

Intertwinement of stress response regulons in *Bifidobacterium breve* UCC2003

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Bifidobacteria constitute an important component of the microbiota of the gastrointestinal tract of humans and other mammals. Various bifidobacterial strains are commercially exploited because of their perceived beneficial role in the maintenance of gut homeostasis. We have determined the response of *B. breve* UCC2003, a Gram-positive bacterium originally isolated from the nursing stool of a breast-fed infant, to several stresses (heat, osmotic, solvent) using transcriptomics, classical techniques and in silico analysis, as well as the transcriptional response of *B. breve* UCC2003 to oxidative stresses caused by the exposure to diamide, peroxide and environmental oxygen. Integration of these results allowed the formulation of a model for an interacting regulatory network for stress response in *B. breve* UCC2003, where HspR controls SOS response and the ClgR regulon, which in turn regulates and is regulated by HrcA. This model of an interacting regulatory network is believed to represent the paradigm for stress adaptation in bifidobacteria.

Bifidobacteria represent one of the most common inhabitants of the animal gastrointestinal tract (GIT). From a phylogenetic perspective the genus *Bifidobacterium* belongs to the subclass Actinobacteridae of the phylum Actinobacteria of high GC-content, Gram-positive bacteria.¹ In the human GIT their presence has been associated with beneficial effects, which have led to the widespread use of many bifidobacterial strains as components of health-promoting or probiotic foods.²

From production of the probiotic to storage and digestion, bifidobacteria, which are strict anaerobes, encounter a plethora of environmental stresses. In particular, exposure to oxygen or other oxygen-derived radicals, organic acids, osmotic stress, heat and cold have a major negative impact on bifidobacterial viability. To cope with these life-threatening challenges they can employ a variety of coping and survival strategies.

Like other bacteria, bifidobacteria are capable of synthesizing a particular set of proteins protecting the cell from damage caused by the accumulation of unfolded and/or misfolded proteins. Several of these protective proteins act as molecular chaperones, such as GroEL (Hsp60), DnaK (Hsp70) and ClpB (Hsp100), or as proteases, such as Lon, ClpP and ClpC, and play key roles in several posttranslational events to prevent protein denaturation, aggregation and misfolding.^{3,4}

Because of their strict anaerobic life style, bifidobacteria are exquisitely sensitive to oxidative stress. Both proteins and DNA suffer from oxidative damage, and in order to alleviate this damage, bifidobacteria, like other bacteria, can upregulate systems that reduce reactive oxygen species, or reverse or remove the damage. To reduce organic peroxides it can upregulate peroxidoredoxins, such as that specified by the AhpC/F system, which has also been shown to function as a chaperone in *H. pylori*.⁵ Additionally, it can protect DNA by upregulating the expression of the so-called DPS proteins, which sequester iron and protect DNA from damage by forming a crystalline structure around the DNA.^{6,7}

Key words: bifidobacterium, stress response, transcriptomics

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Table 1. Stress induced genes of *B. breve* UCC2003

