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Transcription Factor Rex in Regulation of Pathophysiology in Oral Pathogens

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SUMMARY

The NAD⁺ and NADH-sensing transcriptional regulator Rex is widely conserved across Gram-positive bacteria. Rex monitors cellular redox poise and controls the expression of genes/operons involved in diverse pathways including alternative fermentation, oxidative stress responses, and biofilm formation. The oral cavity undergoes frequent and drastic fluctuations in nutrient availability, pH, temperature, oxygen tension, saliva, and shear forces. The oral streptococci are major colonizers of oral mucosa and tooth surfaces and include commensals as well as opportunistic pathogens, including the primary etiological agent of dental caries, *Streptococcus mutans*. Current understanding of the Rex regulon in oral bacteria is mostly based on studies in *S. mutans* and endodontic pathogen *Enterococcus faecalis*. Indeed, other oral bacteria encode homologs of the Rex protein and much is to be gleaned from more in-depth studies. Our current understanding has Rex positioned at the interface of oxygen and energy metabolism. In biofilms, heterogeneous oxygen tension influences the ratio of intracellular NADH and NAD⁺, which is finely tuned through glycolysis and fermentation. In *S. mutans*, Rex regulates the expression of glycolytic enzyme NAD⁺-dependent glyceraldehyde 3-phosphate dehydrogenase, and NADH-dependent fermentation enzymes/complexes lactate dehydrogenase, pyruvate dehydrogenase, alcohol-acetylaldehyde dehydrogenase, and fumarate reductase. In addition, Rex controls the expression of NADH oxidase, a major enzyme used to eliminate oxidative stress and regenerate NAD⁺. Here, we strive to summarize recent studies carried out on the Rex regulators in *S. mutans* and *E. faecalis*. This research has important implications for understanding how Rex monitors redox balance and optimizes fermentation pathways for survival and subsequent pathogenicity.

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Keywords

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INTRODUCTION

The oral cavity houses over 700 different bacterial species (Jenkinson and Lamont 2005), many of which live in communities of heterologous biofilms on the tooth surface called dental plaque. The complexity of oral biofilms prevents homogenous diffusion of environmental compounds including antimicrobials, oxygen and reactive oxygen species (ROS), and metabolic byproducts including organic acids. Thus, biofilms can be considered heterogeneous in terms of genetic regulation at the species level depending on localized conditions (Son *et al.* 2012). Previous studies have demonstrated that reduction potential values of supragingival plaque drops from +294 to −141 mV over the course of seven days (Kenney and Ash 1969) and oxygen tension ranges from 5 to 27 mm Hg depending on the depth of periodontal pockets (Mettraux *et al.* 1984). Oxidative stress occurs when ROS, including superoxide, hydrogen peroxide (H₂O₂), and the hydroxyl radical accumulate at a faster rate than the rate at which they are detoxified (Imlay 2013). Researchers have shown that endogenous H₂O₂ is generated in oral biofilms through metabolic oxygen reduction by some streptococci including *Streptococcus sanguinis*, *Streptococcus sobrinus*, and *Streptococcus gordonii* (Ryan and Kleinberg 1995). In oral streptococci, fermentation pathways and ultimately, acidogenesis are linked to oxygen and ROS through changes in the redox poise (Baldeck and Marquis 2008). Bacteria can sense oxygen tension through monitoring the accumulation of metabolites or the redox state of specific compounds as a result of changes in cellular homeostasis (Wang *et al.* 2008).

Gram-positive oral streptococci nearly ubiquitously express homologs of the NADH- and NAD⁺-sensing transcriptional regulator Rex (Ravcheev *et al.* 2012). Rex was first identified in *Streptomyces coelicolor* for being a redox-responsive transcriptional regulator capable of controlling the transcription of NADH dehydrogenase operon *nuoA-nuoN*, the heme biosynthesis genes *hemACD*, and the cytochrome *bd* terminal oxidase operon *cydABCD* (Brekasis and Paget 2003). Rex proteins have since been characterized in several model microorganisms including *Bacillus subtilis*, *Staphylococcus aureus*, *S. mutans*, and *E. faecalis*. Brekasis and Paget first showed Rex-regulated genes were found to have a consensus *cis* binding site 5'-TGTGNNC NNNTTCACA-3' called the Rex operator (ROP) sites (Brekasis and Paget 2003). More recently, a bioinformatics study has identified optimized ROP sites for eleven phylogenetic taxa comprising more than 110 genomes (Ravcheev *et al.* 2012). Moreover, Rex-mediated transcriptional regulation has been implicated in species-specific genetic targets of diverse organisms (Ravcheev *et al.* 2012). In *S. aureus*, Rex controls the expression of genes linked to nitrate/nitrite respiration, fermentation, and amino acid metabolism (Pagels *et al.* 2010). In cariogenic *S. mutans*, Rex controls the expression of genes linked to fermentation, oxidative stress response, and biofilm formation (Baker *et al.* 2014; Bitoun *et al.* 2012; Bitoun *et al.* 2011). Rex-deficiency in *S. mutans* causes increased sensitivity to exogenous H₂O₂ and increased end-point pH values of stationary phase culture medium. Indeed other commensals commonly found in

