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EXPERIMENTAL ARTICLES

Involvement of Alkylhydroxybenzenes, Microbial Autoregulators, in Controlling the Expression of Stress Regulons

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Abstract—Alkylhydroxybenzenes (AHB) were found to control the activation of protective functions of microorganisms by inducing stress gene expression and increasing the frequency of the intrapopulation phase transitions which are responsible for the phenotypic variability of bacteria. We established the dependence of the regulatory effects of AHB on their structure (alkyl radical length) and concentration. A reversion assay using the tryptophan auxotrophic strain *Bacillus subtilis trpA5* B 1733 indicated a relationship between the reversion frequency that was 40–120 times higher than the background value and phase transition's intensity (with R ⟶ S transition rates up to 87% in contrast to 2% in the control experiment) induced by specific doses (5–100 mg/ml) of long-chain AHB such as C_{12}-AHB acting for a short time (1 h) on vegetative (dividing or stationary-phase) *B. subtilis* cells. Using four test strains constructed from *Escherichia coli* C600 *thi, thr, leu* Δ*(pro-lac)* with transcriptional or translational vectors containing the hybrid *umuD-lacZ* or *osmE-lacZ* operons, we demonstrated that AHB perform the regulatory functions involved in controlling the SOS response gene expression and the general *rpoS*-dependent stationary-phase regulon, respectively. The dose-dependent effect of long-chain AHB (within the 50–100 μg/ml range) resulting in a two- to threefold increase in the stress gene expression, similar to the effect of natural stress factors such as UV irradiation and starvation, provides evidence that AHB function as alarmones (danger signals). From the fact that the *osm E* gene is upregulated by 35–70 μg/ml C_{12}-AHB (its regulation level is increased up to twofold), it follows that C_{12}-AHB controls *rpoS*-dependent regulation and the transition to the stationary phase. The effect of the short-chain homologue C_7-AHB substantially differs from that of C_{12}-AHB. C_7-AHB in concentrations of 10–100 μg/ml causes a significant decrease in *osmE* and *umuD* expression. A 30-min preincubation of cells with 10–100 μg/ml C_7-AHB protected them from UV irradiation, as was observed as a 3.6-fold decrease in *umuD* expression. Comparative analysis of the marker gene's expression values in the strains with the transcriptional and translational vectors demonstrates that AHB nonspecifically activate stress regulons at the transcription level.

Key words: alkylhydroxybenzenes, regulation, adaptive mutations, stress genes, hybrid operons, SOS response, *rpoS* regulon, *E. coli, B. subtilis.*

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The adaptive potential of microorganisms that manifests itself under stress and during environmental changes includes alterations in the metabolic activity of their cells, cell division arrest, stress gene expression, and an increase in adaptive mutation frequency. In total, these processes provide for the adaptability and viability of microbial populations under novel or unfavorable conditions [1–4]. In response to the factors causing single-strand breaks in the DNA or disrupting its replication, the SOS response system is induced. It involves the expression of over 40 genes including those responsible for recombinational repair, cell division control, and DNA synthesis bypassing defective nucleotides [5]. In *Escherichia coli*, these functions are performed by the DNA polymerases Pol IV, the *dinP* gene product, and Pol V, the product of the RecA-dependent *umuD* gene. These polymerases are characterized by low precision, resulting in a 100-fold increase in the number of faulty repair events. This causes the hypermutability effect that promotes survival of the population. The nature of the SOS response-inducing signal operating on the population level has not yet been elucidated.

Apart from external stress factors, adaptive responses are initiated by "programmed" stress (starvation or high cell density) and the transition to the stationary phase involving the onset of expression of the general stationary-phase *rpoS* regulon [6]. The communicative signal for the transition to the stationary phase has not yet been detected. Presumably, density-dependent autoregulators such as homoserine lactones [7]

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and alkylhydroxybenzenes (AHB) [8] may perform these functions.

The adaptive potential of microorganisms also includes their capacity for intrapopulation phenotypic variability that is caused by reversible recombination processes in the genetic material of their cells [2, 4, 9]. In accord with a number of hypotheses, phenotypic (phase) transitions involve (i) cell division arrest, particularly during the onset of the stationary phase, and (ii) the effects of the regulatory systems controlling the expression of stationary-phase regulons [10]. A relationship between phenotypic transitions and an increased concentration of non-species-specific anabiosis inducers represented by AHB in a number of bacteria and yeast species was revealed earlier [8, 11]. The Ames test with the *Salmonella typhimurium* TA-100 strain also demonstrated the mutagenic properties of long-chain AHB and the induction of the transition of R-type cells to the S form [12]. It was established that AHB are accumulated in stationary-phase or dormant microbial cells (starvation stress), whose germination results in an increase in the percentage of minor phenotypes [8, 11, 13]. In addition, AHB biosynthesis is stimulated in exponentially growing bacterial cultures by nonlethal stress, as studies on heat shock in *Micrococcus luteus* revealed [14]. The protective effect of AHB produced upon an increase in extra- and intracellular AHB concentration in a microbial culture manifests itself in an enhanced resistance of its cells and the retention of their proliferation capacity. The mechanism of the protective effect of AHB is based, inter alia, upon their antioxidant activity [15] and capacity for physical and chemical interactions (including hydrophobic, electrostatic, and hydrogen bonds) with cell biopolymers, such as proteins and DNA, resulting in their structural modification and changes in functional activity [8, 16–18].

