Myrosinase and sulforaphane, an overview

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Purpose: This document is a collation of the research findings for Myrosinase producing bacteria and was compiled when developing the Myrosinase (Longevity) section for www.biomesight.com

What is Myrosinase?
Myrosinase is a family of enzymes most commonly found in plant defences against herbivores. This enzyme works by catalysing the hydrolysis of a class of compounds called 'glucosinolates' into another class of compounds called 'isothiocyanates'. An example of this process can be seen in Figure 1.

How does this work?
This glucosinolate-myrosinase defence system is stored via compartmentalisation, that is to say, they are inert and stored adjacent to one another but in separate cells. This means that when the plant experiences tissue damage and these cells break down, they release their contents so the glucosinolate and myrosinase come into contact with each other to activate the defence system, deterring insect predators (Bones & Rossiter, 1996, Andréasson *et al.,* 2001; Sulforaphane glucosinolate. Monograph, 2010)

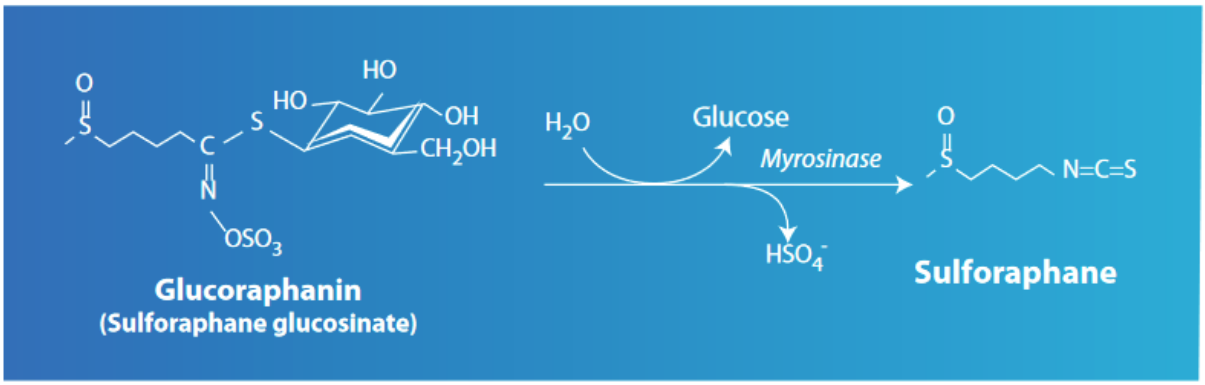

Figure 1: The glucosinolate-myrosinase defence system, where the glucosinolate is glucoraphanin, which is being hydrolysed into the isothiocyanate sulforaphane. Taken from Sulforaphane glucosinolate. Monograph (2010)

Where can this be found?
Almost all plants of the order Brassicales contain glucosinolates. Within this order are the families Brassicaceae as well as Capparaceae and Caricaceae, where Brassicaceae is particularly important since this family contains various edible plants such as the cruciferous vegetables: broccoli, Brussels sprouts, cabbage, and capers. The presence of glucosinolates is often easily perceived since the breakdown products contribute to its distinctive bitter taste (Ishida *et al.,* 2014).

The cooking method of these vegetables is vital to the amount of glucosinolates remaining since these compounds are water-soluble, meaning that they will leach into surrounding water when the cell walls are broken down by boiling. Additionally, heat from boiling or frying will cause thermal

degradation of glucosinolates. Because of this, steaming these vegetables has been found to be the best method to cook them since it causes the lowest loss of glucosinolates (Yuan *et al.,* 2009). Furthermore, these glucosinolates cannot be metabolised by the vegetable's myrosinase since the heat partially inactivates or denatures the myrosinase enzymes. Despite this, the leftover glucosinolates are still metabolised to isothiocyanates in humans. This is explained by the gut microflora because within the gut of humans are myrosinase-producing bacteria (Getahun & Chung, 1999; Sulforaphane glucosinolate. Monograph, 2010). Naturally, different individuals possess varying amounts of these myrosinase-producing bacteria, which explains the variation of isothiocyanates levels between individuals (Conaway *et al.,* 2000; Liou *et al.,* 2020). Additionally, gender may have a weak (Fahey *et al.,* 2012) or no (Kensler *et al.,* 2005; Egner *et al.,* 2011) effect on the bacteria present in the microflora.

