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Original Article

Reduction of the sulfoxide in glucoraphanin and sulforaphane by *E. coli* VL11 and BL21 (DE3)

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Abstract

Sulfoxide reductase activity of two *Escherichia coli* strains VL11 from human feces and BL21(DE3) from a molecular cloning host was expressed upon glucoraphanin induction for 16 hrs at 37°C under aerobic conditions. Bacterial induced cultures were able to convert the sulfoxide in glucoraphanin to the sulfide and thus produced glucoerucin. Only *E. coli* VL11 produced 30 μM erucin and 51 μM erucin nitrile as degradation products from 1 mM glucoraphanin metabolism whereas BL21(DE3) only biotransformed glucoraphanin to glucoerucin with 52% conversion without metabolizing it. Cell-free extracts of each *E. coli* strain from glucoraphnin-induced cells in citrate phosphate buffer pH 7.0 were able to convert the sulfoxides in both glucoraphanin and sulforaphane to the sulfides and thus produced glucoerucin and erucin, respectively at 4 h under the same incubation conditions. Sulfoxide reductases from two *E. coli* strains required Mg^{2+} ions and NADPH reducing reagents for the activity.

Keywords: *E. coli*, glucosinolate, isothiocyanate, nitrile, sulfoxide reductase

1. Introduction

Regular consumption of broccoli has been shown to lower the risk of cancers (Ambrosone & Tang, 2009). Broccoli is rich in glucosinolates (GSLs), secondary metabolites, that upon hydrolysis gives products such as nitriles (NITs) and/or isothiocyanates (ITCs) with possible chemopreventive benefits by plant myrosinase (thioglucohydrolase; E.C. 3.2.1.147) (Zhang *et al*., 2007). The most abundant GSL in broccoli is glucoraphanin, which gives rise to the most potent ITC sulforaphane. Glucoerucin and glucoiberin are also present in broccoli and are hydrolysed to erucin and iberin, respectively as ITC products (Ye *et al*., 2002). Bioactivities of ITC products depend on side groups of ITCs which in turn influenced by side groups of GSL precursors and also enzymes that can modify the side groups of GSLs/ITCs. Thus far, only biotransformation from sulfoxide GSLs/ITCs i.e. methylsulfinylalkyl group to sulfide analogues i.e. methylthioalkyl group has been documented e.g. sulforaphane was interconverted to erucin *in vivo* in humans (Clarke *et al*., 2011), but the reverse conversion has never been detected. Sulforaphane N-acetyl cysteine conjugate, free sulforaphane, sulforaphane nitrile, and erucin were produced in the urine after glucoraphanin administered intravenously into caecal microbiota in F344 rats nevertheless both glucoraphanin and glucoerucin were detected in bile (Bheemreddy & Jeffery, 2007). In addition, glucoraphanin produced only erucin nitrile instead of sulforaphane during *in vitro* fermentation of ceacal microbiota from male F344 rats (Lai *et al*., 2010). Likewise, human gut microbiota was able to bioconvert glucoraphanin

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to glucoerucin and yield sulforaphane, erucin and corresponding NITs (Saha *et al*., 2012).

To date, there are only two *Escherichia coli* strains identified to exhibit sulfoxide reductase activity, namely *E. coli* Nissle 1917 (Mullaney *et al*., 2013) and *E. coli* VL8 (Luang-In *et al*., 2014). Both were able to bioconvert glucoiberin and glucoraphanin to glucoiberverin and glucoerucin, respectively and both were isolated from the human gut. The sequence of sulfoxide reductase gene or protein for this activity is yet to be determined and there is no data on sulfoxide reductase activity from other *E. coli* strains from other sources. Thus the aims of this work were (1) to determine whether another *E. coli* from human gut and other *E. coli* that is not derived from human gut i.e. from molecular biology work were able to reduce the sulfoxide on glucoraphanin and/or sulforaphane after GSL induction during their aerobic incubation using HPLC and GC-MS analyses, (2) to determine the capacity to biotransform glucoraphanin and sulforaphane by bacterial sulfoxide reductases in cell-free extracts, (3) to determine whether a sulfoxide reductase from each *E. coli* strain required Mg^{2+} and/or reducing equivalent for its activity and to determine whether they were similar. These *E. coli* sulfoxide reductases may be of industrial use or laboratory use in chemical biotransformation in the future. The findings will also help us to better understand the occurrence of bacterial sulfoxide reductases in nature.

