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A Double-Blind, Randomized, Crossover Study to Examine the Effect of a Probiotic on the Production and Bioavailability of Sulforaphane in Healthy Adults

The health benefits of a diet rich in cruciferous plants is due not only to the vitamins, minerals and fiber found in the plants but also to compounds such as glucosinolates. Glucosinolates such as glucoraphanin, found in foods like broccoli, kale, and cabbage are well known for providing health benefits such as reduced risk of certain types of cancer as well as neurodegenerative disorders (Mullaney et al., 2013; Zhang and Tang, 2007). However, multiple studies demonstrate that many of these glucosinolate compounds are poorly absorbed into the body (Marín et al., 2015). Recent work demonstrates that the majority of benefits from phytochemicals may come from the smaller, more well absorbed bacterial metabolites rather than from the parent compound (Selma et al., 2009).

While highly abundant, glucoraphanin provides little benefit until broken down by an endogenous plant enzyme, myrosinase. Myrosinase is released upon rupture of the plant cells such as chopping or chewing, and glucoraphanin is hydrolyzed into sulforaphane (Fahey, et al., 2015 and Atwell, et al., 2015). Sulforaphane not only benefits the plant by providing a defense system against insects, but also provides many health benefits to humans. Sulforaphane induces Phase II enzymes, promoting carcinogen metabolism and antioxidant activities (Mullaney, et al., 2013). In addition, more recent work has shown that sulforaphane has the potential to inhibit histone deacetylases (HDACs) by competitive inhibition (Atwell, et al., 2015). HDAC inhibition was followed by the induction of G2/M phase cell cycle arrest and apoptosis of the cancer cells. Sulforaphane has both a chemoprotective effect through phase II enzyme induction and promotes cancer cell death through apoptosis. Sulforaphane also has demonstrated effects in inducing detoxifying enzymes.

While glucoraphanin conversion to sulforaphane is done by the myrosinase enzyme present in the vegetable, cooking of the vegetable causes denaturation of the enzyme and glucoraphanin can no longer be hydrolyzed into sulforaphane. Therefore, a probiotic bacterium containing a naturally occurring microbial myrosinase enzyme would be an effective probiotic to produce sulforaphane from dietary ingredients. Research at Arm & Hammer Co., Inc. has identified *B. velezensis* 839, a unique bacterial strain capable of metabolizing glucoraphanin to sulforaphane. To test the ability of the bacterial strain to biotransform glucoraphanin in the human body, a pilot trial was conducted and levels of sulforaphane were quantified in urine of individuals who ingested a commercially available broccoli seed extract in combination with *B. velezensis* 839 and a placebo.

Methods.

Participants.

Nine healthy adults (18-40 years) were recruited in Waukesha, WI. The study was conducted at the Church & Dwight Microbial Center of Excellence, Waukesha, WI. Exclusion criteria

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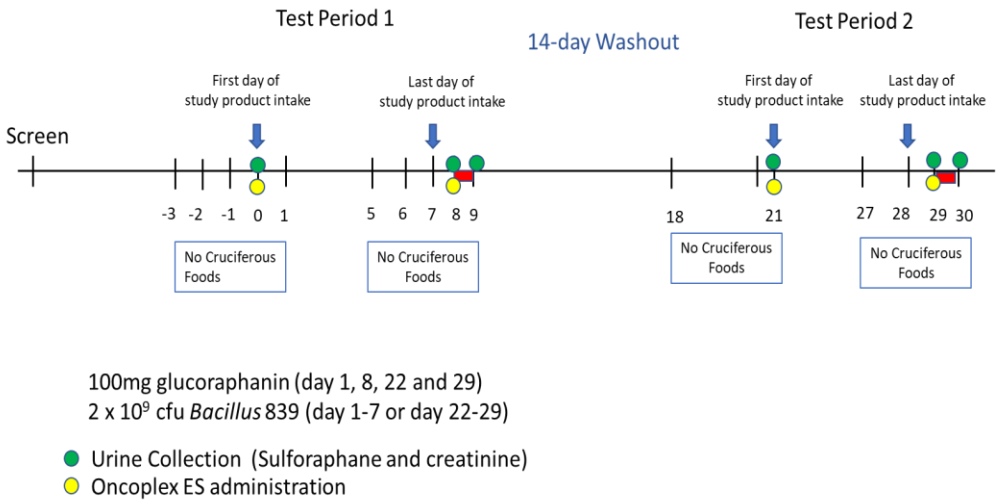
included: Women who are nursing or pregnant, individuals allergic to broccoli, have urinary or kidney maladies, urinary tract infections, have recently or are currently taking antibiotics, or those who cannot refrain from eating cruciferous vegetables.