the oral cavity encode homologs of Rex (Table 1). In *E. faecalis*, Rex-deficiency causes impaired growth of aerated cultures, which can be rescued by the addition of catalase to the medium suggesting the *rex*-deficient mutant has an impaired ability to cope with oxidative stress (Mehmeti *et al.* 2011; Vesic and Kristich 2013). Recently, a Rex-regulated gene product of *Streptococcus pneumoniae*, AdhE, was shown to increase pneumolysin production under alcohol stress conditions, thereby increasing pneumococcal virulence (Luong *et al.* 2015).

Rex proteins are homodimers with N-terminal winged helix DNA-binding motifs and C-terminal Rossmann fold NADH/NAD⁺-binding motifs. The Rex protein of *S. aureus* was shown to have a K_d of 95 nM for NADH and 150 μ M for NAD⁺ (Pagels *et al.* 2010). The higher binding affinity of Rex to NADH suggests that Rex is poised to modulate gene expression when subtle changes are made to the redox poise. Under basal growth conditions, the high cellular NAD⁺ concentration outcompetes NADH for Rex-binding. Rex-NAD⁺ complex binds the *cis* ROP sites of regulated promoters and negatively regulates the transcription of target genes including *adhE*, *ldh*, and *frdC*, for alcohol-acetylaldehyde dehydrogenase (AdhE), lactate dehydrogenase (Ldh), and fumarate reductase (FrdC), respectively. On the other hand, when the NADH concentration rises against the threshold, NADH outcompetes NAD⁺ for Rex-binding, changes the allosteric conformation of Rex protein, and alleviates genetic repression of target genes (McLaughlin *et al.* 2010; Wang *et al.* 2011). Rex homologs from select oral cocci show species-specific differences in primary amino acid sequence (Figure 1). As seen, helix-turn-helix and Rossmann fold motifs are conserved in Rex homologs among the oral cocci. However, differences in primary amino acid sequences suggest that Rex has evolved to regulate the expression of different genes/operons among the oral cocci. When Rex primary amino acid sequences of select Gram-positive cocci were parsed using Rossmann-fold prediction algorithm Cofactory (Geertz-Hansen *et al.* 2014), there were notable differences in NAD(H) binding potential among Rex homologs (Table 1). Understanding differences in NAD(H) binding to Rex homologues of oral streptococci may help researchers fine-tune species-specific Rex regulons.

FERMENTATION IN *STREPTOCOCCUS MUTANS*

The oral streptococci predominate in dental plaque and use fermentation pathways to generate ATP (Cole 1977). The oral streptococci metabolize a wide range of sugars and sugar alcohols into pyruvate through glycolysis (Abranches *et al.* 2004; Grossiord *et al.* 2003; Lemos *et al.* 2005). NAD⁺ and NADH are the gatekeepers of glycolysis and fermentation, respectively, in the oral streptococci. Recently, glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase was shown to be regulated by Rex in *S. mutans* (Ravcheev *et al.* 2012). In addition, Rex regulates the expression of genes encoding proteins that use the reducing power of NADH during fermentation, including lactate dehydrogenase, pyruvate dehydrogenase, alcohol-acetylaldehyde dehydrogenase, and anaerobic fumarate reductase.

Like other lactic acid bacteria, the default fermentation pathway of *S. mutans* is the catabolism of excess glucose through lactate dehydrogenase to form lactic acid (Korithoski *et al.* 2008). Lactate dehydrogenase uses NADH as an electron donor to convert pyruvate

into lactic acid, thus priming the next cycle of glycolysis by oxidizing NADH back into NAD⁺. The lactate generated is the culprit in the dissolution of the tooth enamel leading to dental caries. However, under conditions of anaerobiosis, glucose limitation, or low dilution rates in continuous culture, fermentation products shift from mainly lactate towards a mixture of lactate, formate, acetate, and ethanol (Yamada and Carlsson 1975). When grown in galactose, *S. mutans* yields a blend of formate, acetate, and ethanol end-products (Abbe *et al.* 1982). *S. mutans* also ferments sugar alcohols, mannitol and sorbitol, into a large quantity of ethanol (Brown and Patterson 1973).