Below is a table of some of the gut microflora that have been shown to degrade glucosinolates via myrosinase:

Highest taxonomy	Species
Bacteroides - species	*Bacteroides thetaiotaomicron* (Elfoul *et al.,* 2001; Liou *et al*., 2020) *Bacteroides vulgatus* (Rabot *et al.,* 1993)
Bifidobacterium - species	*Bifidobacterium adolescentis* (Cheng *et al.,* 2004) *Bifidobacterium longum* (Cheng *et al.,* 2004) *Bifidobacterium pseudocatenulatum* (Cheng *et al.,* 2004)
Enterobacter cloacae - species	*Enterobacter cloacae* (Tani *et al.,* 1974; Mullaney *et al.,* 2013; Wasserman *et al.,* 2017)
Enterococcus casseliflavus - strain	*Enterococcus casseliflavus* CP1 (Luang-In *et al.,* 2013; 2016)
Escherichia coli - strain	*Escherichia coli* (Oginsky *et al.,* 1965) *Escherichia coli* Nissle 1917 (Mullaney *et al.,* 2013) *Escherichia coli* O157:H7 (Herzallah *et al.,* 2011; Luciano *et al.,* 2011) *Escherichia coli* VL8 (Luang-In *et al.,* 2013; 2016),
Lactobacillus - species	*Lactobacillus acidophilus* (Lai *et al.,* 2009) *Lactobacillus agilis* R16 (Palop *et al.,* 1995; Luang-In *et al.,* 2013; 2016) *Lactobacillus casei* (Lai *et al.,* 2009) *Lactobacillus gasseri* (Lai *et al.,* 2009) *Lactococcus lactis* subsp. *lactis* KF147 (Mullaney *et al.,* 2013) *Lactobacillus plantarum* (Lai *et al.,* 2009) *Lactobacillus plantarum* KW30 (Mullaney *et al.,* 2013) *Lactobacillus plantarum* UM131L, UM135L (Luciano *et al.,* 2011)
Staphylococcus - species	*Staphylococcus aureus* (Herzallah *et al.,* 2011) *Staphylococcus carnosus* (Herzallah *et al.,* 2011) *Staphylococcus carnosus* UM123M, UM13SM, UM136M (Luciano *et al.,* 2011)

What are isothiocyanates/sulforaphane?
Isothiocyanates are compounds that contain the chemical group -N=C=S, which come under the umbrella term of organosulfur compounds, which are organic compounds that contain sulfur. The phytochemical sulforaphane is an isothiocyanate and is the end product of the glucosinolate-myrosinase reaction, specifically when the glucosinolate is glucoraphanin (see Figure 1).

How does sulforaphane work?
It seems widely accepted that the consumption of vegetables from the Brassicaceae family, and therefore the isothiocyanate, sulforaphane, and its precursor glucosinolate, glucoraphanin, are associated with a decreased risk of developing cancer (Fahey *et al.,* 2002; Clarke *et al.,* 2008; Herr & Büchler, 2010; Li *et al.,* 2010), being anti-pathogenic against fungi, bacteria, or viruses such as SARS-CoV-2 and *H. pylori* (Fahey *et al.,* 2002; Johansson *et al.,* 2008), as well as being effective against neurological disorders (Santín-Márquez *et al.,* 2019; Uddin *et al.,* 2020), just to name a few.

Anti-carcinogenic
Sulforaphane has been shown to help prevent cancer during the initiation phase regulating transcription or blocking and inhibiting Phase 1 enzymes, more specifically, cytochrome P450 enzymes. It is important that these enzymes are being interacted with since they are responsible for converting procarcinogens to carcinogens (Myzak & Dashwood, 2006; Clarke *et al.,* 2008).

This is coupled with sulforaphane upregulating gene expression for Phase 2 enzymes which are responsible for the detoxification and excretion of carcinogens. This upregulation is being done via antioxidant response element (ARE)-driven gene expression. This upregulates genes for the enzymes NAD(P)H:quinone reductase (NQO1), heme oxygenase 1 (HO1) and gamma-glutamylcysteine synthetase (γ-GCS), a rate-limiting enzyme in glutathione (GSH) synthesis. And this is all mediated by nuclear factor E2-factor related factor (Nrf2) binding to ARE transcription sites. However, Nrf2 in its default state is bound to Kelch-like ECH-associated protein 1 (Keap1). But sulforaphane can bind to Keap1, releasing Nrf2 from Keap1, allowing Nrf2 to bind to ARE transcription sites to upregulate gene expression (Myzak & Dashwood, 2006; Clarke *et al.,* 2008).

