14]. Thus, it is critical to find new methods for effective control of *E. faecalis* infection in root canal therapy.

Researchers have synthesized new type of antimicrobial agents that show good antimicrobial activity towards Gram-positive bacteria, but the effect against *E. faecalis* remains further study [15, 16]. It has been shown that silver nanoparticles, as medicament and not an irrigant,

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have potential to eliminate residual bacterial biofilms during root canal disinfection [17]. Silver (Ag)-loaded mesoporous bioactive material shows a potent antibacterial activity against *E. faecalis* in root canalsof human teeth [18, 19]. Moreover, silver nanoparticles have been used as a vehicle and exhibited anti-biofilm efficacy for calcium hydroxide medicament against *E. faecalis* [20]. Silver nanoparticles can therefore be used to effectively disinfect *E. faecalis*. However, the actual mechanism for silver nanoparticles in *E. faecalis* disinfection during root canal treatment remains unclear.

In this study, we applied RNA-sequencing technology to examine the transcriptome changes of *E. faecalis* after silver nanoparticle treatment. We found that about 100 genes were differentially expressed in *E. faecalis* after silver nanoparticle treatment. The GO terms and KEGG pathways that these genes may belong were also analyzed.

2. MATERIALS AND METHODS

2.1. Synthesis and Characterization of Silver Nanoparticles

Oligonucleotide 5′-$[C_3TAA]_3C_3T$-3′ (>98%, HPLC) was purchased from Takara Bio (Dalian, China). Silver nanoparticles were synthesized through reduction of $AgNO_3$ aqueous solution by $NaBH_4$ in the presence of DNA template according to the method described in published reports [21].

Oligonucleotides 5′-$[C_3TAA]_3C_3T$-3′ (13.6 μM) dissolved in 10 mM PBS buffer solution (pH 5.0) were first annealed by heating at 95 °C for 5 min and cooling down to 4 °C. 600 μM $AgNO_3$ aqueous solution was then added into above annealed DNA sample at $[Ag^+]$/[base] molar ratio of 2.0, followed by stirring for 1 min. 600 μM $NaBH_4$ solution was added into the above annealed $AgNO_3$ solution at $Ag^+/NaBH_4$ ratio of 1.0 under stirring. Biocompatible silver nanoparticles were then prepared.

The DNA-templated silver nanoparticles were characterized using transmission electron microscopy (TEM) on a JEM-2010FEF model (JEOL Ltd., Tokyo, Japan). UV spectra were performed on a Cary 300 UV/vis spectrophotometer equipped with a digital circulating water bath (Varian Medical Systems, Palo Alto, CA).

2.2. Culture of *Enterococcus faecalis*

E. faecalis strain ATCC 29212 was cultured using Brain-Heart infusion (BHI) medium at 37 °C in an anaerobic environment, and in the absence or presence of silver nanoparticles. The growth of *E. faecalis* was evaluated using spectrophotometry.

2.3. Preparation and Sequencing of RNA Samples

E. faecalis was cultured in triplicate (S1, S2, and S3) in BHI medium containing 90 Mm silver nanoparticles for 3.5 h (treatment group); or cultured in triplicate (WA, WB, and WC) in BHI medium for 2.5 h (control group). Total RNA was extracted from each culture using TRIzol reagent (Invitrogen, Carlsbald, CA) according to the manufacturer's protocol. After quality assurance, the RNA samples were processed and sequenced using Illumina Hi-Seq™ 2500 according to the standard procedure. Raw data were obtained after base calling and conversion of sequenced reads. The raw data were filtered using Trimmomatic v0.32 [22]. After removal of reading with <30 quality or one end, the remaining data were used for the transcriptome analysis.

2.4. Transcriptome Analysis

The structure and levels of transcripts were analyzed by aligning the filtered readings to the reference genome (*E. faecalis* ATCC 29212, NCBI) using Rockhopper software [23]. The differential gene expression was analyzed according to the levels of transcripts using edgeR (v2.2.5) and TMM normalization software [24]. $P < 0.05$ and FDR < 0.1 were considered statistically significant. Hierarchical clustering was performed according to the transcript levels of the differentially-expressed genes using R.

2.5. Gene Ontology (GO) Analysis

To reveal the function of the genome of *E. faecalis* and differentially-expressed genes, we performed Gene Ontology analysis (GO, http://www.geneontology.org/) [25–27]. The genome of *E. faecalis* and differentially-expressed genes were first annotated through alignment to bacterial proteins whose sequence and function were known in the SWISSPORT database using BLAST. The GO annotation was downloaded from pertinent Biomart databases (Ensembl). GO term enrichment analysis was performed using topGO [28].

2.6. Pathway Analysis

The genome of *E. faecalis* and the differentially-expressed genes were annotated through alignment to bacteria proteins whose sequence and function were known in KEGG database using BLAST. Based on these annotations, an analysis of the KEGG pathway in *E. faecalis* was performed.