The goal of this work was to elucidate the role of AHB as a communicative signal involved in (i) regulating the expression of the stress genes of the SOS system and the *rpoS* regulon, and (ii) increasing the frequency of intrapopulation phase transitions.

MATERIALS AND METHODS

The AHB alkylresorcinols C_7- and C_{12}-AHB synthesized in the Lomonosov Institute of Fine Chemical Technology (purity index 99.9%) that differ in their alkyl radical length, hydrophobicity, and biological effects [8, 19] were used as microbial autoregulators. The stock AHB solutions were prepared in 30% ethanol, with the 1.5% (vol/vol) solvent concentration in the samples.

The studies were conducted with two types of subjects.

(i) The mutant *Bacillus subtilis* B 1733 *trpA5*, an auxotroph for tryptophan, was obtained from the collection of the State Research Institute of Genetics and Selection of Industrial Microorganisms. The bacteria were cultivated in a liquid medium [11] in 250-ml flasks with 50 ml of the medium at 30°C on a shaker (160 rpm). The inoculum (suspension of 7-day-old endospores) was added to the medium to the initial optical density (OD) of 0.2 measured with a Specord spectrophotometer (λ = 660 nm and l = 10 mm). The number of colony-forming units (CFU) was determined by plating *B. subtilis* cell suspensions on the agar-supplemented Spitzeisen medium containing the following (g/l): $(NH_4)_2SO_4$, 2.0; KH_2PO_4, 6.0; K_2HPO_4, 14.0, sodium citrate, 1.0; $MgSO_4 \cdot 7H_2O$, 0.2; glucose, 5.0; agar 20; and tryptophan, 50 μg/ml. Tryptophan was omitted from the medium used for detecting the prototrophic revertants. AHB were added to the aliquots of cultures during the exponential (6 h, OD = 1.5) and stationary (12 h, OD = 2.3) growth phases; after incubation for 1 h, the cultures were transferred to the tryptophan-containing and tryptophan-lacking medium. The tested substance was considered a mutagen if the reversion frequency determined in its presence was two or more times higher than the spontaneous background level.

(ii) The other group of subjects included four genetic models constructed from the bacterium *E. coli* C600 *thi*, *thr*, *leu* Δ(*pro-lac*) and containing the hybrid operons *osmE-lacZ* (the *osmE* gene coding for the stress-inducible lipoproteins of the outer membrane forms part of the *rpoS* regulon) or *umuD-lacZ* (the *umuD* gene coding for the low precision polymerase Pol V is involved in the SOS response). For this purpose, the leader domain of the *osmE* gene including the promoter was cloned by amplifying it using the PCR assay with the flanking primers E1 (5'-cccgaattccttaaagctaaccgttg) and E2 (5'-cccggatccatattcttgttcatattccg). In a similar fashion, the leader domain of the *umuD* gene was cloned using the flanking primers D1 (5'-cccgaattctgctggatgagcgtgc) and D2 (5'-cccggatccgcaggcttgataacaac). The PCR assay was performed with a Gene Amp PCR System 2400 device (Perkin-Elmer Cetus). The temperature conditions were adjusted taking into account the length and the composition of the amplified fragment and the primers. The PCR products were isolated and purified using the GFX™ PCR DNA and the Gel Band Purification Kit (GE Healthcare). The primers of the *osmE* and *umuD* genes contained recognition sites for restriction endonucleases *Eco*RI and *Bam*HI, respectively. The PCR fragments obtained were cloned into the poly linker sequence of the expression multicopy cloning vectors pJEL250 (a transcriptional vector) and pJEL246 (a translational vector), which contain the *lacZ* reporter gene and the ampicillin resistance determinant (Amp^R) [20].

The vectors were transferred to the recipient strain via transformation. The following strains were obtained:

um250—E. coli C600 *thi, thr, leu* Δ(*pro-lac*)/pJEL250Amp^R*umuD-lacZ*;

um246—E. coli C600 *thi, thr, leu* Δ(*pro-lac*)/pJEL246Amp^R*umuD-lacZ*;

os250—E. coli C600 *thi, thr, leu* Δ(*pro-lac*)/pJEL250Amp^R*osmE-lacZ*;

os246—E. coli C600 *thi, thr, leu* Δ(*pro-lac*)/pJEL246Amp^R*osmE-lacZ*.

The transformed bacteria grown at 30°C contained one or two copies per cell of each plasmid.