After the cancer initiation phase, sulforaphane can also help suppress cancer development by regulating the cell cycle, and therefore cell proliferation, cell differentiation, and apoptosis.

Cell cycle regulation
One of the defining properties of cancer is unregulated hyperproliferation due to the loss of regulation of the cell cycle. The regulators of the cell cycle are primarily cyclin-dependent kinases (CDKs), cyclins, and CDK inhibitors, where CDK complexes and cyclins induce cell cycle progression while CDK inhibitors induce cell cycle arrest (Myzak & Dashwood, 2006; Clarke *et al.,* 2008). Sulforaphane's effect on these CDKs, cyclins and CDK inhibitors is complex and varied depending on the cell type, dose, and time of treatment. Gamet-Payrastre *et al.* (1998, 2000) tested the effect of sulforaphane on cancer cells using HT-29 human colon cancer cells and CaCo-2 cells. In these tests, it was found that sulforaphane seems to selectively arrest the undifferentiated cells over differentiated cells, helping to stop the rapid hyperproliferation that would have otherwise occurred. This was also found by Ho *et al.* (2009). Although not always, sulforaphane induced cell cycle arrest seems to mostly happen around the G2/M phase, eliminating these cells from the general population. If the cell cycle were to progress, it would require an active cyclinB/CDK1 complex (Clarke *et al.,* 2008). This explains sulforaphane's effect on cell cycle arrest in Acute lymphoblastic leukaemia (ALL) cells found

by Suppipat *et al.* (2012). This is because sulforaphane was found to be associated with the upregulation of the cell cycle inhibitor proteins p53 and p53-independent upregulation of protein $p21^{CIP1/WAF1}$, and inhibition of the cyclinB/CDK1 complex.

Apoptosis

Apoptosis is programmed cell death that can be triggered through death-receptor caspase cascades or the mitochondria caspase cascades. Alongside the other anti-carcinogenic mechanisms, apoptosis helps reduce cancer since it kills off the carcinogenic cells. Cytoplasmic histone associated DNA fragments, poly (ADP-ribose) polymerase (PARP) cleavage, changes in Bcl-2 protein family ratios (increased pro-apoptotic proteins and decreased anti-apoptotic proteins), and cytochrome C release from the mitochondrial membrane are all associated with apoptosis (Clarke *et al.,* 2008). Sulforaphane can be seen as an inducer of apoptosis when used in the following cancer cells: in DU145 prostate cancer cells there is an increase in PARP cleavage and increased release of histone associated DNA fragments (Cho *et al.,* 2005), in HTC116 colon cancer cells there is induced activation of caspase 7 and caspase 9 causing apoptosis independent of p53, increase in PARP cleavage, and increase in pro-apoptotic factors (Pappa *et al.,* 2006), in HT-29 colon cancer cells there is an increase in pro-apoptotic factors, increase in PARP cleavage, and release of cytochrome C from mitochondria (Gamet-Payrastre *et al.,* 1998; 2000), in PC3 prostate cancer cells there is an increase in sub-G0/G1 DNA content, PARP cleavage, increased release of cytoplasmic histone associated DNA fragments, increase in pro-apoptotic factors, and an increased activation of caspase 3, 8, and 9 (Singh *et al.,* 2004), and in ALL cells where there is dose-dependent apoptosis and G2/M cell cycle arrest, which is associated with the activation of caspases (3, 8, and 9), inactivation of PARP, p53-independent upregulation of $p21^{CIP1/WAF1}$, and inhibition of the cyclinB/CDK1 complex (Suppipat *et al.,* 2012).

