Technical Bulletin

Production of a dietary based anti-inflammatory compound from *Bacillus subtilis* 1579

Introduction

The health benefits of a diet rich in plants, fruits and vegetables is due not only to the vitamins, minerals and fiber found in the plants but also to compounds such as polyphenols. Polyphenols such as quercetin, catechins and anthocyanins, found in foods like apples, onions, and berries, are well known for providing health benefits. However, multiple studies demonstrate that many of these polyphenol compounds are poorly absorbed into the body (Marín et al., 2015). Recent work demonstrates that the majority of benefits may come from the smaller, more well absorbed bacterial metabolites rather than from the parent compound (Selma et al., 2009).

Phenolic acids, such as protocatechuic acid (PCA), can exert significant benefits to health through antioxidant and anti-inflammatory activity, cardiovascular benefits, antiglycemic and anticarcinogenic activity and modulation of neuroinflammation (Krzysztoforska et al., 2019; Masella et al., 2012).

While small quantities of PCA are found naturally in plants, polyphenols, the precursors of PCA, are found in high quantities in the plants we eat such as berries, fruits and grains. When these polyphenols are metabolized by specific bacteria, higher levels of PCA are released from the diet. PCA has greater bioavailability and tissue distribution than the parent polyphenol yet still exerts benefits such as anti-inflammatory effects through down regulation of NF-κB and MAPK transduction pathways (Wang et al., 2010), the central regulators of inflammation. This decrease in signaling can dampen inflammation by decreasing cytokine and cyclooxygenase (COX2) production, and cellular signaling (Farombi et al., 2016). Research at Arm & Hammer Co., Inc. has identified *Bacillus subtilis* 1579, a unique bacterial strain capable of metabolizing polyphenols to PCA in order to help modulate the immune system and benefit health and performance.

Bacillus subtilis 1579 increases PCA production from dietary polyphenols

Bacillus subtilis 1579 was identified from over 4000 *Bacillus* strains in our library as a unique strain that can produce PCA from quercetin, a phytogenic anti-inflammatory compound found in dietary items such as apples, berries and grains.

Scope of Investigation

Rodent and human studies were conducted at Arm & Hammer Co. Inc. to substantiate the effect of *Bacillus subtilis* 1579 on PCA production.

Study One: Mice were maintained on standard rodent chow supplemented with 0.1% rutin (quercetin glycoside) The diet for mice in the experimental group (n=3) was supplemented with *Bacillus subtilis* 1579 spores in the feed, while control mice (n=4) received only rutin supplemented feed. After 7 days, the levels of PCA and rutin in the feces were assessed by Ultra High Performance Liquid Chromatography (Fig 1.).

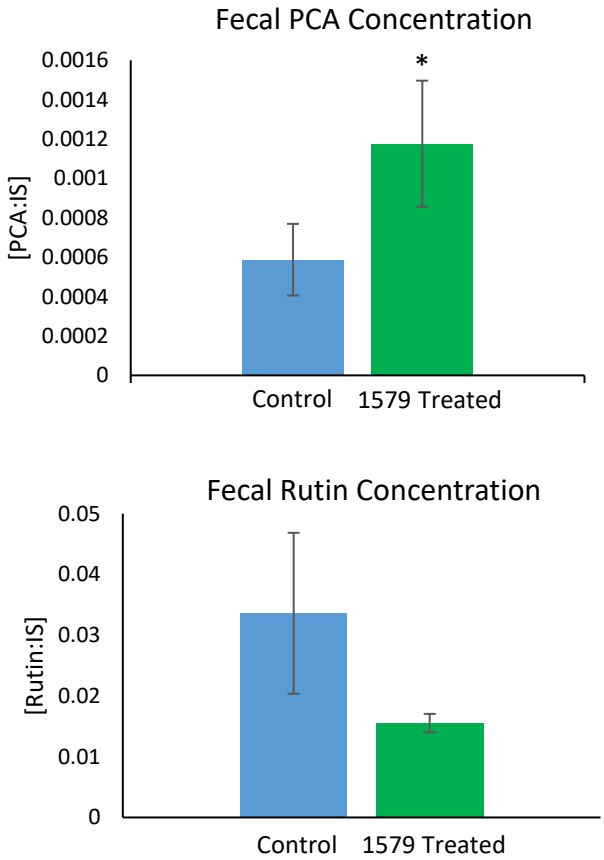

Figure 1. PCA and rutin concentrations in mouse feces. Animals fed *Bacillus* 1579 showed a significant increase in PCA production in the feces compared to control animals. Consistent with rutin acting as the source of PCA there is a trend toward less rutin in 1579 treated animal feces. * Significant difference from control ($p < 0.05$)

Study Two: A proof of principle study in human participants was conducted to substantiate the effect of *Bacillus* 1579 on PCA production from oral supplementation with quercetin. Participants (n=3) were

asked to minimize or refrain from foods high in quercetin for 24 hours leading up to fecal sample collections. Participants consisted of an individual who was mostly vegetarian and two individuals on a typical western diet. 24 hours prior to each fecal sample collection, a 500mg capsule of quercetin (quercetin dihydrate) was taken orally. On day 0, a fecal sample was collected. Following the initial fecal sample, participants began oral dosing of *Bacillus* 1579. Oral dosing continued daily for 10 days. On day 9, participants again received a 500mg tablet of quercetin along with the final *Bacillus* 1579 dose. On day 10, a final fecal sample was collected. Fecal samples were extracted by liquid-liquid extraction and the levels of PCA in the feces were quantified by UHPLC (Fig 2).

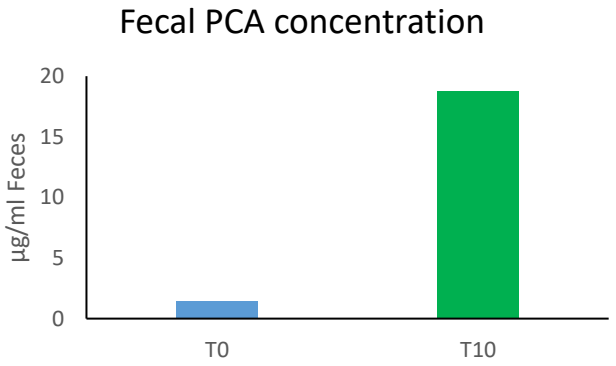

Figure 2. PCA concentrations in human feces. People treated with *Bacillus* 1579 for 10 days (T10) demonstrated greater PCA concentrations in feces than when they had quercetin alone (T0).

Conclusion

These findings provide proof of principle and support the conclusions that *Bacillus subtilis* 1579 produced protocatechuic acid from a dietary quercetin source in both humans and animals. These results suggest that taking *Bacillus subtilis* 1579 along with a healthy diet may optimize use of dietary phytochemicals through bacterial biotransformation resulting in improved health in people.

References

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