The specific activity of β-galactosidase (per unit of culture OD) was determined in the cells by the standard technique of degrading *o*-nitrophenol-β-galactopyranoside. An activity unit corresponded to the enzyme amount that increased the OD value at 420 nm by 1 unit in 1 min [21].

The test strains of *E. coli* were grown in the LB (Luria–Bertani) medium with ampicillin (50 μg/ml) in 250-ml flasks (50 ml of the medium) at 30°C on a shaker (160 rpm). The inoculum was a stationary-state culture. Its amount added to the medium yielded an initial optical density of 0.2.

In experimental studies, solutions with the required AHB concentrations were added to aliquots of exponential- or stationary-phase cultures of the test strains and the system was incubated under static conditions for 1 h. The specific quantity of β-galactosidase in the cells was determined after washing them twice with a Z buffer [21]. In control studies, the sample was supplemented with an equivalent amount of the solvent (1.5% ethanol) that did not influence the reporter gene expression according to the results of preliminary studies.

The regulation level in the hybrid *osmE-lacZ* operon-containing strains was determined from the ratio between β-galactosidase activity in AHB-treated exponential-phase cells to that in the control stationary-phase cells without AHB.

The regulation level in the hybrid *umuD-lacZ* operon-containing strains was determined from the ratio between β-galactosidase activity in AHB-treated cells to that in UV-irradiated cells.

UV irradiation of the cell suspensions was performed using a DB 30-1W UV lamp. The distance from the radiation source was 0.8 m.

Statistical analysis was carried out using standard techniques and the Microsoft Excel XP software package. The required parameter value was the average of three independent experiments, taking into account the standard deviation value. Differences between the data groups were considered significant at P values below 0.05.

RESULTS

The impact of AHB on the genome stability of bacterial cells of different physiological age was investigated using the reversion assay with the tryptophan auxotrophic strain *Bacillus subtilis trpA5*. Microbial AHB act, inter alia, as growth autoinhibitors [8]. Therefore, adequate data on the influence of C_7- and C_{12}-AHB on the bacterial genome stability were obtained using concentration ranges (up to 100 μg/ml C_7-AHB and 50 μg/ml C_{12}-AHB), within which the growth-inhibiting activity of the AHB did not override their predicted mutagenic effect.

Our studies revealed an influence of the growth phase of the culture on the ratio between the subpopulations of phenotypically distinct cells after removal of the inducing factor, i.e., the AHB effect. For example, the hydrophobic C_{12}-AHB in a concentration of 5 μg/ml increased 43-fold the reversion frequency in cell populations of the exponential-phase of the *B. subtilis trpA5* culture, with concomitant induction of the phase transition from the original R phenotype to the S phenotype (the S phenotype percentage was 87% in contrast to 2% in the control study, Table 1). Stationary-phase cells were more resistant to C_{12}-AHB: The reversion frequency increased 4- and 125-fold with 10 and 50 μg/ml C_{12}-AHB, respectively, while the S variant's percentage among the revertants increased to 50 and 84%, respectively (the control value was 5%). The more hydrophilic C_7-AHB exerted a weak mutagenic influence that only manifested itself in the exponential-phase cells. Its high concentrations (50 μg/ml) caused a 2.5-fold increase in the reversion frequency and brought the percentage of the minor S variant up to 40% (in contrast to 5% in the control study). C_7-AHB displayed antimutagenic activity with respect to stationary-phase cells. The reversion frequency was significantly below that in the control sample in all tested autoregulator concentrations.

Hence, a relationship was revealed between the reversion frequency and the intensity of R ⟶ S phase transitions induced by specific AHB doses acting for a short time (1 h) on vegetative (dividing and stationary-phase) cells of the auxotrophic *B. subtilis* strain.

The AHB involvement in regulating the expression of the SOS response genes (*umuD*) and the stationary-phase's *rpoS* regulon (*osmE*) was investigated using the genetically modified strains *um250, os250, um246*, and *os246* by determining the expression level of the hybrid operons under stress and in the presence of exogenous AHB. The activity of β-galactosidase increased twofold in the cells of the strains *um250* and *um246* upon an increase in the UV dose that caused single-strand breaks in the DNA without decreasing the CFU number. This indicated that the SOS response was carried out (Table 2). In stationary-phase cells of the strains *os250* and *os246*, the β-galactosidase level was two times higher than in the cells from an exponentially growing culture (Fig. 1), indicative of *rpoS* activation during starvation stress. The data obtained supported the idea that the genetic models developed by us could be used in the main series of experiments.