2. Materials and Methods

2.1 Materials

Glucoraphanin (ca. 99% purity) was obtained from Teelasu Co. Ltd (Thailand). Other compounds; sinigrin, sulforaphane, erucin, erucin nitrile, $MgCl_2$, reduced β-nicotinamide adenine dinucleotide phosphate (NADPH) were purchased from Sigma-Aldrich Co. (Singapore). *E. coli* VL11 was isolated from human fecal sample as the previous method (Luang-In *et al*., 2014) with 99% genome identity to *E. coli* UMNF18 (Altshul *et al*., 1999), BL21(DE3) was obtained from Dr. P. Klanrit from Khon Kaen University, Thailand.

2.2 Bacterial cultures and resting cells

Individual *E. coli* strains from glycerol stocks were aerobically sub-cultured in 5 mL LB media for 16 h at 37°C at 200 rpm. One hundred µl of overnight cultures were sub-cultured into 900 µl fresh LB media containing glucoraphanin (1 mM) for 16 hrs at 37°C at 200 rpm and these cells were referred to as 'GSL-induced cells'. The negative controls were carried out in parallel without addition of glucoraphanin (1 mM) in LB media and these cells were referred to as 'non-induced cells'. After 16 h, their OD_{600nm} and pH were recorded. The clear cell-free supernatant from each culture was collected for HPLC and GC-MS analyses after centrifugation of the cultures at 16,100 x g for 5 min. The cell pellets were washed with 0.1 M citrate phosphate buffer pH 7.0 twice and incubated with 1 mM glucoraphanin or 1 mM sulforaphane in 1 ml of this buffer for 4 h at 37°C at 200 rpm. These cells were referred to as 'resting cells'. Biological triplicates were used for all experiments. After 4 h, the resting cells were centrifuged at 16,100 x g for 5 min and clear supernatants were analysed for degradation of GSL and GSL biotransformation by HPLC and for the presence of GSL hydrolysis products and ITC biotransformation by GC-MS. Controls were included to test the stability of glucoraphanin and sulforaphane in LB media and buffer in the absence of bacterial cells/extracts under the same conditions. The same experiment in anaerobic conditions (Anaerogen system, Oxoid) were performed with *Enterococcus casseliflavus* CP1 (a Gram-positive facultative anaerobe gut bacteria) and *Acetobacter tropicalis* (a Gram-negative aerobic bacteria isolated from Thai water kefir). Neither bacterium did not exhibit sulfoxide reductase activity towards glucoraphanin suggesting reductase activity was common in *E. coli*.

2.3 Cell-free extract experiments

These experiments were performed as previously as described by Luang-In *et al*. (2014).

2.4 HPLC analysis and GC-MS analysis

Sample preparation and analytical conditions for HPLC and GC-MS analyses were carried out as the previous report (Luang-In *et al*., 2014). LC-20 AD (Shimadzu, Japan) equipped with SPD-M20 a diode array detector, Synergi 4 µm Hydro-RP 80A (150 x 4.6 mm) column (Phenomenex, USA) fitted with security guard column AQ C18 (4 x 3 mm) was used for HPLC analysis. Quantification of GSL was analysed using external standard method. GC-MS QP2010 (Shimadzu, Japan) with a capillary column, Agilent HP-5MS (5% Phenylmethylsiloxane, 30 m×0.25 mm id; film thickness, 0.25 µm) was used with helium as the carrier gas (split mode, 25:1; splitter inlet pressure, 40 kPa) was used for GC-MS analysis. Quantitative analysis of degradation products was performed using an external standard method.

3. Results and Discussion

3.1 Biotransformation of glucoraphanin by *E. coli* strains

To date, it has been reported that only *E. coli* VL8 (Luang-In *et al*., 2014) and *E. coli* Nissle 1917 (Mullaney *et al*., 2013), both isolated from the human gut, were able to metabolize glucoraphanin to erucin and erucin nitrile, and erucin nitrile only, respectively. There is limited data on GSL metabolism and sulfoxide reductase activity from other *E. coli* strains. Thus, we used two *E. coli* strains from different sources in this work for the study of their metabolic capacity on glucoraphanin. The results showed that only *E. coli* VL11 from human feces was able to metabolize glucoraphanin to