Histone Deacetylase (HDAC) inhibition

HDAC activity is commonly found in many cancers, resulting in repression of transcription. This repression of transcription causes deregulation of differentiation, upregulation of the cell cycle, and apoptosis. Naturally, this would mean that inhibitors of HDAC would result in upregulation of these mechanisms, therefore reducing the risk of cancer. It is important to note that this manner of HDAC inhibition only affects neoplastically transformed cells, meaning that normal cells will be unaffected (Brinkmann *et al.,* 2001; Ho *et al.,* 2009). Sulforaphane has since been found to act as an HDAC inhibitor via its metabolites in the mercapturic acid pathway, these metabolites namely being Sulforaphane-cysteine and Sulforaphane-N-acetylcysteine, both acting as competitive inhibitors for HDAC (Myzak *et al.,* 2004). This proof of concept was shown in prostate cancer cells, where the inhibition of HDAC resulted in an increase of acetylation, and induction of cell cycle apoptotic mechanisms. This has also been found in other cancer cell lines such as breast, and colon cancer cells, suggesting that this effect of HDAC inhibition is not limited to only one type of cancer cell line (Myzak *et al.*, 2006).

Anti-pathogenic

Whilst being anti-carcinogenic in terms of bodily functions, sulforaphane's anti-pathogenic properties also contribute to the decreased cancer risk via its bactericidal effect on *Helicobacter pylori* since this is stopping the shown link between *Helicobacter pylori* and the risk of gastric cancer (Fox & Wang, 2001; Uemura *et al.,* 2002; Hsu *et al.,* 2007; Moss, 2017). Naturally, this anti-pathogenicity also extends to pathogens other than *Helicobacter pylori*. One such example is SARS-CoV-2. Sulforaphane freeing up Nrf2 has been shown to also combat COVID-19 since Nrf2 can now block the angiotensin II receptor type 1 (AT_1R) axis associated with oxidative stress. With no Nrf2 present,

SARS-CoV-2 would bind to angiotensin-converting enzyme 2 (ACE2), downregulating it. As a result, this would enhance the AT_1R axis associated with oxidative stress, causing insulin resistance as well as lung and endothelial damage (Bousquet *et al.,* 2020).

Against cardiovascular disease and ageing

Furthermore, Nrf2 has been found to reduce oxidative stress. This is because Nrf2 can block the promotion of inflammation and foam cell formation which would normally be caused by the oxidation of low-density lipoproteins. This results in blocking inflammation which could have led to atherosclerosis and cardiovascular disease (Zakkar *et al.,* 2009; Evans, 2011; Bai *et al.,* 2015). On top of this, it may also reduce blood pressure, although this has primarily been tested in rats (Wu & Juurlink, 2001; Senanayake *et al.,* 2012), with little done on human subjects. One such study has been done on pregnant women and has found that sulforaphane decreases pregnancy hypertension (Langston-Cox *et al.,* 2021). Alongside these benefits is that the reduction in oxidative stress also reduces skin damage associated with ageing caused by UV radiation by inhibiting UVB-induced AP-1 activation. Moreover, sulforaphane is also effective against neurological disorders associated with ageing, this being also done through Nrf2 upregulation (Zhu *et al.,* 2004; Talalay *et al.*, 2007; Santín-Márquez *et al.,* 2019; Uddin *et al.,* 2020).

Anti-diabetic

Lastly, sulforaphane has also been found to help improve glucose control in patients with type 2 diabetes. It does this by releasing Nrf2, allowing Nrf2 to repress gene transcription of three of the four key enzymes used in gluconeogenesis, these being pyruvate carboxylase, phosphoenolpyruvate carboxykinase 1, fructose-1,6-bisphosphatase 1, and glucose-6-phosphatase, catalytic subunit. Only pyruvate carboxylase was not significantly downregulated in H4IIE cells. When done on human subjects, the findings were similar, where the subjects who had poor diabetes control had significantly reduced fasting blood sugar levels (Axelsson *et al.,* 2017). Additionally, with sulforaphane freeing up Nrf2, it is suggested that this will help reduce diabetes and its complications by activating the ARE-mediated antioxidant response. Furthermore, it has been suggested that Nrf2 activation during the prediabetic state may be an emerging strategy to avoid diabetes development (Jiménez-Osorio *et al.,* 2015).

Summary

In summary, sulforaphane can be derived from vegetables from the Brassicaceae family such as broccoli, Brussels sprouts, cabbage, and capers. This compound has been found to have various health benefits such as being anti-carcinogenic, anti-pathogenic, and anti-ageing. However, the majority of this research has been done primarily on cell lines or animals, urging the need for more research to be conducted on humans.

Adding more sulforaphane to people's diets via consumption of vegetables from the Brassicaceae family is very easily done and will provide a wide range of benefits to add to the longevity of the consumer.

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