In preliminary studies, we determined the AHB concentration ranges, within which the growth-inhibiting activity of the AHB homologues, did not manifest itself

Table 1. Increase in reversion frequency relative to the spontaneous background level and the R ⟶ S phase transition for the dominant variant in the *B. subtilis* B 1733 *trpA5* strain under the influence of AHB

AHB concentration, μg/ml	Increase in reversion frequency relative to the spontaneous background level, times	Phase transition index of the revertants, %	
		R	S
In the presence of C_{12}-AHB			
Exponential growth phase			
0	1.0	98	2
1	1.3	95	5
5	43.0	13	87
50*	1650.0	31	69
Stationary growth phase			
0	1.0	95	5
1	1.1	92	8
5	1.2	88	12
10	4.0	50	50
50	125.0	16	84
In the presence of C_7-AHB			
Exponential growth phase			
0	1.0	98	2
5	1.0	100	0
10	2.0	72	28
50	2.5	60	40
Stationary growth phase			
0	1.0	95	5
5	0.74	100	0
10	0.34	84	16
50	0.62	95	5

* C_{12}-AHB inhibited growth of the exponential-phase cells in concentrations over 5 μg/ml. Hence, the data are inadequate and are given here for comparison.

in the tested *E. coli* strains. This condition was satisfied at C_{12}-AHB and C_7-AHB concentrations up to 100 and 600 μg/ml, respectively.

The effect of the hydrophobic C_{12}-AHB in concentrations of 50 and 100 μg/ml on the cells of the strains used for testing the SOS response resulted in a proportional (maximally twofold) increase in β-galactosidase activity, both in the transcriptional and the translational fusion, to the same value as under the influence of UV irradiation, a natural stress factor, pointing to a SOS response-activating function of long-chain AHB (Figs. 2, 3).

Table 2. Activity of β-galactosidase in the cells of the *um250* and *um246* strains containing the transcriptional and the translational fusions of the *umuD* gene, respectively, after UV irradiation

Irradiation time, s	β-galactosidase activity, U (baseline activity level change, times)	
	Transcriptional fusion	Translational fusion
0	133.2	51.5
60	206.5 (1.55)	87.6 (1.70)
120	293.0 (2.19)	123.6 (2.40)

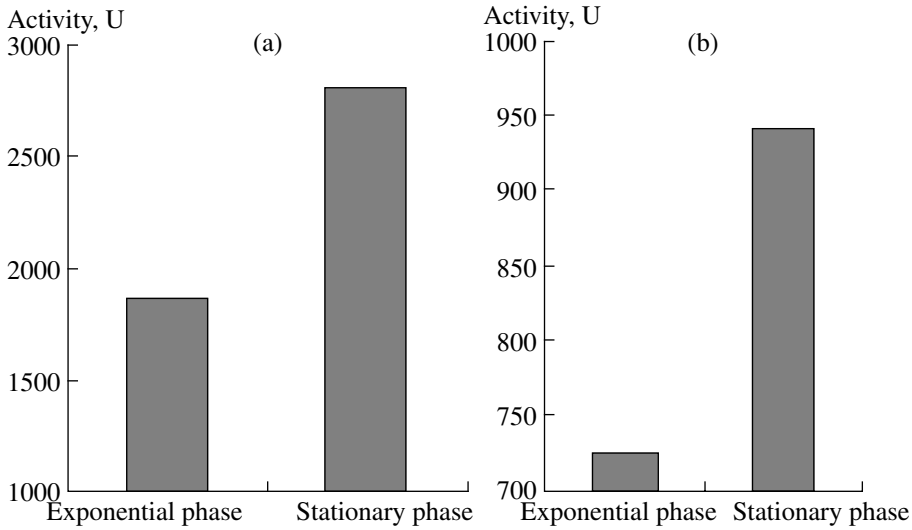

Fig. 1. Expression of the hybrid *osmE-lacZ* operon in bacterial cells during various growth phases: (a), the *os250* strain with the transcriptional fusion; (b), the *os246* strain with the translational fusion (*osmE-lacZ*).

A dose-dependent (twofold and above) increase in the expression level of the hybrid *osmE-lacZ* operon over its baseline value occurred upon the addition of C_{12}-AHB to the exponential- or stationary-phase cultures of the strains used to test the *rpoS* regulon (Tables 3, 4). Importantly, the dose dependence of the C_{12}-AHB effects was observed after reaching a threshold concentration that was 35 and 10 μg/ml for the exponential- and stationary-state cells, respectively. The β-galactosidase activity expressed by the translational fusion was below the control level of enzyme activity (without AHB) expressed by the transcriptional fusion at all C_{12}-AHB concentrations (Tables 3, 4). This points to the conclusion that C_{12} acts at the transcriptional level.