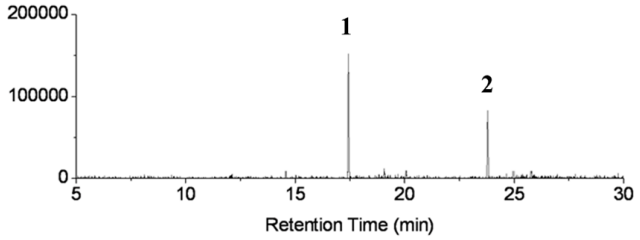

Figure 1. GC-MS chromatogram of products from glucoraphanin metabolized by *E. coli* VL11 at 16 h. Glucoraphanin was metabolized to erucin nitrile 1, peak at 17.4 min and erucin 2, peak at 23.8 min.

produce erucin and erucin nitrile (Figure 1). However, *E. coli* BL21 (DE3) was able to convert glucoraphanin substrate to glucoerucin at 52% conversion (Figure 2a, 2b) without degrading glucoraphanin and thus no degradation products were detected (Table 1). *E. coli* VL11 was also able to transform glucoraphanin to glucoerucin via reduction of the sulfoxide group and thus the degradation products were reduced analogues erucin and erucin nitrile (Figure 2c, Table 1). No substrate interconversion was observed in the controls indicating that bacteria must have mediated the reduction of sulfoxides in glucoraphanin. This finding indicated that two *E. coli* bacteria tested exhibited sulfoxide reductase activity to reduce the sulfoxide of glucoraphanin, but only *E. coli* VL11 expressed myrosinase-like enzyme to metabolise glucoraphanin and produce ITC and NIT as degradation products. Interestingly, these two *E. coli* bacteria grew well on glucoraphanin whilst the pH of 5.80 in *E. coli* VL11 incubation at 16 h was lower than the pH of 7.32 observed in BL21(DE3) incubation. The results of pH, degradation products during glucoraphanin incubation by *E. coli* VL11 were similar to those found in *E. coli* VL8 with 98% identity to *E. coli* O83:H1 NRG 857C (Luang-In *et al*., 2014). The presence of the products erucin and erucin nitrile in *E. coli* VL11 may contribute to lowering pH values whereas increased pH in BL21 (DE3) is unknown. Since both *E. coli* VL11 and VL8 from human gut bacteria were able to metabolize glucoraphanin as opposed to *E. coli* BL21(DE3) used in molecular cloning, it was thought that only human gut *E. coli* strains have evolved myrosinase-like enzyme to utilize and metabolize GSLs from cruciferous vegetables upon consumption and digestion in human gastrointestinal tract. However, GSL-reducing sulfoxide reductase enzyme may be conserved among all *E. coli* strains regardless of their origins. More experiments with more different *E. coli* strains are needed to confirm this hypothesis.

