## SI-3-2

The Study of Co-relationship between Polyphosphate and Oral Microorganism in terms of Metal Tolerance

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It has been reported that polyphosphate is a strong chelator of metal ions. Small amount of nickel, mainly used as a alloy, and cadmium are capable of chelation by polyphosphate and the capability of polyphosphate produce by oral microorganisms may not be excluded. Nickel resistance strain was isolated from gingival crevicular fluid, which obtained after removal of non-precious metal crown or bridge from two patients, and identified *Enterococcus faecalis*. Testing antibiotic sensitivity using Sensi-Disc(BBL) and nickel resistance, this strain showed sensitivity only to tetracycline and resistance to nickel up to the concentration of 100 mM. In *E. coli*,  $ppk/ppx(\triangle)$  reduced the degree of 0.2 mM in their resistance against cadmium than wild type strain and restored by ppk plasmid. These results implicated that polyphosphate played a important role in heavy metal resistance.

## SI-3-3

Dna2 of Saccharomyces cerevisiae is a single-stranded DNA specific endonuclease that is able to act on double-stranded DNA in the presence of ATP

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The recombinant Dna2 protein of Saccharomyces cerevisiae, previously described as a cellular replicative helicase, was overexpressed and purified to homogeneity from insect cells in order to define its biochemical properties and gain insights into its biological functions. We detected ssDNA-dependent ATPase activity of Dna2 as previously reported, but not any explicit DNA helicase activity. Interestingly, we found an endonuclease activity associated with Dna2, which utilizes (1) ssDNA and (2) dsDNA in an ATP-dependent fashion. ATP (2-4 mM) inhibited ssDNA-specific endonuclease activity, whereas it stimulated the nuclease activity that degrades dsDNA. Only ATP and dATP were active for the enzyme to act on duplex DNA, indicating that their hydrolysis is required for this reaction, being consistent with the fact that ATP and dATP are the only nucleoside triphosphates that can be hydrolyzed by Dna2. Both of these activities were resistant to separation from ssDNA-dependent ATPase under a variety of different purification conditions and could be depleted coincidentally by antibodies specific to Dna2. These results demonstrate that all of these activities reside in the Dna2 protein. By virtue of its nucleolytic activities, the Dna2 protein may play roles in maintenance of chromosomal integrity such as repair or other related process rather than in propagation of cellular replication forks.