3. RESULTS AND DISCUSSION

3.1. Synthesis of Silver Nanoparticles and Effects on *Enterococcus faecalis* Growth

To examine the effect of silver nanoparticles on *E. faecalis* growth, we synthesized and characterized the DNA-templated silver nanoparticles first. These particles were uniform with an average diameter of 5.2 nm (Figs. 1(a and b)). The typical absorbance peaks at 260 nm and 450 nm (Fig. 1(c)). We then treated *E. faecalis* with silver nanoparticles and determined the growth of *E. faecalis* using spectrophotometry. Results showed that silver

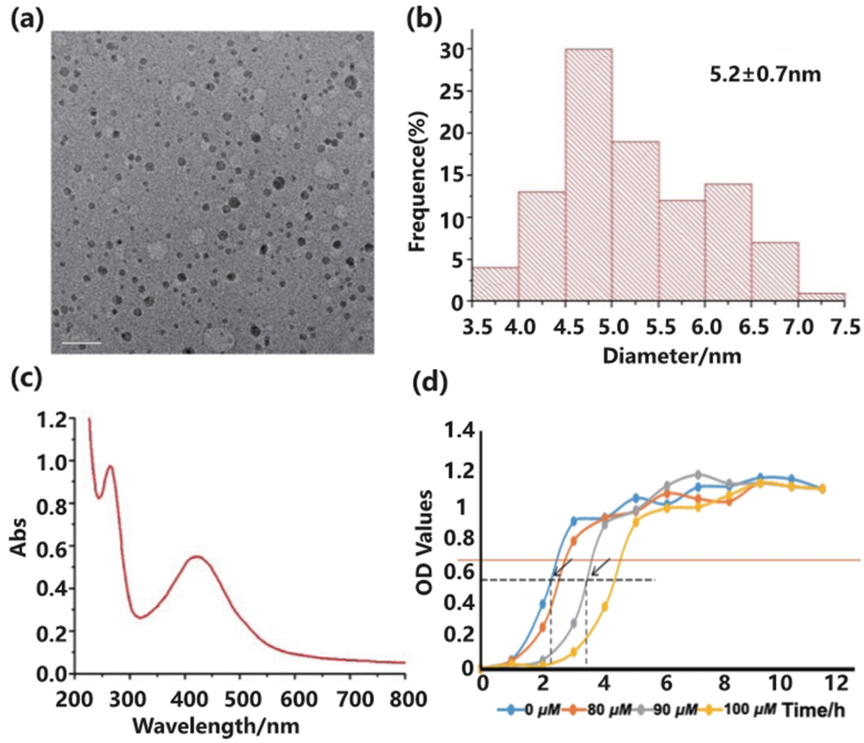

Figure 1. (a) TEM images of DNA-templated silver nanoparticles, the scale bar is 20 nm. (b) Histogram for particle size. (c) UV spectra for DNA-templated silver nanoparticles. (d) Effects of silver nanoparticles on *Enterococcus faecalis* growth.

nanoparticles significantly inhibited the growth of *E. faecalis* by prolonging the lag phases in a dose dependent manner (Fig. 1(d)). And our study found that the silver nanoparticles significantly inhibited the growth of *E. faecalis* by prolonging the lag phases in a dose dependent manner. This was consistent with previous studies supporting application of silver nanoparticles for disinfection of *E. faecalis* in root canal therapy [17–20].

3.2. RNA-Sequencing and Identification of DEGs in *Enterococcus faecalis* Treated with Silver Nanoparticles

To further characterize the suppressive effect mechanism for silver nanoparticles on *E. faecalis*, we extracted RNA samples from *E. faecalis* cultures growing in the presence of silver nanoparticles (90 μM) for 3.5 h or growing without silver nanoparticles for 2.5 h (when the OD values of *E. faecalis* cultures between these two groups was about the same). In total, $>1.8 \times 10^7$ clean reads were generated from all the samples, and the mapping rates ranged from 93%–97%. The distribution of read counts are shown in Figures 2(a) and (b).

Using $P < 0.05$ and FDR < 0.1 significance criterion, we detected a total of 100 differentially-expressed genes (3 upregulated and 97 downregulated differentially-expressed genes) in the *Enterococcus faecalis* Treated with Silver Nanoparticles groups versus the untreated group (Fig. 2(c)). Top 10 significantly differentially-expressed genes in *E. faecalis* after silver nanoparticle treatment are listed in Table I. Hierarchical clustering of differentially-expressed genes revealed a distinct expression signature of mRNAs in the silver-treated groups compared to untreated group. The results showed that the differentially-expressed genes were divided into multiple hierarchical clusters (Fig. 2(d)).

3.3. Functional Annotation of Differentially Expressed Genes

According to the GO enrichment analysis, differentially-expressed genes were significantly enriched in 20 biological processes, 3 cellular components, and 20 molecular functions (Table II). The enriched GO terms are shown in Table II and Figure 3.

Carbohydrate metabolic process ($P = 2.10$E–05), cell adhesion ($P = 1.40$E–04), biological adhesion ($P = 1.40$E–04), peptide transport ($P = 2.00$E–04), and amide transport ($P = 2.00$E–04) were the most significantly enriched in the biological process; 6-phospho-beta-glucosidase activity ($P = 5.30$E–04), beta-glucosidase activity ($P = 9.10$E–04), glucosidase activity ($P = 5.11$E–03), active transmembrane transporter activity ($P = 1.71$E–02), carbohydrate transmembrane transporter activity ($P = 2.12$E–02), carbohydrate transporter activity ($P = 2.12$E–02), were significantly enriched in the