The effect of C_7-AHB, the other tested AHB homologue, substantially differed from the effect produced by C_{12}-AHB. Within the physiological concentration range (10–100 μg/ml), it caused a significant decrease in the expression levels of the hybrid *umuD-lacZ* and *osmE-lacZ* operons relative to their baseline values in the cells of the *E. coli* test strains (Tables 5–7). In both

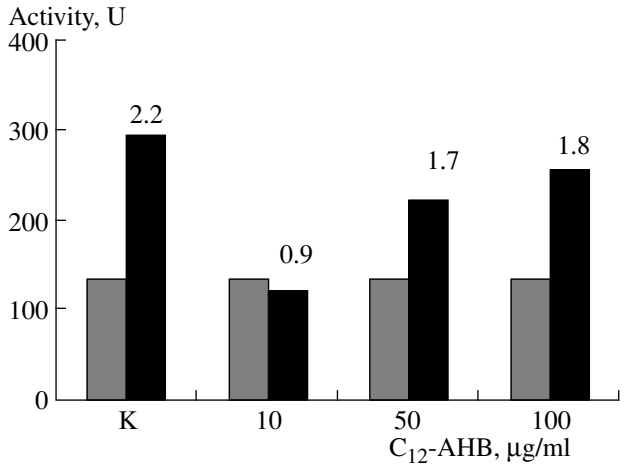

Fig. 2. β-galactosidase activity (U/min) in proliferating cells of the strain *um250* containing the transcriptional fusion (*umuD-lacZ*) after UV irradiation (K) or the treatment with C_{12}-AHB (in concentrations of 10, 50, and 100 μg/ml). In this figure, and in Figs. 3–5 below, grey bars refer to the baseline activity level and digits indicate baseline activity changes (times).

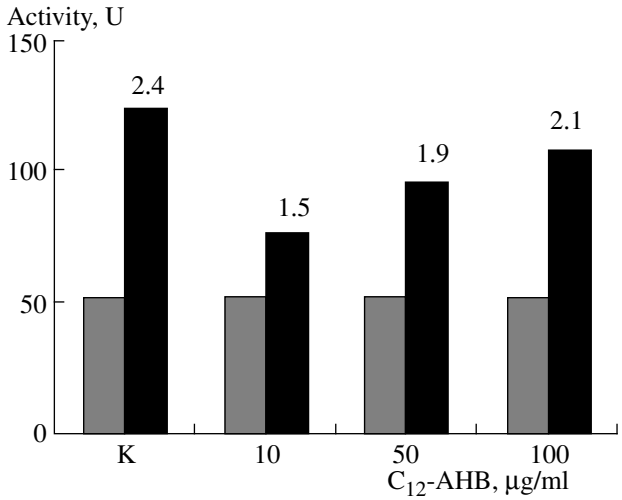

Fig. 3. β-galactosidase activity (U/min) in proliferating cells of the *um246* strain containing the translational fusion (*umuD-lacZ*) after UV irradiation (K) or the treatment with C_{12}-AHB (in concentrations of 10, 50, and 100 μg/ml).

Table 3. Activity of β-galactosidase in the cells of the *os250* strain containing the transcriptional fusion of the *osmE* gene, after treatment with C_{12}-AHB

C_{12}-AHB concentration, μg/ml	β-galactosidase activity, U (baseline activity level change, times)		Regulation level
	Exponential phase	Stationary phase	
0	1882.0	2800.0	0.67
1	1660.0 (0.88)	2408.0 (0.86)	0.59
5	1675.0 (0.89)	2660.0 (0.95)	0.60
10	1693.0 (0.89)	3266.0 (1.16)	0.60
35	2512.0 (1.33)	4049.0 (1.44)	0.90
70	3636.0 (1.93)	6022.0 (2.15)	1.30

Table 4. Activity of β-galactosidase in the cells of the *os246* strain containing the translational fusion of the *osmE* gene, after the treatment with C_{12}-AHB

C_{12}-AHB concentration, μg/ml	β-galactosidase activity, U (baseline activity level change, times)		Regulation level
	Exponential phase	Stationary phase	
0	812.0	940.0	0.86
1	844.5 (1.04)	1137.0 (1.20)	0.90
5	982.5 (1.20)	1128.0 (1.20)	1.05
10	998.8 (1.23)	1410.0 (1.50)	1.06
35	1232.2 (1.51)	1692.0 (1.80)	1.31
70	1538.0 (1.89)	2800.5 (2.97)	1.64

test strains (*os250* and *os246*), an increase in β-galactosidase activity expressed by the hybrid's *osmE-lacZ* operon occurred only in the experiments with stationary-phase cells upon addition of very high C_7-AHB concentrations ranging from 200 to 600 μg/ml (Tables 6, 7). However, the regulation level in the strains with both the transcriptional and the translational fusion remained virtually unchanged within the whole concentration range. Therefore, C_7-AHB cannot be considered as a stress response-inducing substance.

Hence, the regulatory effects of AHB depend on their concentration rather than structure.

It should be noted that, apart from C_7-AHB, C_{12}-AHB applied in low concentrations (up to 10 μg/ml) also caused a reduction in the expression of both *umuD–lacZ* and *osmE–lacZ* test operons in the strains with the transcriptional fusion (Fig. 2, Table 3).

