Brindhalakshmi Balasubramanian, oral, Antibiofilm efficacy of eugenol nanoemulsion against *Listeria monocytogenes*. Major Advisor- Abhinav Upadhyay.

Antibiofilm efficacy of eugenol nanoemulsion against *Listeria monocytogenes*.

Listeria monocytogenes (LM) is a major food borne pathogen that is known to form sanitizer tolerant biofilms leading to food contamination and subsequent human infections. Eugenol is a Generally Recognized as Safe (GRAS) status phytochemical that exhibit significant antimicrobial activity against LM. However, low solubility of eugenol thwarts its application as a sanitizer in the industry. This study investigated the efficacy of eugenol nanoemulsion (EGNE) in reducing LM biofilm formation and inactivating mature LM biofilm on steel surface. In addition, the effect of EGNE on LM motility, biofilm architecture, and proteome critical for biofilm formation was studied using standard bioassays and LC-MS/MS analysis, respectively. EGNE were prepared using food grade emulsifiers (Gum Arabic, lecithin) and high energy sonication. For the biofilm inhibition assay, LM was allowed to form biofilm on steel, either in the presence or absence of sub-MIC concentrations of EGNE (600, 700 ppm) at 25°C (for 4 days) or 10°C (for 24 days), respectively. Biofilm-associated LM were enumerated at 24 h intervals using a glass-beads based biofilm quantification assay. For the inactivation assay, mature LM biofilm developed at 25℃ or 10℃ were treated with MBC and two higher concentrations of EGNE (2300, 2750 and 3500 ppm) for a period of 1, 5, 15, or 30 min. Surviving LM were quantified as described above. The entire study was conducted on two strains of LM. All experiments had completely randomized design with duplicate samples and repeated at least three times. Data was analyzed using one-way ANOVA at $P<0.05$.LM population in the biofilm developed in presence of sub-MICs of EGNE was reduced by ~1.5 log CFU/ml as compared to control by 4 days (25℃) and 24 days (25℃) ($p<0.05$). Complete inactivation of mature biofilm was observed as early as 1 min of treatment with 2750 and 3500 ppm EGNE at both temperatures of incubation ($p<0.05$). LC-MS/MS analysis identified ~ 1000 LM proteins. Exposure to 700 ppm eugenol or EGNE for 24 h reduced the expression of major LM proteins involved in flagellar synthesis (FlgE, FlgK, FlaA), motility (MotB), attachment (InlA), and quorum sensing (LuxS) when compared to control ($P<0.05$).Results indicate that eugenol nanoemulsion could be used as a safe, effective and easy to use disinfectant in the food industry to eliminate LM biofilms.