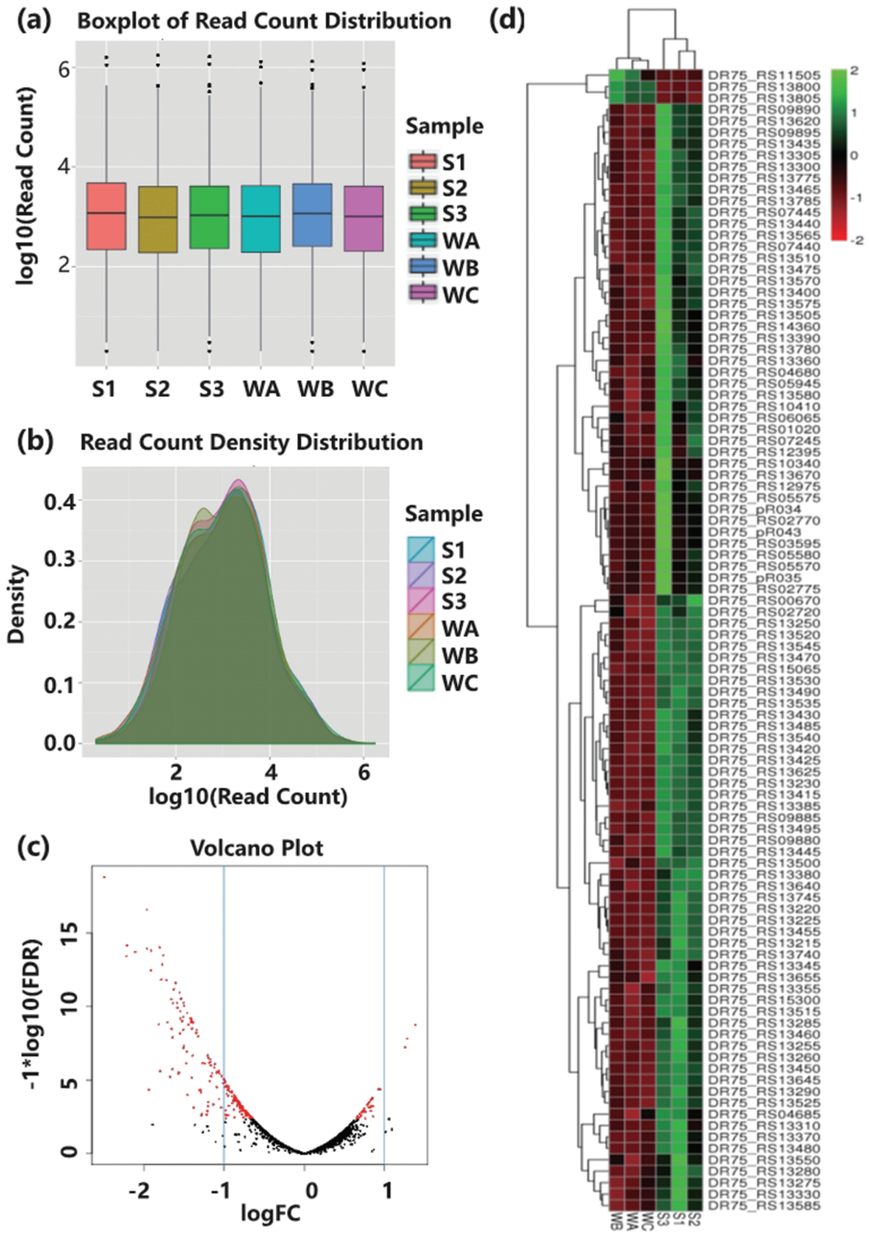

Figure 2. (a) Read count distribution. (b) Boxplot and density distribution. (c) Volcano plot of differentially-expressed genes. Red dots: Differentially-expressed genes. Horizontal axis: Fold changes of gene expression. Vertical axis: Statistical significance of expression changes of gene. (d) Hierarchical clustering of differential gene expression.

molecular function; and membrane (P = 1.00E–02), plasma membrane (P = 1.40E–02), cell periphery (P = 1.80E–02), were significantly enriched in the cellular component. The most significant biological processes were related to transport and metabolism, such as single-organism carbohydrate metabolic process, single-organism transport, organic substance transport, carbohydrate metabolic process, transport, localization, and establishment of localization. The molecular function GO terms were also related to transport and metabolism, such as intramolecular transferase activity, 6-phospho-beta-glucosidase activity, beta-glucosidase activity, glucosidase activity, carbohydrate transmembrane transporter activity, carbohydrate transporter activity, and active transmembrane transporter activity.

Based on the KEGG enrichment analysis, the differentially-expressed genes in the silver-treated groups belonged to two pathway classes (environmental information processing and metabolism) and were involved in 19 pathways (Table III). ABC transporters and

Table I. Top 10 differentially-expressed genes in *Enterococcus faecalis* after silver nanoparticles treatment.

GID	logFC	logCPM	*P* value	FDR	Trend
DR75_RS13565	−2.492564664	2.091191183	1.56E–19	4.77E–16	Down
DR75_RS13225	−1.963257124	7.530019664	2.65E–17	4.04E–14	Down
DR75_RS13570	−2.211214909	1.591405627	7.04E–15	7.12E–12	Down
DR75_RS13540	−1.802793445	5.595959519	9.45E–15	7.12E–12	Down
DR75_RS13220	−1.960847525	2.765621708	1.17E–14	7.12E–12	Down
DR75_RS13435	−1.908130143	3.13300728	1.59E–14	8.07E–12	Down
DR75_RS13445	−2.107121625	1.83670944	1.91E–14	8.34E–12	Down
DR75_RS13775	−1.757372336	5.998373055	3.38E–14	1.25E–11	Down
DR75_RS13230	−2.21699092	1.313750832	3.70E–14	1.25E–11	Down
DR75_RS13450	−1.769951233	4.001502818	1.38E–13	4.22E–11	Down
DR75_RS13565	−2.492564664	2.091191183	1.56E–19	4.77E–16	Down

Table II. Enriched GO terms of differentially-expressed genes in *Enterococcus faecalis* after silver nanoparticle treatment.