The major point of divergence in *S. mutans* fermentation comes at the point of pyruvate (Guest *et al.* 1989) (Figure 2). Pyruvate can be metabolized into organic acids lactate, acetate, and formate and non-acid ethanol. The differences in byproduct pKa significantly contribute pathogenicity. For example, if pyruvate is metabolized exclusively by pyruvate dehydrogenase (PDH) and bifunctional alcohol/acetylaldehyde dehydrogenase (AdhE), major quantities of ethanol would be formed, reducing *S. mutans* acidogenicity. Under strictly anaerobic conditions, pyruvate-formate lyase (PFL) converts a portion of pyruvate to formate and acetyl-CoA. Acetyl-CoA catabolism can yield ethanol or acetate through the enzymatic activities of alcohol-acetylaldehyde dehydrogenase (AdhE) or phosphotransacetylase (PTA) and acetate kinase (ACK), respectively. Pyruvate-formate lyase activating enzyme (PFL-AE) contains an extremely oxygen-labile iron-sulphur cluster and is inactivated under aerobic conditions (Takahashi-Abbe *et al.* 2003). On the other hand, under aerobic conditions, pyruvate is catabolized to lactate, acetate, or ethanol via the activities of lactate dehydrogenase (LDH), pyruvate oxidase (POX), or the pyruvate dehydrogenase (PDH) complex (Korithoski *et al.* 2008). Previous studies have shown that pyruvate dehydrogenase is regulated by redox potential (Snoep *et al.* 1992). Interestingly, *pdhA* expression was more highly induced in an anaerobic environment in *S. mutans* (Korithoski *et al.* 2008).

REX-DEFICIENCY IN *STREPTOCOCCUS MUTANS*

S. mutans contains a single *rex* gene (SMU.1053) that encodes for a protein of high similarity to the Rex family of NADH- and NAD⁺ transcription factors. In *S. mutans*, microarray experiments comparing gene expression of a *rex*-deficient mutant to the wild-type showed Rex-deficiency up-regulated 25 genes at $P < 0.001$, when the cultures were grown statically at 37°C in an aerobic incubator with 5% CO₂ (Bitoun *et al.* 2011). As shown in other organisms, many of the up-regulated genes/operons in response to Rex-deficiency in *S. mutans* were part of fermentation pathways. *S. mutans* encodes multiple fermentative genes/operons including lactate dehydrogenase (*ldh*, SMU.1115), acetoin dehydrogenase (*adhABCD*, SMU.127-SMU.130), pyruvate dehydrogenase (*pdhABCD*, SMU.1421-SMU.1424), alcohol-acetylaldehyde dehydrogenase (*adhE*, SMU.148), pyruvate-formate lyase (*pfl*, SMU. 402 and *pfl-2*, SMU.493) and fumarate dehydrogenase (*frdC*, SMU.490). Based on microarray data, *pdhA*, *adhE*, *adhA*, and *frdC* were up-regulated by 2.7-fold, 10.0-fold, 2.9-fold, and 4.7-fold, respectively, in a *rex*-deficient mutant as compared to wild-type. In a subsequent study, researchers used electrophoretic mobility binding assays to show Rex binds specifically to the promoters of *rex*, *ldh*, *frdC*, and *adhE* (Bitoun *et al.* 2012). Researchers demonstrated downstream *rex* operon member *guaA* was

also up-regulated in response to Rex-deficiency, suggesting auto-regulation of the two gene *rex* operon (Bitoun *et al.* 2012). A model is shown in Figure 3 with the ROPs of the Rex-regulated genes *adhE*, *rex*, and *ldh* demonstrating the ROPs are found within the -10 and -35 regions of the respective operators. Interestingly, *ldh* was not shown to be differentially regulated in the microarray study despite having two ROP sites upstream of the transcription start site.