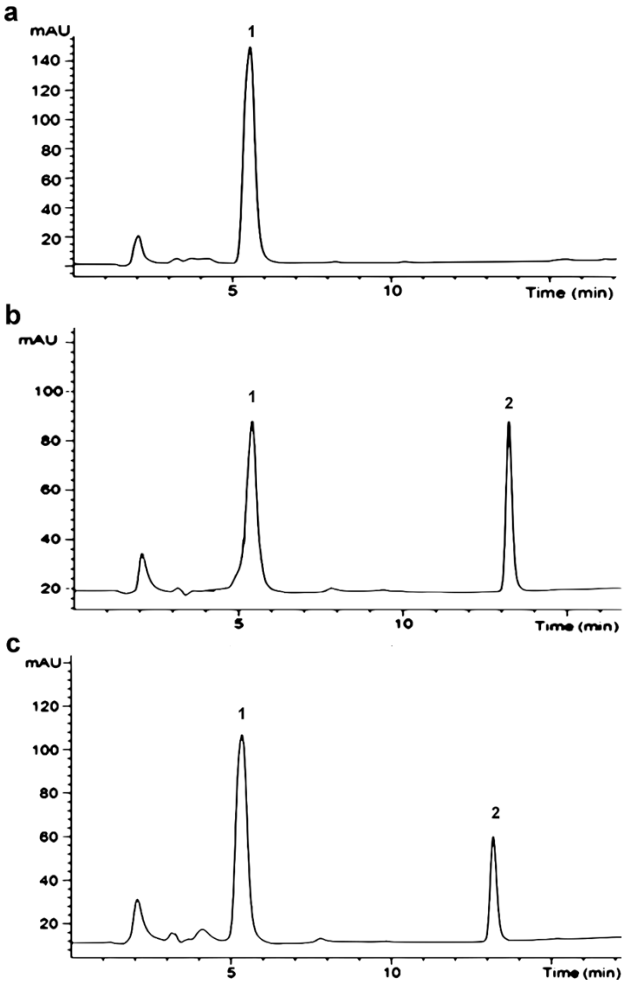

Figure 2. HPLC chromatograms of glucoraphanin converted to glucoerucin by *E. coli* cell-free extract in 0.1 M citrate phosphate buffer at 4 hrs aerobic incubation at 37°C. (A) Glucoraphanin, 1 (5.33 min) at 0 hrs. (B) Glucoraphanin was converted to glucoerucin, 2 (13.8 min) by *E. coli* BL21(DE3). (C) Glucoraphanin was converted to glucoerucin, 2 (13.8 min) by *E. coli* VL11. These figures are representatives of triplicates.

Table 1. Metabolism of 1 mM glucoraphanin by *E. coli* in LB media at 37°C for 16 hrs.

Strain	pH	OD_{600nm}	GRP remained (mM)	GER remained (mM)	Degradation products (μM)
VL11	5.80	0.643	0.39 ± 0.03	0.12 ± 0.03 51 ± 5 (Erucin nitrile)	30 ± 4 (Erucin)
BL21(DE3)	7.32	0.651	0.48 ± 0.04	0.52 ± 0.02	ND

Values are means ±SD, n=3, but only means are shown for pH and OD_{600nm}. ND, Not Detected; GRP, Glucoraphanin; GER, Glucoerucin. Initial pH was 6.64.

3.2 Sulfoxide reductases in cell-free extracts of induced *E. coli* cells

To determine whether sulfoxide reductase enzymes of *E. coli* strains were inducible by GSL, the two *E. coli* bacteria were grown overnight without glucoraphanin supplementation in LB media and the following day the cell-free extracts were obtained and aerobically incubated with either 1 mM glucoraphanin in 0.1 M citrate phosphate buffer pH 7.0 at 37 °C for 4 hrs. There was no reduction of glucoraphanin from non-induced *E. coli* cultures. However, when bacterial cultures of *E. coli* strains were supplemented with 1 mM glucoraphanin in LB media for 16 hrs before isolating the cell-free extracts in the buffer, the sulfoxide group of glucoraphanin was reduced to produce glucoerucin at 4 hrs incubation from both bacteria (Table 2). The reduction bioconversion (%) from glucoraphanin to a reduced analogue glucoerucin by each *E. coli* cell-free extracts with similar isolated protein quantity of 1,000 µg was found to be different (35% and 51%) at 4 h (Table 2) with higher sulfoxide reductase activity found in BL21(DE3). These results strongly suggest the existence of active, GSL-inducible, soluble, cytosolic sulfoxide reductase enzymes in both *E. coli* strains tested. These characteristics are the same to the previous report of sulfoxide reductase from *E. coli* VL8 (Luang-In *et al*., 2014). The cytosolic sulfoxide reductases from these *E. coli* bacteria seem to be different from dimethyl sulfoxide (DMSO) reductase of *E. coli,* which is a membrane enzyme composed of three subunits (Sambasivarao and Weiner, 1991).

To test whether these *E. coli* cell-free extracts were able to biotransform the sulfoxide group on sulforaphane to a reduced analogue i.e. erucin, similar experiments were carried out using *E. coli* cell-free extracts (obtained from glucoraphanin-induced overnight cultures) incubated with 1 mM sulforaphane in 0.1 M citrate phosphate buffer pH 7.0 at 37°C under aerobic conditions for 4 h. Cell-free extracts from glucoraphanin-induced *E. coli* cells biotransformed initial sulforaphane (1 mM) to erucin at 4 hrs and the remaining of 21-25% of sulforaphane was observed (Table 3). The control sample containing only 1 mM sulforaphane with no cell-free extracts in the same buffer showed the decline of 45% in sulforaphane level at 4 hrs (Table 3). This was possibly due to the instability of sulforaphane that may be conjugated to the buffer components or sulforaphane degradation or further metabolism to unknown and/or undetected metabolites. This result was in accordance with the previous reports of unstable ITCs in media and buffers (Kawakishi and Namiki, 1969; Tang *et al.*, 1972). The sulfoxide reductase from *E. coli* BL21(DE3) in cell-free extract was able to reduce the sulfoxides in sulforaphane to erucin within 4 h (Figure 3). This activity was very similar to that in *E. coli* VL11. Interestingly Ando *et al*. (1957) found that all *E. coli* strains they tested were able to reduce DMSO, but this test was not studied on our bacteria. In this work, both *E. coli* tested regardless of their origins exhibited sulfoxide reductase activity upon glucoraphanin induction to reduce the sulfoxide in glucoraphanin or sulforaphane. This led us to think that glucoraphanin or sulforaphane in the sulfoxide form may be toxic to *E. coli* bacteria, and bacterial reduction by a sulfoxide reductase may serve to make glucoraphanin or sulforaphane less toxic. In literature, sulforaphane was reported to be the most toxic to cancer cells and infectious bacteria and much more potent than its reduced form, erucin (Melchini and Traka, 2010).