GO. ID	Term	No. of DEGs	*P* value (classic Fisher test)	Gene list
Biological process				
GO:0005975	Carbohydrate metabolic process	15	2.10E–05	DR75_RS00670, DR75_RS01020, DR75_RS02770, DR75_ RS02775, DR75_ RS04685, DR75_RS07245, DR75_RS12395, DR75_ RS13300, DR75_ RS13370, DR75_RS13400, DR75_RS13440, DR75_ RS13450, DR75_ RS13455, DR75_RS13520, DR75_RS13645
GO:0007155	Cell adhesion	3	1.40E–04	DR75_ RS05580, DR75_RS13575, DR75_RS13655
GO:0022610	Biological adhesion	3	1.40E–04	DR75_RS05580, DR75_RS13575, DR75_RS13655
GO:0015833	Peptide transport	4	2.00E–04	DR75_RS09880, DR75_RS09885, DR75_RS09890, DR75_RS09895
GO:0042886	Amide transport	4	2.00E–04	DR75_RS09880, DR75_RS09885, DR75_RS09890, DR75_RS09895
GO:0015796	Galactitol transport	2	1.12E–03	DR75_RS13440, DR75_RS13450
GO:0019402	Galactitol metabolic process	2	1.12E–03	DR75_RS13440, DR75_RS13450
GO:0044765	Single-organism transport	14	1.19E–03	DR75_RS02720, DR75_RS05575, DR75_RS05580, DR75_RS07440, DR75_RS09880, DR75_RS09885, DR75_RS09890, DR75_RS09895, DR75_RS13305, DR75_RS13440, DR75_RS13445, DR75_RS13450, DR75_RS13575, DR75_RS13655
GO:0006810	Transport	16	1.70E–03	DR75_RS02720, DR75_RS05570, DR75_RS05575, DR75_RS05580, DR75_RS07440, DR75_RS09880, DR75_RS09885, DR75_RS09890, DR75_RS09895, DR75_RS13305, DR75_RS13440, DR75_RS13445, DR75_RS13450, DR75_RS13565, DR75_RS13575, DR75_RS13655
GO:0051179	Localization	16	1.70E–03	DR75_RS02720, DR75_RS05570, DR75_RS05575, DR75_RS05580, DR75_RS07440, DR75_RS09880, DR75_RS09885, DR75_RS09890, DR75_RS09895, DR75_RS13305, DR75_RS13440, DR75_RS13445, DR75_RS13450, DR75_RS13565, DR75_RS13575, DR75_RS13655
GO:0051234	Establishment of localization	16	1.70E–03	DR75_RS02720, DR75_RS05570, DR75_RS05575, DR75_RS05580, DR75_RS07440, DR75_RS09880, DR75_RS09885, DR75_RS09890, DR75_RS09895, DR75_RS13305, DR75_RS13440, DR75_RS13445, DR75_RS13450, DR75_RS13565, DR75_RS13575, DR75_RS13655
GO:0008104	Protein localization	4	2.44E–03	DR75_RS09880, DR75_RS09885, DR75_RS09890, DR75_RS09895
GO:0015031	Protein transport	4	2.44E–03	DR75_RS09880, DR75_RS09885, DR75_RS09890, DR75_RS09895
GO:0045184	Establishment of protein localization	4	2.44E–03	DR75_RS09880, DR75_RS09885, DR75_RS09890, DR75_RS09895

Table II. Continued.