Taking into account the data obtained, we tested the protective properties of C_7-AHB in respect to the induction of the SOS response in the UV-irradiated exponential-phase's *E. coli* cells. Activity of β-galactosidase was significantly lower with C_7-AHB (10–100 μg/ml) added 30 min before irradiation than in the control system (without C_7-AHB pretreatment) at all tested autoregulator concentrations (Figs. 4, 5). It follows that the homologue C_7-AHB exerted a protective influence on *E. coli* cells, abolishing the SOS-dependent regulation that is characteristic of *umuD*.

Table 5. Activity of β-galactosidase in the cells of the *um250* and *um246* strains containing the transcriptional and the translational fusion of the model *umuD* gene, respectively, after the treatment with C_7-AHB

C_7-AHB concentration, μg/ml	β-galactosidase activity, U (baseline activity level change, times)	
	Transcriptional fusion	Translational fusion
0	133.2	51.5
10	85.3 (0.64)	32.5 (0.63)
50	79.9 (0.59)	36.1 (0.70)
100	113.2 (0.84)	36.1 (0.70)

The protective influence of C_7-AHB (and C_{12}-AHB in low concentrations) can be due to their antioxidant activity that was demonstrated earlier [15]. AHB quench reactive oxygen species (ROS) formed under UV-induced oxidative stress. This also accounts for the

Table 6. Activity of β-galactosidase in the cells of the *os250* strain containing the transcriptional fusion of the *osmE* gene, after treatment with C_7-AHB

C_7-AHB concentration, μg/ml	β-galactosidase activity, U (baseline activity level change, times)		Regulation level
	Exponential phase	Stationary phase	
0	1882.0	2800.0	0.67
10	1599.7 (0.85)	2016.0 (0.72)	0.57
50	1656.2 (0.88)	2324.0 (0.83)	0.59
100	1656.2 (0.88)	2464.0 (0.88)	0.59
200	1825.5 (0.97)	3889.0 (1.38)	0.65
300	1904.0 (1.01)	3977.0 (1.42)	0.68
600	2098.0 (1.10)	4810.0 (1.70)	0.75

Table 7. Activity of β-galactosidase in the cells of the *os246* strain containing the translational fusion of the *osmE* gene, after the treatment with C_7-AHB

C_7-AHB concentration, μg/ml	β-galactosidase activity, U (baseline activity level change, times)		Regulation level
	Exponential phase	Stationary phase	
0	812.0	940.0	0.86
10	787.6 (0.96)	1128.0 (1.20)	0.84
50	852.6 (1.05)	846.0 (0.90)	0.90
100	830.8 (1.02)	864.8 (0.92)	0.88
200	878.3 (1.08)	1386.0 (1.47)	0.93
300	954.6 (1.17)	1659.0 (1.76)	1.01
600	1045.0 (1.28)	1778.0 (1.90)	1.11

dose-independent effects produced by C_7-AHB within a wide concentration range (10–100 μg/ml).

DISCUSSION

This work provides evidence that AHB function as communicative signals in microbial populations, inducing a stress response coupled to the stress regulon expression and intragenomic alterations resulting in phenotypic phase transitions. Taken together, these processes secure the survival of the population (the species).

An artificial increase in the AHB level in a *B. subtilis* B 1733 *trpA5* culture auxotrophic for tryptophan simulated a nonlethal and non-DNA-impairing stress in bacteria, similar to a moderate heat shock that stimulates AHB biosynthesis, according to [14, 15]. The fact that auxotrophic cells of bacilli revert to prototrophs in the presence of C_{12}-AHB confirmed the mutagenic effect of long-chain AHB that was demonstrated earlier using an Ames test with *Salmonella typhimurium* TA-100 [12]. In contrast, the amphiphilic homologue C_7-AHB exhibited protective properties. Research on a new subject (*B. subtilis* B 1733 *trpA5*) provided addi-

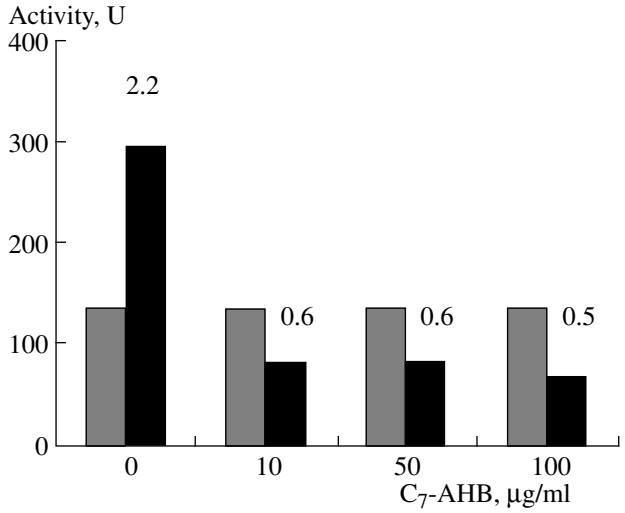

Fig. 4. β-galactosidase activity (U/min) in proliferating cells of the *um250* strain containing the transcriptional fusion (*umuD-lacZ*) that were pretreated with C_7-AHB in concentrations of 10, 50, and 100 μg/ml for 30 min, and thereupon, irradiated with UV.