Table 2. Reduction bioconversion of glucoraphanin to glucoerucin by cell-free extracts of *E. coli* (obtained from glucoraphanin-induced cells) at 37°C for 4 hrs.

Strain	Bioconversion from GRP to GER (%)
VL11	35 ± 3
BL21(DE3)	51 ± 2

Values are means ± SD, n = 3. Experiments were carried out in 0.1 M citrate phosphate buffer pH 7.0 at 37°C under aerobic condition with 200 µL cell-free extracts and 1 mM glucoraphanin as a substrate. *Protein quantity of 1,000 µg was used in each sample determined by Bradford assay using bovine serum albumin as a protein standard. GRP, Glucoraphanin; GER, Glucoerucin.

Table 3. Reduction bioconversion of sulforaphane to erucin by cell-free extracts of *E. coli* (from cells induced with 1 mM glucoraphanin overnight) at 37 °C for 4 hrs.

Strain	Time (h)	Cell-free extract[a]	
		SFN (µM)	ERN (µM)
Control[b]	0	1000 (100)	ND
(No bacteria)	4	550 ± 15 (55)	ND
VL11	4	213 ± 10 (21)	274 ± 13 (27)
BL21(DE3)	4	245 ± 13 (25)	322 ± 9 (32)

[a]Cell-free extracts (200 µL) was added to a 1 mL reaction containing 1,000 ìM sulforaphane at 0 hrs. [b]The control sample containing only 1 mM sulforaphane without cell-free extracts in the buffer. Values representing the ITC concentrations remained in the solution are means±SD, n=3. Values in brackets are remaining percentages of each ITC. This experiment was carried out in 0.1 M citrate phosphate buffer pH 7.0 at 37°C under aerobic conditions. ND, Not detected; SFN, Sulforaphane; ERN, Erucin.

3.3 Conditions for sulfoxide reductase activity of *E. coli* bacteria

The previous report showed that bacterial sulfoxide reductase from *E. coli* VL8 required Mg^{2+} and either NADH/NADPH as electron donor for its activity in the reduction of sulfoxide groups (Luang-In *et al*., 2014). In this work, sulfoxide reductases from *E. coli* cell-free extracts of two bacteria

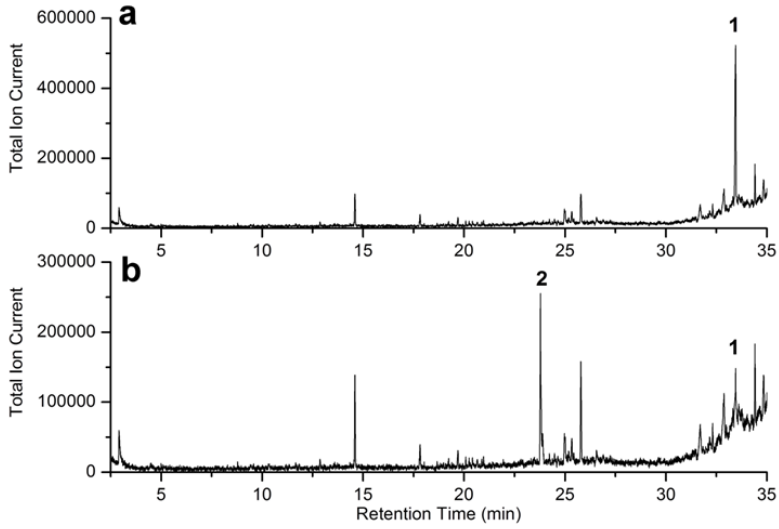

Figure 3. GC-MS chromatograms showing reduction bioconversion of sulforaphane to erucin by *E. coli* BL21(DE3) cell-free extracts. (A) Sulforaphane, 1 (33.40 min) at 0 hrs. (B) A decline of sulforaphane, 1 led to an increase of erucin, 2 (23.86 min) after 4 hrs aerobic incubation at 37°C in citrate phosphate buffer. These figures are representatives of triplicates.