GO. ID	Term	No. of DEGs	*P* value (classic Fisher test)	Gene list
GO:0071702	Organic substance transport	10	2.99E–03	DR75_RS02720, DR75_RS07440, DR75_RS09880, DR75_RS09885, DR75_RS09890, DR75_RS09895, DR75_RS13305, DR75_RS13440, DR75_RS13445, DR75_RS13450
GO:0044723	Single-organism carbohydrate metabolic process	9	3.15E–03	DR75_RS02770, DR75_RS02775, DR75_RS04685, DR75_RS13370, DR75_RS13400, DR75_RS13440, DR75_RS13450, DR75_RS13455, DR75_ RS13520
GO:0042732	D-xylose metabolic process	2	3.28E–03	DR75_RS13370, DR75_RS13400
GO:0006059	Hexitol metabolic process	2	6.42E–03	DR75_RS13440, DR75_RS13450
GO:0015791	Polyol transport	2	6.42E–03	DR75_RS13440, DR75_RS13450
GO:0015850	Organic hydroxy compound transport	2	6.42E–03	DR75_RS13440, DR75_RS13450
Cellular component				
GO:0016020	Membrane	17	1.00E–02	DR75_RS02720, DR75_RS05570, DR75_RS05575, DR75_RS05580, DR75_RS07440, DR75_RS09880, DR75_RS09885, DR75_RS09890, DR75_RS09895, DR75_RS13305, DR75_RS13450, DR75_RS13490, DR75_RS13570, DR75_RS13575, DR75_RS13645, DR75_RS13655, DR75_RS13780
GO:0005886	Plasma membrane	16	1.40E–02	DR75_RS02720, DR75_RS05570, DR75_RS05575, DR75_RS05580, DR75_RS09880, DR75_RS09885, DR75_RS09890, DR75_RS09895, DR75_RS13305, DR75_RS13450, DR75_RS13490, DR75_ RS13570, DR75_RS13575, DR75_RS13645, DR75_RS13655, DR75_RS13780
GO:0071944	Cell periphery	16	1.80E–02	DR75_ RS02720, DR75_RS05570, DR75_RS05575, DR75_RS05580, DR75_RS09880, DR75_RS09885, DR75_RS09890, DR75_RS09895, DR75_RS13305, DR75_RS13450, DR75_RS13490, DR75_RS13570, DR75_RS13575, DR75_RS13645, DR75_RS13655, DR75_RS13780
Molecular function				
GO:0008706	6-phospho-beta-glucosidase activity	3	5.30E–04	DR75_RS01020, DR75_RS07245, DR75_RS12395
GO:0008422	Beta-glucosidase activity	3	9.10E–04	DR75_ RS01020, DR75_RS07245, DR75_RS12395
GO:0090584	Protein-phosphocysteine-galactitol-phosphotransferase system transporter activity	2	9.50E–04	DR75_RS13440, DR75_RS13450
GO:0015926	Glucosidase activity	3	5.11E–03	DR75_RS01020, DR75_RS07245, DR75_RS12395
GO:0090563	Protein-phosphocysteine-sugar phosphotransferase activity	2	5.48E–03	DR75_RS13440, DR75_RS13450
GO:0022804	Active transmembrane transporter activity	8	1.71E–02	DR75_RS05575, DR75_RS07440, DR75_RS13305, DR75_ RS13440, DR75_RS13445, DR75_RS13450, DR75_RS13490, DR75_RS13570
GO:0015144	Carbohydrate transmembrane transporter activity	4	2.12E–02	DR75_RS13305, DR75_RS13440, DR75_RS13445, DR75_RS13450
GO:1901476	Carbohydrate transporter activity	4	2.12E–02	DR75_RS13305, DR75_RS13440, DR75_RS13445, DR75_RS13450
GO:0016868	Intramolecular transferase activity, phosphotransferases	2	2.98E–02	DR75_RS00670, DR75_RS13520
GO:0004742	Dihydrolipoyllysine-residue acetyltransferase activity	1	3.12E–02	DR75_RS02775
GO:0008869	Galactonate dehydratase activity	1	3.12E–02	DR75_RS13455
GO:0008962	Phosphatidylglycerophosphatase activity	1	3.12E–02	DR75_RS13785
GO:0009045	Xylose isomerase activity	1	3.12E–02	DR75_ RS13400
GO:0015195	L-threonine transmembrane transporter activity.	1	3.12E–02	DR75_RS13490
GO:0015562	Efflux transmembrane transporter activity	1	3.12E–02	DR75_RS13490

Table II. Continued.

GO. ID	Term	No. of DEGs	*P* value (classic Fisher test)	Gene list
GO:0015565	Threonine efflux transmembrane transporter activity	1	3.12E–02	DR75_RS13490
GO:0015577	Galactitol transmembrane transporter activity	1	3.12E–02	DR75_RS13450
GO:0016418	S-acetyltransferase activity	1	3.12E–02	DR75_RS02775
GO:0016990	Arginine deiminase activity	1	3.12E–02	DR75_ RS11505
GO:0022889	Serine transmembrane transporter activity	1	3.12E–02	DR75_RS13490

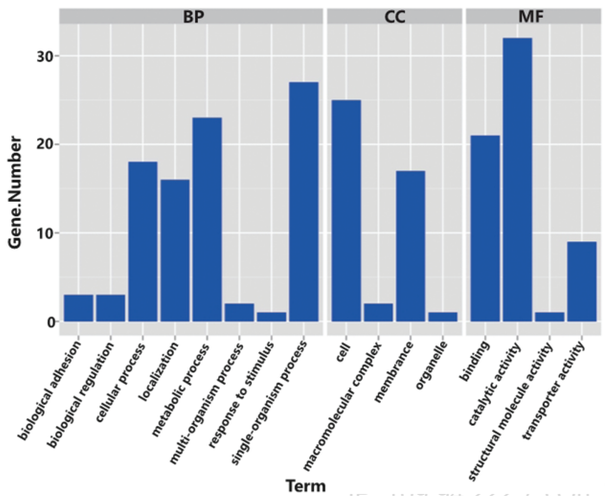

Figure 3. GO enrichment of DEGs in *Enterococcus faecalis* treated with silver nanoparticles.

phosphotransferase system (PTS) were the most involved pathways, followed by glycolysis/gluconeogenesis, amino sugar and nucleotide sugar metabolism, fructose and mannose metabolism, galactose metabolism, arginine and proline metabolism, ascorbate and aldarate metabolism, citrate cycle (TCA cycle), pyruvate metabolism, starch and sucrose metabolism, HIF-1 signaling pathway, glycine, serine and threonine metabolism, butanoate metabolism, glyoxylate and dicarboxylate metabolism, pentose and glucoronate interconversions, pentose phosphate pathway, methane metabolism, and purine metabolism. According to the KEGG enrichment analysis, pathways related to carbohydrate and energy metabolism were mostly involved. Citrate cycle (TCA cycle) is a key metabolic pathway that connects carbohydrate, fat, and protein metabolism. Some of DEGs, like DR75_RS02770 and DR75_RS02775 were enriched in Citrate cycle (Fig. 4). The TCA cycle maybe a major mechanism for *E. faecalis* with silver nanoparticles

Table III. KEGG pathways enrichment analysis for differentially expressed genes.

