Isolation and Characterization of Nitrate Reducing Bacteria (NRB) to Generate Vegetable-Derived 'Natural Nitrite' and Validation of its Effectiveness against *Clostridium* Spores in Low- and High-Fat Hotdogs.

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Introduction. In the US, sodium nitrate is used as a curing agent in processed meats and is therefore a regulated ingredient. Nitrate reducing bacteria (NRB) can convert vegetable nitrate into nitrite allowing green/clean label status in the US as per USDA-FSIS definition of 'natural nitrite'.

Objectives. Our objectives were to isolate and characterize new nitrate reducing bacteria that that can generate nitrite from vegetable extracts and examine celery-derived nitrite to inhibit spore germination by *Clostridium sporogenes* (surrogate for pathogenic *Clostridium* spp.) in processed meats.

Materials and Methods. The traditional qualitative in-liquid nitrite broth test has been used to detect nitrite in individual culture tubes is further not suitable for Gram(+)/lactic acid bacteria (LAB) nor for testing mixtures of bacteria from various food/animal samples. We developed an in-liquid version of nitrate broth using M17 broth (containing 0.1% potassium nitrate) that was suitable for Gram(+)/lactic acid bacteria and further developed an M17-based 'on-agar' colony-screening plate assay to detect the conversion of nitrate to nitrite by bacterial colonies using multiple soft agar overlays. Nitrate to nitrite conversion was quantified by C8 reversed-phase ion-pairing HPLC analysis. Celery-nitrite powder was also used to manufacture low- and high-fat hotdogs inoculated with spores of *Clostridium sporogenes* and incubated at various temperatures.

Results. Several strains isolated using our on-agar nitrate assay were selected and compared to *Staphylococcus carnosus*, a strain commonly used for nitrate reduction. *S. carnosus* was able to convert 1100 ppm M17-nitrate broth to 917 ppm nitrite. *Staphylococcus caprae* and *Panteoa agglomerans*, several NRB isolates, were also able to ferment the same broth to 916 ppm and 867 ppm nitrite, respectively. Vegetable nitrite (celery) was able to prevent spore germination in equivalent fashion as sodium nitrite in both low- and high-fat hotdogs. Although no germination was observed at low temperature, the use of high temperature ('permissive conditions') allows potential *Clostridium* spore activation to be observed.

Significance. We have demonstrated that vegetable/celery-nitrite provides equivalent inhibition of *Clostridium* spore germination as does sodium nitrite when standardized to common levels in hotdogs. This is the first report of an on-agar colony screening assay for the detection of NRBs allowing them to be readily isolated from various mixed bacterial sources. This will allow the identification of new bacteria that may generate nitrite an produce additional inhibitory factors (i.e., bacteriocins) that may contribute to pathogen suppression in foods.

Keywords: Vegetable nitrite, *Clostridium*, spores, nitrate reducing bacteria, hotdogs, sodium nitrite.