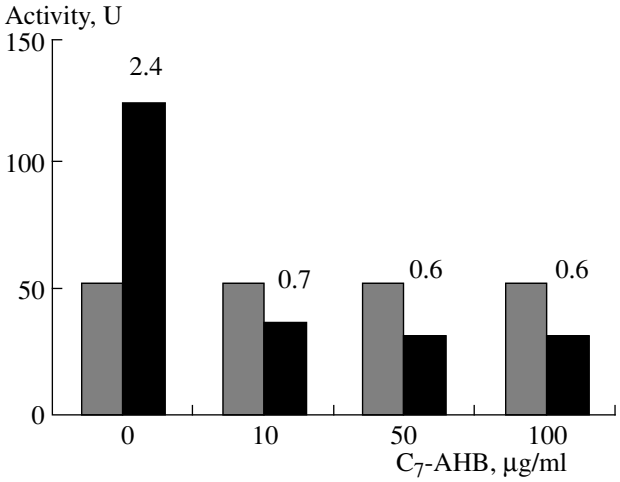

Fig. 5. β-galactosidase activity (U/min) in proliferating cells of the *um246* strain containing the translational fusion (*umuD-lacZ*) that were pretreated with C_7-AHB in concentrations of 10, 50, and 100 μg/ml for 30 min, and thereupon, irradiated with UV.

tional evidence of the AHB effect on the bacterial genome and demonstrated (i) a correlation between mutation (reversion) frequency and the phase transition intensity at the same C_{12}-AHB concentrations; (ii) a genome transition-inducing AHB effect not involving its selective pressure because the R ⟶ S phenotypic phase transition occurred if inoculum cells were treated with AHB for a short time, in contrast to the growth of bacteria on an AHB-containing medium described in [12]; and (iii) the influence of the growth phase on the ratio between the subpopulations of phenotypically distinct cells after removing the inducer (C_{12}-AHB), which may be due to a higher sensitivity of dividing cells to stress factors [12]. Reversible gene activity changes mostly result from the interactions between genetic (transpositions and inversions) and epigenetic (modification of DNA and DNA-associated proteins) events that are subject to control by regulatory proteins [2]. The involvement of AHB in controlling the phase transitions can be due to their interactions both with the DNA and the DNA-binding or regulatory proteins. In vitro studies revealed the capacity of AHB for direct interactions with the DNA, which results in changes in its topology and physical and chemical properties [18], and may influence the efficiency of the processes that cause intragenomic transitions. According to [18, 20], DNA–AHB interactions can involve (i) bonds between the alkyl radicals of AHB (C_{12}-AHB, not C_7-AHB) and hydrophobic macromolecular domains or DNA-bound lipids [22], and (ii) intramolecular hydrogen bonds between the DNA phosphate groups and the hydroxyl groups of the aromatic nuclei of AHB (both C_{12}-AHB and C_7-AHB) that are intercalated between the turns of the helix. This mechanism accounts for the differences between the mutagenic effects of the C_7- and C_{12}-AHB homologues related to their interactions with the DNA because these substances differ in terms of hydrophobicity. Low concentrations of AHB that display antioxidant activity (particularly C_7-AHB) should neutralize the effects of the ROS accumulated in stressed cells [15]. With an increase in AHB concentration, the hydrophobic interactions of AHB (chiefly C_{12}-AHB) with the DNA should cause changes in DNA topology, increasing the probability of genetic events resulting in a mutagenic effect in conjunction with reversible intragenomic transitions. However, it seems more likely that AHB exert a controlling influence on the functional activity of DNA-associated or regulatory proteins, taking into account the AHB involvement in modification of protein macromolecules that was demonstrated in a large number of enzyme models [16, 17].

Using other experimental models developed for these studies, viz., the *E. coli* strains *um250, um246, os250*, and *os246*, we established that AHB perform a signal function in the regulation of stress responses involving the induction of the SOS system and the general stationary-phase *rpoS* regulon. The results obtained revealed that long-chain AHB (C_{12}-AHB) play priority roles in activating the stress gene expression, whereas the short-chain C_7-AHB homologue, in contrast, secures DNA protection from the destructive effect of UV radiation within a wide concentration range (up to 100 μg/ml).