Locus	Product	Description	42°C	44°C	47°C	50°C	EtOH	Osmo	Diamide	H ₂ O ₂	Shaking
Bbr_0038	AhpC	Alkyl hydroperoxide reductase	-2.0	-3.2	0.3	-10.2	3.9	-1.3	-1.1	-1.1	4.1
Bbr_0039	AhpF	Flavoenzyme	-1.8	-2.3	-1.2	-3.2	3.6	1.2	2.9	1.7	7.0
Bbr_0076	Hsp20	Chaperone	19.4	26.7	51.8	119.3	1.8	5.4	66.1	8.0	1.8
Bbr_0124	DnaK	Chaperone protein	3.5	7.0	10.2	29.3	3.6	16.7	20.3	2.7	1.3
Bbr_0125	GrpE	GrpE protein	3.2	6.7	7.8	22.8	3.6	13.1	15.5	2.2	1.2
Bbr_0126	DnaJ1	Chaperone protein	3.6	8.2	10.2	24.9	1.6	16.2	22.5	1.7	1.3
Bbr_0127	HspR	Regulator	2.6	6.1	8.8	23.8	1.9	15.4	9.0	1.8	-1.1
Bbr_0216		Modification methylase	-1.3	1.3	5.3	3.1	-1.1	1.9	1.0	3.7	2.4
Bbr_0496	MutY	DNA glycosylase	1.1	2.3	2.5	-1.7	3.9	1.7	1.9	-2.4	2.1
Bbr_0585	ImpB	DNA repair	1.3	4.3	33.0	18.3	1.9	4.7	1.2	6.3	2.3
Bbr_0794	ClpPI	Protease	1.0	1.2	1.0	-1.3	3.4	-2.3	1.0	-1.1	1.1
Bbr_0795	ClpP2	Protease	-1.1	1.2	-1.4	-3.5	3.3	-1.5	0.9	1.3	1.1
Bbr_1004	HrcA	Regulator	2.0	3.0	11.2	3.5	5.7	6.9	3.7	2.0	1.5
Bbr_1005	DnaJ2	Chaperone protein	1.4	2.4	2.0	-4.4	3.8	1.5	1.8	1.9	0.9
Bbr_1013	RuvA	Holliday junction DNA helicase	1.1	2.0	5.7	6.7	2.6	1.5	1.6	1.8	1.1
Bbr_1014	RuvB	Holliday junction DNA helicase	1.0	1.9	2.2	-2.4	2.4	1.0	-2.2	2.2	1.4
Bbr_1046	Nfo	Endonuclease IV	1.1	1.7	8.9	18.1	5.0	1.9	2.4	1.3	1.8
Bbr_1171	HrdB	principal sigma factor	1.1	1.7	5.8	13.1	1.7	1.3	1.0	-2.4	-1.6
Bbr_1180	RecX	Recombination regulator	1.2	2.9	17.7	10.9	3.5	2.6	5.7	3.6	2.1
Bbr_1181	RecA	Recombinase	1.4	3.3	10.2	12.0	4.3	2.4	8.6	5.8	2.1
Bbr_1182	-	-	1.0	1.7	9.1	66.9	3.6	1.2	4.7	2.9	1.1
Bbr_1183	ClgR	Regulator	1.6	3.6	57.2	416.3	7.1	3.3	28.9	7.2	2.2
Bbr_1271	LexA	Regulator	1.1	1.9	2.8	7.0	3.4	6.8	1.7	6.2	3.6
Bbr_1356	ClpC	Protease	-1.1	1.2	-1.2	-2.7	2.4	-1.6	-1.8	1.0	1.1
Bbr_1364	GroEL	Chaperone protein	2.1	3.6	3.8	1.1	3.8	1.1	1.5	2.1	1.4
Bbr_1668	GroES	Chaperone protein	1.5	2.3	3.3	3.3	3.3	1.8	4.1	3.3	1.2
Bbr_1781	ClpB	Chaperone protein	4.2	12.2	27.3	94.6	4.4	8.0	36.0	13.1	2.2

The respective stresses are given, 42°–50°, 1 hour exposure to the temperature given; EtOH, 1 hour exposure to 8% ethanol; Osmo, 1 hour exposure to 0.5 M NaCl; Diamide, 1 hour exposure to 8 mM diamide; H₂O₂, 1 hour exposure to 1.2 mM H₂O₂; Shaking, 1 hour exposure to environmental oxygen by shaking at 200 rpm. Micro array fold upregulation is given as compared to an unstressed *B. breve* UCC2003 culture.