Dietary Intervention:

Subjects were randomized to consume *B. velezensis* 839 (2 x10^9 cfu) or a placebo of maltodextrin daily. All subjects were instructed to avoid consumption of cruciferous vegetables in any form for three days leading up to the trial and each urine collection. They were provided with a list of foods and supplements to avoid. On the days of the glucoraphanin consumption, subjects were administered two capsule of OncoPlex ES and asked to collect urine at three timepoints (2, 6 and 24hrs) over the next 24 hours. Baseline sulforaphane was assessed in urine taken on day 0. For 7 consecutive days subjects were asked to orally ingest a test product of either *B. velezensis* 839 or placebo. Again, on the 3 days prior to urine collection subjects were to refrain from consuming foods with glucoraphanin. On day 8 after ingestion of test product, participants were administered two OncoPlex ES capsules and urine was again collected at 2-, 6- and 24-hour timepoints post ingestion. Following the 7 days of probiotic or placebo ingestion, a two-week washout period was imposed, and the participants entered the cross-over treatment for an additional 7 days with urine collections at 2, 6 and 24 hour after final ingestion of broccoli supplements.

Urinary sulforaphane concentrations were measured using the cyclocondensation reaction. Briefly, 0.5ml of urine were incubated in 500ml of buffer and 1ml of 1,2-benzenedithiol in a sealed 2ml tube for 2 hours at 65C. After reaching room temperature the samples were centrifuged, 1 ml aliquots were transferred to HPLC vials and samples were assessed by UHPLC for sulforaphane quantification. Urinary creatinine was also assessed at each time point as a comparator for sulforaphane concentrations using an ELISA.

Sulforaphane Production



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In the experiment, urine analysis was performed on coded samples, and investigators were blind to the participant identity. Sulforaphane values were normalized to creatinine values of the same participant and time point to account for varying hydration levels of participants. Data was analyzed using a two-tailed t-test using unequal variance. Box and whisker plots identify median (center line), mean (x), second and third quartile, and the maximum and minimum values (whiskers).

Results.

The peak sulforaphane recovery from participants supplemented for 7 days with *B. velezensis* 839 demonstrated a 1.75-fold increase in the amount of sulforaphane produced in urine at peak production on the final day of probiotic intake compared to the same timepoint on the first day of probiotic intake (Fig. 1). Participants taking the placebo for 7 days demonstrated a slight decrease in sulforaphane production (-0.49 fold) at the same time point on the final day of placebo intake compared to the first day of placebo intake (Fig. 1). The bioavailability was variable both within and between participants, consistent with results reported by others (Fahey et al., 2015, 2019; Shapiro et al., 2006).

Participants reported that they did not experience any adverse events during participation in the trial. There were two reports of minor borborygmus and flatulence in one of the participants, but the participant did not report any pain or bloating.

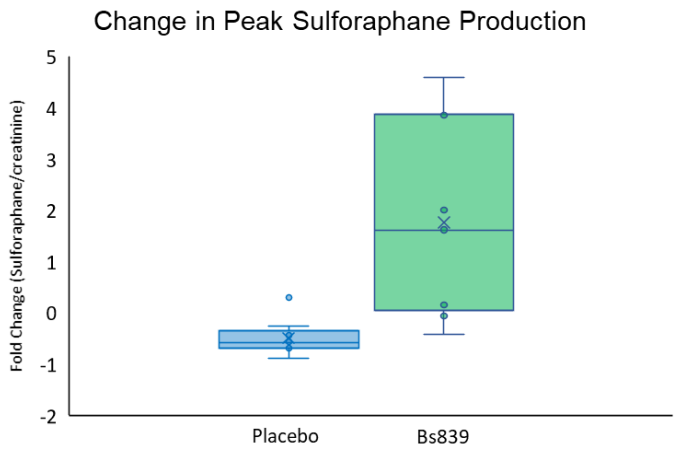

Figure 1. Peak production of sulforaphane metabolites. Placebo and Bv839 were given daily for 7 consecutive days. Prior to urine collection ~150mg glucoraphanin was given and levels of sulforaphane were determined by UHPLC and normalized against creatinine levels. Boxes around data points delineate the 25th and 75th percentiles of values and whiskers delineate the 5th and 95th percentiles. The solid lines in the center represent the median, x represents the mean value. The Bv839 cohort demonstrated a statistically significant increase in the fold change of sulforaphane production from initial to final values within treatment tests compared to the placebo cohort (1.75 fold vs -0.49 fold, $P < 0.008$).

Conclusion.

In this trial, we have provided an avenue for enhanced sulforaphane production from dietary glucoraphanin sources through microbial myrosinase activity. Addition of *B. velezensis* 839 provides an active myrosinase enzyme which can convert glucoraphanin to sulforaphane in the intestine. This study demonstrates that a roughly doubling of the sulforaphane concentration can be achieved at the current dosage of *B. velezensis* 839 allowing for greater availability to the body while ingesting the same amount of glucoraphanin.

While the current trial demonstrated a significant and substantial increase in the production of sulforaphane, the study also demonstrated a large variability within individuals and between individuals to biotransform glucoraphanin to sulforaphane as would be expected from the literature. Due to limitations in the trial, a future study will collect all urine over the 24-hour period after glucoraphanin administration. This will help to alleviate issues of variability as well as provide more accurate determination of changes in the individual participant between the cross over groups.

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