tested required both Mg^{2+} and NADPH for their activities in 0.1 M citrate phosphate buffer at pH 7.0 and at 37°C as their optimal conditions for sulfoxide reductase activity (Table 4) with higher bioconversion (35%) from BL21(DE3) desalted cell-free extracts. The reduction activities from both bacterial desalted cell-free extracts with both 1 mM Mg^{2+} and 1 mM NADPH were lower than those of non-desalted cell-free extracts suggesting the used conditions of 1 mM Mg^{2+} and 1 mM NADPH may be not optimal for their activities. These bacterial sulfoxide reductases did not work without NADPH or Mg^{2+}. It is important to note that other metal ions other than Mg^{2+} or NADH were not tested in the current study. From this finding, we concluded that bacterial sulfoxide reductases involved in the reduction of the sulfoxides in glucoraphanin and sulforaphane from these two *E. coli* bacteria share a similarity in the requirement of Mg^{2+} and NADPH as electron donor for their activities. This was partly similar to DMSO sulfoxide reductase found in *E. coli* K-12 that needed either NADH or NADPH however it did not need Mg^{2+} for its activity to reduce DMSO (Zinder and Brock, 1978). This emphasizes the differences between DMSO sulfoxide reductase in *E. coli* and GSL/ITC sulfoxide reductases from two *E. coli* strains in this work.

Thus far, there were only three *E. coli* bacteria reported to exhibit either myrosinase activity and/or sulfoxide reductase activity (Table 5). Only *E. coli* strains isolated from human gut or food source namely, *E. coli* 1917 Nissle (Mullaney *et al*., 2013), *E. coli* VL8 (Luang-In *et al*., 2014) and *E. coli* O157:H7 (Luciano *et al*., 2009), possess myrosinase-like activity to metabolize GSLs to ITC and/or NIT as degradation products. *E. coli* O157:H7 was able to metabolize sinigrin and sinalbin to allyl ITC and ñ-hydroxybenzyl ITC, respectively during dry sausage manufacture (Luciano *et al*., 2009). Interestingly, *E. coli* 1917 Nissle produced only NIT product from GSL metabolism (Mullaney *et al*., 2013) whereas *E. coli* VL8 (Luang-In *et al*., 2014) and VL11 in this work,

Table 4. Parameters for reductase activity from desalted cell-free extracts of three *E. coli* strains at 37°C for 4 hrs.

Strain	Bioconversion from GRP to GER (%)		
	1 mM NADPH	1 mM Mg^{2+}	1 mM NADPH + 1 mM Mg^{2+}
VL11	ND	ND	23 ± 3
BL21(DE3)	ND	ND	35 ± 2

Desalted cell-free extracts (200 µL) was added to a 1 mL reaction containing 500 µM glucoraphanin. Values represented the amount of glucoraphanin converted to glucoerucin at 4 hrs divided by the initial amount of glucoraphanin at 0 hrs (in % conversion). Values are means ±SD, n=3. This experiment was carried out in 0.1 M citrate phosphate buffer pH 7.0 at 37°C under aerobic conditions. ND, Not detected; GRP, Glucoraphanin; GER, Glucoerucin.

Table 5. Myrosinase and sulfoxide reductase activity in different *E. coli* strains.