Details concerning Rex-mediated transcriptional activation have remained elusive. Using microarray analysis, researchers have shown at least 28 genes down-regulated in *S. mutans* in response to Rex-deficiency. Based on microarray data, *mleS*, *mleP*, *gshR*, and *tpn*, were down-regulated 17.6-fold, 15.4-fold, 18.7-fold, and 40.6-fold respectively, in a *rex*-deficient mutant as compared to wild-type (Bitoun *et al.* 2011). Interestingly, *gshR* encodes a putative glutathione reductase which uses NADPH for the reduction of oxidized glutathione yet Rex has not been shown to be responsive to NADPH. The microarray data was confirmed with Realtime PCR experiments that demonstrated almost 20-fold down-regulation of *gshR* in a *rex*-deficient mutant of *S. mutans* as compared to wild-type (Bitoun *et al.* 2012). In addition, researchers showed that purified recombinant Rex could indeed bind to a region in the promoter of *gshR* (Bitoun *et al.* 2012). No previous Rex-mediated transcriptional activation had since been reported, and further studies are needed to elucidate the *cis* element for Rex-mediated genetic activation.

Researchers also demonstrated that a *rex*-deficient mutant of *S. mutans* was at least 1-log more sensitive to H₂O₂-mediated killing than the wild-type. In addition, the *rex*-deficient mutant displayed an extended lag phase when incubated with paraquat, an oxidative stress inducing agent (Bitoun *et al.* 2011). Researchers have found that the NADH:NAD⁺ ratio is the highest under anaerobic conditions (de Graef *et al.* 1999; Snoep *et al.* 1994). In some oral streptococci, overlapping regulatory mechanisms of NADH oxidase, encoded by the *nox* gene, have linked oxidative stress response with the redox balance (Baker *et al.* 2014; Derr *et al.* 2012; Sheng *et al.* 2010). NADH oxidase is an important enzyme that can restore the basal redox balance by oxidizing existing NADH to NAD⁺ with the concomitant reduction of molecular oxygen to water. This enzyme uses FAD cofactor and cycling disulphides to perform the electron transfer reactions to ultimately replenish the NAD⁺ pools necessary for glycolysis (Derr *et al.* 2012). Researchers tested the ability of Rex to bind the *nox* promoter using electrophoretic mobility shift analysis, and found that Rex can bind the *nox* promoter (*Pnox*) (Baker *et al.* 2014). Co-incubation of Rex with NAD⁺ increased the ability of Rex to bind the *nox* promoter and co-incubation of Rex with NADH resulted in alleviating Rex-*Pnox* interactions. Interestingly, using microarray analysis researchers found that a *nox*-deficient mutant of *S. mutans* had nearly 3-fold elevation in *rex* transcript levels, as compared to wild-type, suggesting NADH oxidase activity changes the NADH:NAD⁺ ratio with downstream alterations in the ability of Rex to regulate itself.

Biofilm formation is a hallmark of *S. mutans* virulence. The glucosyltransferases GtfB, GtfC, and GtfD catalyse extracellular glucose polymer (known as glucan) formation with sucrose as the substrate (Bowen and Koo 2011). The glucans, including both soluble and insoluble ones varying with glycosidic linkages (α -1,3, α -1,4, α -1,6), are used by *S. mutans* to adhere to tooth enamel and the surfaces of other microorganisms in the plaque

community. Researchers noticed that biofilms of the *rex*-deficient strain accumulated 3.5-fold less biomass than wild-type when grown in glucose and 3-fold less biomass than wild-type when grown in sucrose. When grown in glucose and sucrose, biofilms of the *rex*-deficient strain displayed a more porous and rugged architecture than wild-type UA159 with a 2-fold higher glucan to cell ratio. Realtime PCR was carried out on the glucosyltransferase genes, *gtfB*, *gtfC*, and *gtfD* in both the wild-type and *rex*-deficient strain. Studies showed up-regulation of *gtfC* by more than 13-fold in biofilms of the *rex*-deficient strain and subsequent analysis of the *gtfC* promoter revealed a potential ROP site, linking biofilm formation to redox poise (Bitoun *et al.* 2011).

The overlap in the control of redox and oxidative stress response genes is not surprising. Researchers have shown that both hydrogen peroxide and paraquat are more difficult for a *rex*-deficient mutant to overcome as compared to wild-type. In addition, Rex can bind to its own promoter and is subject to auto-regulation.