In high GC-content, Gram-positive bacteria, regulation of the AhpC/F system is likely to occur by OxyR and a CRP/FNR type regulator.^{8,9} Members of these families are commonly associated with the response to changes in intracellular oxygen levels, redox-state or oxidative stresses.^{10,11} Bioinformatic-based analysis of the *B. breve* UCC2003 genome sequence has revealed the presence of just a single regulator-encoding gene annotated as a putative member of the Crp/FNR family. Furthermore, the UCC2003 genome contains putative Crp/FNR type motifs not only in the promoter regions of *ahpC/F*, but also in the promoter region of *hrcA*. Preliminary microarray analysis shows the upregulation of *ahpC/F* as well as *hrcA* under oxidative stress caused

by the exposure of *B. breve* UCC2003 to diamide, peroxide and environmental oxygen. Additionally, other overlaps in upregulated genes between the heat shock response and oxidative response were detected such as *clpB* and *clgR* (Table 1).

To alleviate DNA damage inflicted by these environmental stresses the SOS system is activated.¹² Under normal conditions, expression of genes involved in DNA damage repair is very low as they are under tight negative control. Repression of these genes is managed by the LexA regulator, which binds to the SOS box in the promoter region of these genes.¹³ Activation of the SOS genes occurs when DNA is damaged, which can happen as a result of heat or oxidative stress. This DNA damage results in single stranded

DNA (ssDNA) accumulation at the site of (stalled) replication, which in turn activates RecA. LexA autocleavage is promoted by this activated form of RecA and derepression of the SOS genes takes place as a consequence.¹⁴

In *B. breve* UCC2003, SOS response occurs when the cells are subjected to both heat shock and oxidative stresses (Table 1).¹⁵ Induction of RecA expression is probably also under the control of HspR under heat shock conditions because of the proximity of *recA* and *recX* to the HspR-controlled *clgR* and Bbr_1182 genes, which form a transcriptional unit. Induction of *recA* expression by HspR could consequently activate LexA autoproteolysis and result in upregulation of the SOS genes (Fig. 1) in addition to other targets of HspR.

Stress responses in bifidobacteria are thus governed by a complex interactive network of regulators (ClgR, HspR, HrcA, LexA and others). A model for the stress regulatory network of *B. breve* UCC2003 was constructed using the current knowledge obtained from the research described in the manuscript and other available literature data.¹⁵ A model including the oxidative stress conditions is given in **Figure 1**. Although simplified, this model shows the complexity of stress gene regulation in *B. breve* UCC2003. Connections between the various regulons occur at different levels, indicative of complex interactions. The high level of conservation of the regulons and binding sites between various sequenced bifidobacterial genomes suggest that this model generally applies to all representatives of the Bifidobacterium genus.

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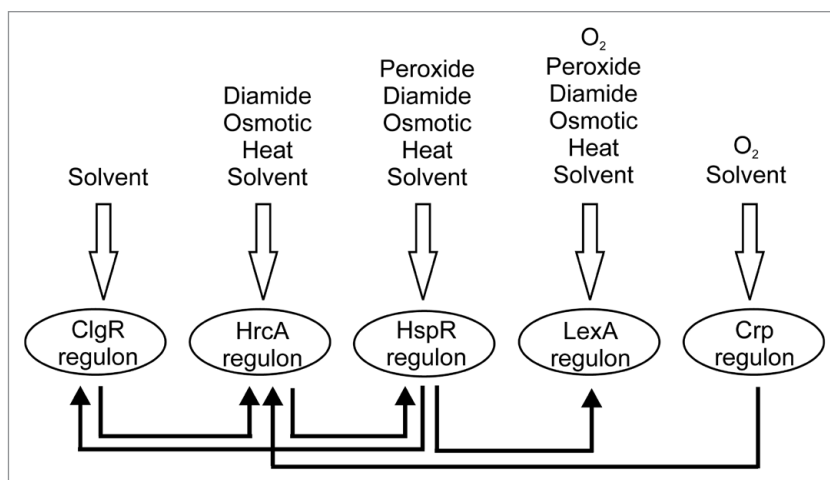


Figure 1. Simplified representation of the stress gene regulatory network of *B. breve* UCC2003. Stress conditions that (partly) activate the regulons are indicated above; lines indicate a (putative) interaction between the regulons.

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