Comparative analysis revealed both similarities and differences between the effects of C_7- and C_{12}-AHB in relation to their concentrations. A common trend was the decrease in the expression of the SOS response gene (*umuD*) and *rpoS* regulon (*osmE*) in the strains with

transcriptional fusions in the presence of C_{12}-AHB at low (10 μg/ml and below) concentrations (Fig. 2, Table 3) and C_7-AHB within the concentration range below 100 μg/ml (Tables 5, 6). This demonstrates the protective function of AHB, due to their antioxidant activity [15], which is peculiar to a large number of phenolic compounds. Differences between the AHB homologues in terms of their effects include a pronounced concentration dependence of the stress-inducing effect of C_{12}-AHB (Fig. 2, Table 3), in contrast to the dose-independent protective effect of C_7-AHB (10–100 μg/ml) (Figs. 4, 5, Tables 5, 6). These results are consistent with the earlier data that the induction of protein RecA (in the SOS chromotest with strain *E. coli* PQ37) is influenced, apart from the AHB dose added, by the AHB structure that depends on the alkyl substituent's length [23].

Although C_{12}-AHB was used in our studies in physiological concentrations that did not inhibit test culture growth, these doses were sufficient to cause significant changes in the stress gene expression level. The stress gene expression increased to a similar extent under the influence of other nonlethal stress factors. For instance, the expression of the hypoxia-related *ole*I gene increased fourfold in *Saccharomyces cerevisiae* upon decreasing the O_2 concentration to the extreme value of 0.5 mM [24]. Importantly, the increase in the stress gene regulation level caused by a shift in C_{12}-AHB concentration is quantitatively concordant to that resulting from the effects of natural stress factors such as UV irradiation (Fig. 2) or starvation (Table 3). The results obtained testify to a signal function of long-chain AHB which operate as alarmones (danger signals) that control the activation of the protective functions of an organism. They are consistent with the earlier data on an increase in extra- and intracellular AHB levels in a microbial culture under sublethal stress caused by starvation, heat shock, and other factors [8, 14, 15]. AHB exerted a significant influence on the test strains with both transcriptional and translational fusions. However, the original *umuD* and *osmE* gene expression level in the system with the transcriptional fusions was higher than in that with the translational fusions even after enhancing the expression of the genes with C_{12}-AHB (Figs. 3–7). For this reason, C_{12}-AHB acting within the physiological concentration range qualifies as an activator of stress regulon transcription, whereas the C_7-AHB homologue is to be considered an inhibitor of this process.

As for the possible mechanisms of C_{12}-AHB action as a regulator of the stress gene expression, we should reemphasize that AHB can directly interact with DNA and, therefore, influence its topology and stability, which may result in the changes in DNA reactivity [18, 25].

Another mechanism of the regulatory influence of AHB on gene expression that may function under stress involves the AHB ability to structurally modify proteins, which was well documented in vitro studies with enzyme proteins [16, 17, 26]. Interactions of AHB with transcription activity-regulating proteins may account for the AHB effects on the regulation of both phase transitions and stress regulon expression. Apparently, this is the most likely mechanism because the influence of AHB on the reporter gene expression level in translational fusions (resulting in its two- to threefold increase) was detected in all our studies. Nevertheless, the resulting expression level was below that in the transcriptional fusions without activation.

Let us consider the following options concerning the AHB involvement in controlling the cellular stress response. By binding as ligands (structural modifiers) to transcription-regulating proteins, long-chain AHB can induce the expression of protein RecA, as shown by us earlier [23]. Alternatively, direct interaction of AHB with RecA resulting in its activation is also possible. Protein RecA in its modified, activated form binds to the single-stranded DNA in the replication fork, inactivating repressor protein LexA and abolishing the repression of the SOS response genes. This results in activating the expression of RecA-dependent genes including *umuD* [27]. In this system, hydrophobic interactions of C_{12}-AHB with the relevant domains of regulatory proteins account for the advantages of long-chain AHB in terms of the stress response activation. Another possible mechanism of controlling the SOS response by AHB implies their involvement in redox regulation of the cell, which plays a major role in stress responses [28]. Redox regulation involves modifying the regulatory proteins by means of (i) ROS and (ii) AHB as low molecular weight antioxidants/oxidants [28, 29]. The results obtained suggest that AHB may operate as redox-sensitive effectors that regulate the activity of protein transcription factors. Long-chain AHB can activate the SOS system, functioning as reductants in such processes as the Fenton reaction, resulting in an increase in the ROS level. ROS can impair the DNA, which is consistent with the data obtained in [20, 25]. C_7-AHB exhibits appreciable antioxidant activity [15]. Therefore, it can quench ROS, decreasing their intracellular level, which provides for DNA protection against damage and prevents oxidative stress and induction of the SOS response in the cell.

Thus, the results of this work in conjunction with the data obtained earlier [8, 11, 12, 23] reveal a novel function of AHB that act as adaptogens at the population level. We present evidence that they function as signals in eliciting a cell's adaptive response to stress factors. An increase in the extracellular concentration of AHB induces the expression of the general *rpoS*-dependent stationary-phase regulon and increases the frequency of phenotypic phase transitions. The feasibility of directed regulation of the stress gene expression is of considerable importance for gene engineering and optimization of protein synthesis. The antimutagenic and protective

properties of C_7-AHB with respect to harmful factors of various types can be used in biotechnological developments and medical practice.

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