Pathway class	Pathway name	KO	Total gene	Target gene	Gene list
Environmental information processing; membrane transport	ABC transporters	ko02010	95	11	DR75_RS06065; DR75_RS07440; DR75_RS10340; DR75_RS13565; DR75_RS13570; DR75_RS13575; DR75_RS15065; DR75_RS02720; DR75_RS05570; DR75_RS05575; DR75_RS05580;
Environmental information processing; membrane transport	Phosphotransferase system (PTS)	ko02060	83	11	DR75_RS10410; DR75_RS13305; DR75_RS13310; DR75_RS13380; DR75_RS13385; DR75_RS13390; DR75_RS13440; DR75_RS13445; DR75_RS13450; DR75_RS13500; DR75_RS13505;
Metabolism; carbohydrate metabolism	Glycolysis/ Gluconeogenesis	ko00010	41	8	DR75_RS07245; DR75_RS12395; DR75_RS13305; DR75_RS13310; DR75_RS13520; DR75_RS02770; DR75_RS02775; DR75_RS01020;
Metabolism; carbohydrate metabolism	Amino sugar and nucleotide sugar metabolism	ko00520	63	7	DR75_RS10410; DR75_RS13300; DR75_RS13305; DR75_RS13310; DR75_RS13380; DR75_RS13385; DR75_RS13390;
Metabolism; carbohydrate metabolism	Fructose and mannose metabolism	ko00051	53	5	DR75_RS10410; DR75_RS13380; DR75_RS13385; DR75_RS13390; DR75_RS13400;
Metabolism; carbohydrate metabolism	Galactose metabolism	ko00052	27	4	DR75_RS13440; DR75_RS13445; DR75_RS13450; DR75_RS13455;

Table III. Continued.

Pathway class	Pathway name	KO	Total gene	Target gene	Gene list
Metabolism; carbohydrate metabolism	Fructose and mannose metabolism	ko00051	53	5	DR75_RS10410; DR75_RS13380; DR75_RS13385; DR75_RS13390; DR75_RS13400;
Metabolism; carbohydrate metabolism	Galactose metabolism	ko00052	27	4	DR75_RS13440; DR75_RS13445; DR75_RS13450; DR75_RS13455;
Metabolism; amino acid metabolism	Arginine and proline metabolism	ko00330	22	2	DR75_RS11505; DR75_RS13435;
Metabolism; carbohydrate metabolism	Ascorbate and aldarate metabolism	ko00053	9	2	DR75_RS13500; DR75_RS13505;
Metabolism; carbohydrate metabolism	Citrate cycle (TCA cycle)	ko00020	10	2	DR75_RS02770; DR75_RS02775;
Metabolism; carbohydrate metabolism	Pyruvate metabolism	ko00620	25	2	DR75_RS02770; DR75_RS02775;
Metabolism; carbohydrate metabolism	Starch and sucrose metabolism	ko00500	17	2	DR75_RS13310; DR75_RS00670;
Environmental information processing; signal transduction	HIF-1 signaling pathway	ko04066	5	1	DR75_RS02770;
Metabolism; amino acid metabolism	Glycine, serine and threonine metabolism	ko00260	19	1	DR75_RS13520;
Metabolism; carbohydrate metabolism	Butanoate metabolism	ko00650	10	1	DR75_RS02770;
Metabolism; carbohydrate metabolism	Glyoxylate and dicarboxylate metabolism	ko00630	11	1	DR75_RS13435;
Metabolism; carbohydrate metabolism	Pentose and glucuronate interconversions	ko00040	11	1	DR75_RS13400;
Metabolism; carbohydrate metabolism	Pentose phosphate pathway	ko00030	28	1	DR75_RS13435;
Metabolism; energy metabolism	Methane metabolism	ko00680	14	1	DR75_RS13520;
Metabolism; nucleotide metabolism	Purine metabolism	ko00230	63	1	DR75_RS13230;