REX-DEFICIENCY IN *ENTEROCOCCUS FAECALIS*

E. faecalis is a facultative anaerobe, low-GC Gram-positive opportunistic pathogen. *E. faecalis* is a natural commensal of the gastrointestinal tract (Nallapareddy *et al.* 2005). However, due to faecal dissemination and its adaptability to different environments, *E. faecalis* can be routinely associated with enterococcal nosocomial infections including urinary tract infections, surgical wound infections, and bacterial endocarditis (Jett *et al.* 1994; Reffuveille *et al.* 2011). In the oral cavity, *E. faecalis* has been shown to be associated with root caries and with periodontal disease (Preza *et al.* 2008; Sun *et al.* 2009). Virulence factors of *E. faecalis* include bile salt hydrolase, cytolysin toxin, capsular polysaccharides, gelatinase, lipoproteins and other surface-associated LPxTG aggregation substances (Nallapareddy *et al.* 2006; Theilacker *et al.* 2009). Recent studies have shown that cell wall lipoteichoic acid of *E. faecalis* can suppress *Aggregatibacter actinomycetemcomitans* lipopolysaccharide-induced IL-8 expression in human periodontal cells (Im *et al.* 2015).

E. faecalis contains two homologs of Rex, encoded at loci *EF2638* and *EF2933* (Mehmeti *et al.* 2011). The interplay of multiple Rex proteins in a host remains unknown. Researchers characterized the transcriptome of a *rex*-deficient mutant (*rex*, *EF2638*) in *E. faecalis* and found that Rex-deficiency up-regulated genes involved in anaerobic fermentation pathways including pyruvate-formate lyase (*EF1613*), pyruvate-formate lyase activating enzyme (*EF1612*), alcohol/aldehyde dehydrogenase (*EF0900*), anaerobic ribonucleotide reductase (*EF2754-5*), and fumarate reductase (*EF1226-7*). In addition, genes encoding abortive infection protein (*EF2637*) and cell envelope-associated acid phosphatase were also up-regulated in response to Rex-deficiency (Vesic and Kristich 2013). Recently, researchers created a strain of *E. faecalis* V583 deficient of both *ldh1* and *ldh2* (*ldh1.2*), both genes encoding lactate dehydrogenases. Under anaerobiosis, they showed changes in the end-product metabolites of *ldh1.2* as compared to wild-type V583. Researchers showed drastic increases in formate, ethanol, pyruvate, and acetoin production as a result of *ldh1.2* lactate dehydrogenase deficiency (Mehmeti *et al.* 2011). In other studies, researchers showed that the NADH:NAD⁺ ratio decreased more than 2-fold in *rex* as compared to wild-type (Vesic and Kristich 2013). On the other hand, deletion of *EF2933* (*rex2*) maintained a

NADH:NAD⁺ ratio similar to wild-type (Vesic and Kristich 2013). In addition, aerobiosis impairs the growth of *rex* through the production of excess hydrogen peroxide and the addition of catalase to the culture medium rescued the aerobic slow-growth phenotype of the *rex* mutant (Vesic and Kristich 2013).

Increased production of ethanol and pyruvate has been shown to correlate to an increased NADH/NAD⁺ ratio in the lactate dehydrogenase-deficient mutant (Snoep *et al.* 1994). Researchers found at least 22 putative ROP sites upstream of 22 genes/operons that were found to be differentially expressed in their proteome or transcriptome studies of the lactate dehydrogenase deficient mutant (Mehmeti *et al.* 2011). Interestingly, virulence plasmid pAD1 mating pheromone cAD1 precursor protein (*EF3256*) and virulence factor LPxTG cell wall surface anchor family protein (*EF3314*) contain ROP sites in their respective promoters, although direct experimental Rex-mediated regulation of these genes has not been studied (An and Clewell 2002; Creti *et al.* 2009). Like in *S. mutans*, lactate dehydrogenase and alcohol-acetylaldehyde dehydrogenase showed the strongest up-regulation in *rex* and are both preceded by two ROP sites (Bitoun *et al.* 2012; Mehmeti *et al.* 2011).