Strains	% genome similarity	Origins	Myrosinase activity	Sulfoxide reductase activity
O157:H7 (Luciano *et al.*, 2009)	NA	Pathogenic, isolated from sausage	1) Sinigrin → Allyl ITC 2) Sinalbin → ρ-hydroxybenzyl ITC	NA
1917 Nissle (Mullaney *et al.*, 2013)	NA	Probiotic, human gut	1) Glucoraphanin → Erucin nitrile 2) Glucoiberverin → Iberverin nitrile	1) Glucoraphanin → Glucoerucin 2) Glucoiberin → Glucoiberverin 3) Iberin nitrile → Iberverin nitrile 4) Sulforaphane nitrile → Erucin nitrile
VL8 (Luang-In *et al.*, 2014)	98%, O183:H1 NRG857C	Human gut	1) Glucoraphanin → Erucin + Erucin nitrile 2) Glucoiberin → Iberverin + Iberverin nitrile	1) Glucoraphanin → Glucoerucin 2) Glucoiberin → Glucoiberverin 3) Sulforaphane → Erucin
VL11(This work)	99%, UMNF18	Human gut	1) Glucoraphanin → Erucin + Erucin nitrile	1) Glucoraphanin → Glucoerucin 2) Sulforaphane → Erucin
BL21(DE3) (This work)	NA	Non-pathogenic, expression host	ND	1) Glucoraphanin → Glucoerucin 2) Sulforaphane → Erucin

ITC, Isothiocyanate; NA, Not available; ND, Not detected.

derived from human gut as 1917 Nissle, produced both ITC and NIT products from the same GSL substrate used with *E. coli* 1917 Nissle suggesting different GSL metabolism mechanisms exist among different *E. coli* strains. It was proposed that all facultative anaerobic bacteria in the gut possess sulfoxide reductase genes responsible for reduction activity (Mullaney *et al.*, 2013). Our result showed otherwise, *E. coli* BL21(DE3) is not a gut bacterium but does exhibit sulfoxide reductase activity. The finding certainly has offered the new knowledge about occurrence of sulfoxide reductases in *E. coli* bacteria. It remains to be determined whether these sulfoxide reductases can also act upon xenobiotics and/or DMSO containing sulfoxide groups or they are specific to the sulfoxide in GSL or ITC. This also demonstrates that *E. coli* BL21(DE3) has endogenous GSL-inducible sulfoxide reductase. The reason that *E. coli* BL21(DE3) sulfoxide reductase had similar activity towards sulforaphane as *E. coli* VL11 however exhibiting higher reductase activity than *E. coli* VL11 towards glucoraphanin is not certain. It was possibly because sulforaphane is molecularly smaller, more hydrophobic and more unstable due to its electrophilic -N=C=S group than glucoraphanin and thus both sulfoxide reductases can access or react upon it more readily than glucoraphanin.

These bacterial reductases may be further tested for dimethyl sulfoxide (DMSO) reductase activity to be potentially used as an enzyme-linked method to determine DMSO in aqueous solutions in seawater, freshwater, food and other industries as an alternative source to DMSO reductase from purified from the bacterium *Rliodobacter capsufatus* (Hatton *et al.*, 1994). In addition, they should be tested for their dissimilatory metal reduction and potential applications in the bioremediation of heavy metal contamination. High oxidation state forms of arsenic and selenium are toxic contaminants derived from a number of human industries; their reduction to less toxic and more easily eliminated states could be a promising way to treat these pollutants (Stolz *et al.*, 2006). These bacterial sulfoxide reductases from *E. coli* may also be useful in biotransformation of sulforaphane/glucoraphanin to erucin/glucoerucin for therapeutic purposes. Although erucin had not been much less widely studied for its bioactivity compared to its oxidized analogue and most studied isothiocyanate sulforaphane, sulforaphane is not necessarily more bioactive than erucin. A recent report showed that therapeutic efficacy of erucin was higher than sulforaphane as demonstrated in a heterotrophic bladder tumor mouse model (Abbaoui *et al.*, 2012). In RT4 cells, erucin was more effective in inducing apoptosis via increasing caspase 3/7 activity than sulforaphane at the same concentration (Azarenko *et al.*, 2014). In addition, a combination of lapatinib (anti-breast cancer drug) with erucin was more effective than that with sulforaphane in decreasing viability of HER2 overexpressing breast cancer cells. These findings might support the optimization of therapy based on lapatinib treatment (Kaczyñska *et al.*, 2015). All these suggest that usage of erucin instead of sulforaphane could be of therapeutic advantage but further investigations are necessary.

To conclude, both *E. coli* VL11 and BL21(DE3) with different origins exhibited sulfoxide reductase activity upon

glucoraphanin induction with the same requirement for Mg^{2+} ion and NADPH. These sulfoxide reductase enzymes may be a common occurrence among all *E. coli* bacteria through evolution regardless of their strains and origins. This work has shed some light on the occurrence of sulfoxide reductases among *E. coli* bacteria.

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