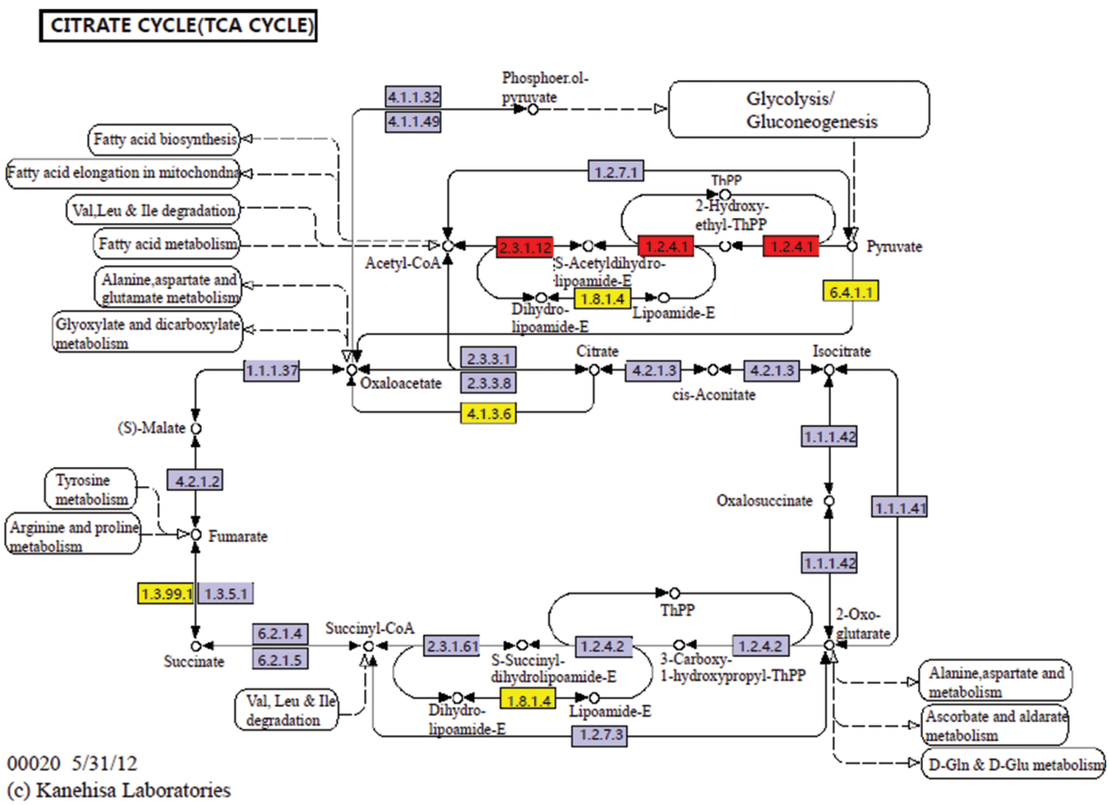

Figure 4. KEGG pathways involved by genes in *Enterococcus faecalis*. Yellow rectangular boxes represent the genes found in *Enterococcus faecalis*. The red stands for differentially-expressed genes.

treatment. These indicated consistent changes in the transcriptome of *E. faecalis* after silver nanoparticles treatment, thus providing a mechanism for supporting the application of silver nanoparticles to disinfect *E. faecalis* during and after dental root canal therapy.

Of the top 10 significantly differentially-expressed genes in *E. faecalis* after silver nanoparticle treatment, 6 genes were involved in 5 pathways: DR7 5_RS13565 (mtsC) and DR75_RS13570 (mtsB) in ABC transporters pathways; DR75_RS13435 (eda ec:4.1.3.16 ec:4.1.2.14) in pentose phosphate pathway; DR75_RS13445 (PTS-Gat-EIIB ec:2.7.1.69) in galactose metabolism pathway; DR75_RS13230 (E2.7.4.3 ec:2.7.4.3) in purine metabolism pathway; and DR75_RS13450 (PTS-Gat-EIIC) in phosphotransferase system (PTS) pathways. No data were found for the rest four genes (DR75_RS13225, DR75_RS13540, DR75_RS13220, and DR75_RS13775), and their involvement in the KEGG pathways deserved further investigation.

These results suggested that the silver nanoparticles treatment of *E. faecalis* may cause significant changes in environmental information processing, including membrane transport and signal transduction, and metabolism including carbohydrate, amino acid, nucleotide, and energy metabolism.

4. CONCLUSIONS

In conclusion, we have confirmed the suppressive effect of silver nanoparticles on *E. faecalis* growth, and identified at least 100 genes that were differentially expressed in *E. faecalis* after treatment. Silver nanoparticles likely suppressed *E. faecalis* growth through affecting the pathways related to environmental information processing, including membrane transport and signal transduction and metabolism including carbohydrate, amino acid, nucleotide, and energy metabolism.