CONCLUSIONS AND FUTURE DIRECTIONS

Accumulating reports in the literature have provided a more complete understanding of Rex-mediated transcriptional regulation in the oral pathogens *S. mutans* and *E. faecalis*. Like other Gram-positive bacteria, in the oral pathogens the Rex transcription factor negatively regulates alternative energy metabolism pathways by monitoring the NADH:NAD⁺ ratio. In *S. mutans*, Rex-deficiency causes a pH increase of stationary phase culture medium, suggesting activation of alternative fermentation pathways generating non-acid byproducts. More studies testing the end-product metabolite ratios in response to Rex-deficiency would provide pertinent information to understanding alternative fermentation in oral pathogens. Unlike previous studies, Rex has also been postulated to activate the transcription of genes in oral pathogens *S. mutans* and *E. faecalis* (Bitoun *et al.* 2012; Mehmeti *et al.* 2011). Further studies are needed to discern the direct mechanism of gene activations. In *S. mutans*, Rex-activated genes *gshR* (SMU.140), *mleR* (SMU.135), and *tpn* (SMU.1363c) have palindromic ROP sites in their promoters of 5'-AAACACCGCCGCGATGA-3', 5'-AAAGTAACAAGATAGTGG-3', and 5'-AACTACTTCGATATTGA-3', respectively (Bitoun, pers. comm.). In addition, other transcription factors encoded at loci SMU.1409, SMU.1419, and SMU.1361c were differentially regulated in response to Rex-deficiency (Bitoun *et al.* 2011). It is also possible that Rex activates gene expression by modulating the expression of other transcription factors. In *S. mutans*, many genes including *ptsG-rgfB*, *pulA*, *gluQHMP*, *mntABC*, and *nox* have been shown to be regulated by more than one transcription factor (Baker *et al.* 2014; Ravcheev *et al.* 2013). Using a combination of mRNA and proteomic analysis, researchers also suggest that Rex may function as a transcriptional activator in *E. faecalis* (Mehmeti *et al.* 2011; Vesic and Kristich 2013). Continued research into Rex-mediated transcriptional modulation of oral pathogens will yield new insights into the pathogenicity and the mechanisms that regulate.

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Figure 1. ClustalW2 multiple alignment of Rex homologs from select Gram-positive cocci. Identical amino acids conserved across all species are labelled with asterisks (*). Sequences were parsed for helix-turn-helix motifs using and Rossmann folds using computer programs GYM 2.0 and Cofactory v.1.0, respectively.

Figure 2.

Pathways of pyruvate catabolism in *Streptococcus mutans*. LDH, L-lactate dehydrogenase; PFL-AE, pyruvate-formate lyase activating enzyme; PFL, pyruvate-formate lyase; PDH, pyruvate dehydrogenase; POX, pyruvate oxidase; PTA, phosphotransacetylase; AdhE, alcohol-acetaldehyde dehydrogenase; ACK, acetate kinase.

Figure 3.

Rex-mediated repression of the *adhE*, *rex*, and *ldh* genes. When NAD⁺ > NADH, transcription is blocked. In contrast, when NADH > NAD⁺, Rex repression is alleviated and transcription is allowed. The -10 and -35 regions of the promoters are aligned and labelled above. The experimentally confirmed ROPs are bolded.

Table 1

Rex Homologs in selected Gram-positive cocci

Bacterial name	Accession number	NAD(H) Score [¶]	Reference(s)
<i>Streptococcus mutans</i>	NP_721445.1 [#]	0.546	Bitoun et al. (2011), Bitoun et al. (2013), Baker et al. (2014)
<i>Streptococcus pyogenes</i>	WP_002984705.1	0.419	-
<i>Streptococcus gordonii</i>	WP_012000628.1	0.873	-
<i>Streptococcus agalactiae</i>	WP_017770057.1	0.905	-
<i>Streptococcus mitis</i>	WP_000653411.1	0.788	-
<i>Streptococcus pneumoniae</i>	WP_000653405.1	0.788	Luong et al. (2015)
<i>Streptococcus equinus</i>	WP_039696977.1	0.392	-
<i>Streptococcus rattus</i>	WP_003088235.1	0.874	-
<i>Streptococcus suis</i>	WP_024413757.1	0.720	-
<i>Streptococcus sanguinis</i>	WP_002909695.1	0.817	-
<i>Streptococcus salivarius</i>	WP_013990215.1	0.760	-
<i>Streptococcus parasanguinis</i>	WP_003007178.1	0.310	-
<i>Streptococcus thermophilus</i>	ETW88492.1	0.782	-
<i>Enterococcus faecalis</i>	NP_816276.1 (EF2638)	0.499	Vesic et al. (2013)
	NP_816551.1 (EF2933)	0.749	Mehmeti et al. (2011)
<i>Lactococcus lactis</i>	WP_023349143.1	0.425	-

[#] *S. mutans* UA159 was used as a template to find Rex homologs in other Gram-positive cocci using BLAST.

[¶] NAD(H) Score was determined using Cofactory v.1.0 with a score > 0.5 indicative of NAD(H) binding specificity.