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Technical Bulletin – *Bacillus velezensis* 839

Introduction

The health benefits of a diet rich in cruciferous plants are due not only to the vitamins, minerals and fiber found in the plants but also to compounds such as glucosinolates. Glucosinolates, like 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 and Zhang, et al., 2017). 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 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 enzyme, myrosinase. Myrosinase is released upon rupture of the plant cells by chopping or chewing, and hydrolyzes glucoraphanin to 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 an active myrosinase enzyme would be an effective probiotic for production of sulforaphane from dietary ingredients. Research at Arm & Hammer Co., Inc. has identified *Bacillus velezensis* 839, a unique bacterial strain capable of metabolizing glucoraphanin to sulforaphane.

Bacillus velezensis 839 converts glucoraphanin to sulforaphane

Bacillus velezensis 839 was identified from over 4000 *Bacillus* strains in the Arm & Hammer library as a unique strain that can produce sulforaphane from glucoraphanin, a chemoprotective compound found in foods such as broccoli, cabbage, and kale.

Scope of Investigation

In vitro studies were conducted at Arm & Hammer Co. to demonstrate the effectiveness of the myrosinase enzyme present in *Bacillus velezensis* 839.

Overnight culture of *Bacillus velezensis* 839 was pelleted at 3000 x g for 10 min. Cells were washed 1x with sterile peptone and spun as above. The pellet was resuspended in 10 ml minimal salts media containing 1 ml 1 mg/ml glucoraphanin and incubated 48hr at 37C in an anaerobic chamber.

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Post incubation, a cyclocondensation reaction was performed on the supernatant. The extracted supernatant was then filter sterilized and assessed by Ultra High-Performance Liquid Chromatography (UHPLC) for detection of sulforaphane as well as reduction of glucoraphanin (Figure 1).

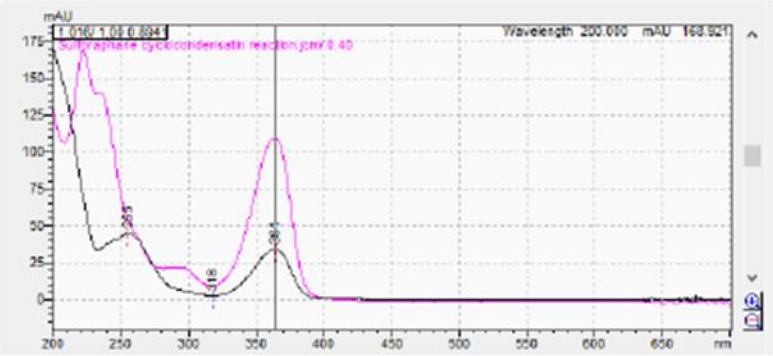

Figure 1. Sulforaphane cyclocondensation reaction ultraviolet spectrum. The ultraviolet spectrum of minimal media after 24 hours with *Bacillus velezensis* 839 in the presence of glucoraphanin (black line) directly correlates with the ultra violet spectrum of the sulforaphane standard after cyclocondensation (pink line).

Additional dichloromethane silica gel column extractions were performed on the 48 hr post-incubation samples as described by Campas-Baypoli, O.N. et al., 2009. The extracted supernatant was then sent to the Shimadzu Lab at the University of Wisconsin-Milwaukee to be run on the mass spectrometer for further confirmation (Figure 2).

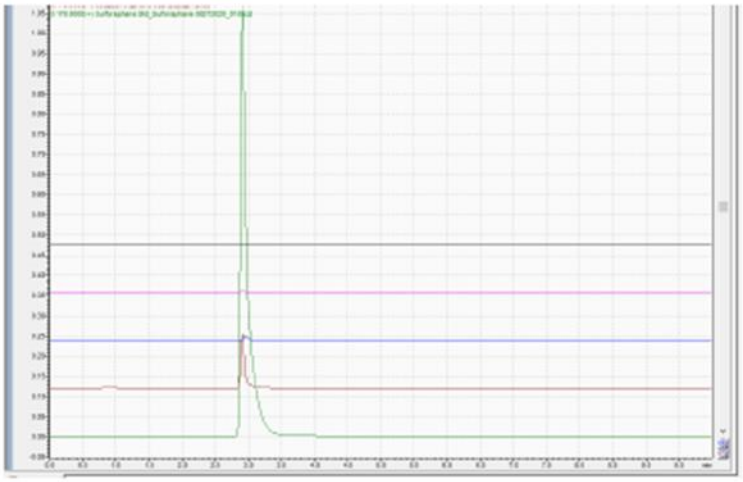

Figure 2. Mass spectrometry chromatograph of sulforaphane. The brown line represents the chromatograph of minimal media after 24 hours with *Bacillus velezensis* 839 in the presence of glucoraphanin. The chromatograph of *Bacillus velezensis* 839 is in alignment with the sulforaphane standard peak (green line).

These findings provide support that the myrosinase enzyme identified in *Bacillus velezensis* 839 is active and converts glucoraphanin to sulforaphane. Further proof of concept studies in humans confirm the ability of *Bacillus velezensis* 839 to convert glucoraphanin from a dietary source.

Gastrointestinal Performance

In order for *Bacillus velezensis* 839 to be effective in human studies, the strain needs to survive passage through the gastrointestinal tract. *Bacillus* are naturally found in the gastrointestinal tract of humans and animals and many species have a long safe history of use in food preservation and production with multiple defined health benefits.

Survival in the gastrointestinal tract requires a variety of attributes, including acid tolerance and the ability to withstand bile salts. *In vitro* assays have demonstrated that *B. velezensis* 839 is largely resistant to low pH conditions (pH 1.5 and 3.0) and survives in the presence of physiological concentrations of bile salts for extended periods of time (Figure 3).

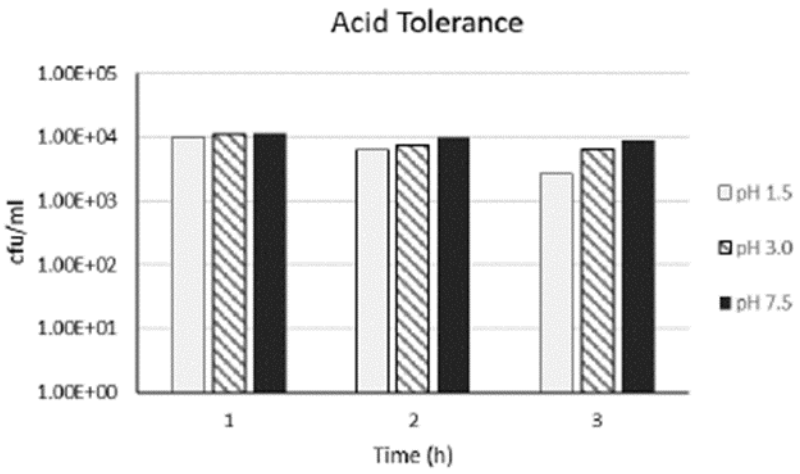

Figure 3. Percent survivability of *Bacillus velezensis* 839 in acid and bile media at 3 hrs.

Conclusion

The ability of *Bacillus velezensis* 839 to convert glucoraphanin to sulforaphane and survive *in vitro* gastrointestinal tract conditions indicate this strain is a good candidate for human studies.

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