References and Notes

1. Pirani, C., Bertacci, A., Cavrini, F., Foschi, F., Acquaviva, G.L., Prati, C. and Sambri, V.S., **2008**. Recovery of *Enterococcus faecalis* in root canal lumen of patients with primary and secondary endodontic lesions. *New Microbiologica, 31*(2), pp.235–240.
2. Chávez de Paz, L.E., Davies, J.R., Bergenholtz, G. and Svensäter, G., **2015**. Strains of *Enterococcus faecalis* differ in their ability to coexist in biofilms with other root canal bacteria. *International Endodontic Journal, 48*(10), pp.916–925.
3. Jr, J.F.S., Rôças, I.N., Souto, R., De, U.M. and Colombo, A.P., **2002**. Actinomyces species, streptococci, and *Enterococcus faecalis* in primary root canal infections. *Journal of Endodontics, 28*(3), pp.168–172.
4. Saleh, I.M., Ruyter, I.E., Haapasalo, M. and Ørstavik D., **2010**. Survival of *Enterococcus faecalis* in infected dentinal tubules after root canal filling with different root canal sealers *in vitro*. *International Endodontic Journal, 37*(3), pp.193–198.
5. George, S., Kishen, A. and Song, P., **2005**. The role of environmental changes on monospecies biofilm formation on root canal wall by *Enterococcus faecalis*. *Journal of Endodontics, 31*(12), pp.867–872.
6. Atilapektas, B., Yurdakul, P., Gülmez, D. and Görduysus, O., **2013**. Antimicrobial effects of root canal medicaments against *Enterococcus faecalis* and streptococcus mutans. *International Endodontic Journal, 46*(5), pp.413–418.
7. Du, T., Wang, Z., Shen, Y., Ma, J., Cao, Y. and Haapasalo, M., **2015**. Combined antibacterial effect of sodium hypochlorite and root canal sealers against *Enterococcus faecalis* biofilms in dentin canals. *Journal of Endodontics, 41*(8), pp.1294–1298.
8. Karkare, S.R., Ahire, N.P. and Khedkar, S.U., **2015**. Comparative evaluation of antimicrobial activity of hydroalcoholic extract of aloe vera, garlic, and 5% sodium hypochlorite as root canal irrigants against *Enterococcus faecalis*: An *in vitro* study. *Journal of Indian Society of Pedodontics & Preventive Dentistry, 33*(4), pp.274–278.
9. Ye, W., Fan, B., Purcell, W., Meghil, M.M., Cutler, C.W., Bergeron, B.E., Ma, J., Tay, F.R. and Niu, L., **2018**. Anti-biofilm efficacy of root canal irrigants against *in-situ Enterococcus faecalis* biofilms in root canals, isthmuses and dentinal tubules. *Journal of Dentistry, 79*, pp.68–76.
10. Li, Y., Sun, K., Ye, G., Liang, Y., Pan, H., Wang, G., Zhao, Y., Pan, J., Zhang, J. and Fang, J., **2015**. Evaluation of cold plasma treatment and safety in disinfecting 3-week root canal *Enterococcus faecalis* biofilm *in vitro*. *Journal of Endodontics, 41*(8), pp.1325–1330.
11. Licata, M.E., Albanese A., Campisi, G., Geraci, D.M., Russo, R. and Gallina, G., **2013**. Effectiveness of a new method of disinfecting the root canal, using Er, CR: YSGG laser to kill *Enterococcus faecalis* in an infected tooth model. *Lasers in Medical Science, 30*(2), pp.707–712.
12. Naghdi, A., Behrooz, E. and Khalilak, Z., **2015**. The minimum residual root thickness after using protaper, race and gates-glidden drills: A cone beam computerized tomography study. *European Journal of Dentistry, 9*(2), pp.228–233.
13. Pladisai, P., Ampornaramveth, R.S. and Chivatxaranukul, P., **2016**. Effectiveness of different disinfection protocols on the reduction of bacteria in *Enterococcus faecalis* biofilm in teeth with large root canals. *Journal of Endodontics, 42*(3), pp.460–464.
14. Ghorbanzadeh, A., Fekrazad, R., Bahador, A., Ayar, R., Tabatabai, S. and Asefi, S., **2018**. Evaluation of the antibacterial efficacy of various root canal disinfection methods against *Enterococcus faecalis* biofilm. An *ex-vivo* study. *Photodiagnosis and Photodynamic Therapy, 24*, pp.44–51.
15. Kang, J., Tangadanchu, V.K.R., Gopala, L., Gao, W.W., and Zhou, C.H., **2017**. Novel potentially antibacterial naphthalimide-derived metronidazoles: Design, synthesis, biological evaluation and supramolecular interactions with DNA, human serum albumin and topoisomerase II. *Chinese Chemical Letters, 28*(7), pp.1369–1374.
16. Zhang, T.Y., Li, C., Tian, Y.S., Li, J.J. and Piao, H.R., **2017**. Synthesis and biological evaluation of dihydrotriazine derivatives as potential antibacterial agents. *Chinese Chemical Letters, 28*(8), pp.1737–1742.
17. Wu, D., Fan, W., Kishen, A., Gutmann, J.L. and Fan, B., **2014**. Evaluation of the antibacterial efficacy of silver nanoparticles against *Enterococcus faecalis* biofilm. *Journal of Endodontics, 40*(2), pp.285–290.
18. Fan, W., Wu, D., Ma, T. and Fan, B., **2015**. Ag-loaded mesoporous bioactive glasses against *Enterococcus faecalis* biofilm in root canal of human teeth. *Dental Materials Journal, 34*(1), pp.54–60.
19. Kung, J.C., Chen, Y.J., Chiang, Y., Lee, C.L., Yang-Wang, Y.T., Hung, C.C. and Shih, C.J. **2018**. Antibacterial activity of silver nanoparticle (AgNP) confined mesoporous structured bioactive powder against *Enterococcus faecalis* infecting root canal systems. *Journal of Non-Crystalline Solids*, 502, pp.62–70.
20. Afkhami, F., Pourhashemi, S.J., Sadegh, M., Salehi, Y. and Fard, M.J., **2015**. Antibiofilm efficacy of silver nanoparticles as a vehicle for calcium hydroxide medicament against *Enterococcus faecalis*. *Journal of Dentistry, 43*(12), pp.1573–1579.
21. Li, W., Liu, L., Fu, Y., Sun, Y., Zhang, J. and Zhang, R., **2013**. Effects of polymorphic DNA on the fluorescent properties of silver

nanoclusters. *Photochemical and Photobiological Sciences, 12*(10), pp.1864–1872.
22. Bolger, A.M., Lohse, M. and Usadel, B., **2014**. Trimmomatic: A flexible trimmer for illumina sequence data. *Bioinformatics, 30*(15), pp.2114–2120.
23. Tjaden, B., **2015**. De novo assembly of bacterial transcriptomes from RNA-seq data. *Genome Biology, 16*(1), pp.1–11.
24. Robinson, M.D., McCarthy, D.J. and Smyth, G.K., **2010**. edgeR: A Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics, 26*, pp.139–140.
25. The Gene Ontology Consortium, **2004**. The gene ontology (GO) database and informatics resource. *Nucleic Acids Research, 32*, pp.258–261.
26. Barrell, D., Dimmer, E., Huntley, R.P., Binns, D., O'Donovan, C. and Apweiler, R., **2009**. The goa database in 2009—An integrated gene ontology annotation resource. *Nucleic Acids Research, 37*(1), pp.396–403.
27. Huntley, R.P., Sawford, T., Mutowomeullenet, P., Shypitsyna, A., Bonilla, C., Martin, M.J. and O'Donovan, C., **2015**. The GOA database: Gene ontology annotation updates for 2015. *Nucleic Acids Research, 43*(Database issue), pp.1057–1063.
28. Alexa, A., Rahnenführer, J. and Lengauer, T., **2006**. Improved scoring of functional groups from gene expression data by decorrelating GO graph structure. *Improved Scoring of Functional Groups from Gene Expression Data by Decorrelating GO Graph Structure*. Oxford University Press.

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