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Overexpression of the ATP-dependent helicase RecG improves resistance to weak organic acids in *Escherichia coli*

Abstract Increased resistance to several weak organic acids was conferred on *Escherichia coli* by overexpression of the ATP-dependent helicase RecG and, to a lesser extent, by overexpressing the helicase RuvAB. This property of helicases was identified by reproducible selection of *recG*-bearing clones from genomic libraries of the acetate-resistant species *Acetobacter aceti* and *Staphylococcus capitis*. We show that overexpression of RecG from both species, but also from *E. coli*, increased the maximum biomass concentration attained by *E. coli* cultures that were grown in the presence of various weak organic acids and uncouplers. Furthermore, overexpression of RecG from *A. aceti* significantly improved the maximum growth rates of *E. coli* under weak organic acid challenge. Based on the known role of RecG in DNA replication/repair, our data provide a first indication that weak organic acids negatively affect DNA replication and/or repair, and that these negative effects may be counteracted by helicase activity.

Introduction

Weak organic acids are typical products of microbial metabolism and have a long history as food preservatives due to their general antimicrobial activity. The weak acid traverses the membrane in its undissociated form and dissociates at the near neutral intracellular pH, liberating an anion and a proton in the cytoplasm (Russell and Diez-Gonzalez 1998). The release of protons decreases the intracellular pH and may dissipate the proton motive force in a process also known as uncoupling (Brul and Coote 1999; Dürre et al. 1988; Russell and Diez-Gonzalez 1998). The concomitant accumulation of anions is likewise toxic (Russell 1992) and has been reported to inhibit metabolic reactions (Krebs et al. 1983; Roe et al. 2002), reduce the synthesis of macromolecules (Cherrington et al. 1991), or disrupt membranes (Freese et al. 1973).

In contrast to *Escherichia coli* and most other microbes, a few bacteria are known to be relatively resistant to high concentrations of the most prominent weak organic acid, acetate. A Gram-negative example would be the acetic acid bacteria such as *Acetobacter aceti* that can grow at acetate concentrations of up to 60 g/l (Park et al. 1991). As an example of resistant Gram-positive bacteria, *Staphylococcus capitis* is capable of growth at acetate concentrations of up to 40 g/l (Lasko et al. 2000), which is well above the level of resistance of acetic acid bacteria that are not adapted to high acetate concentrations (Steiner and Sauer 2003). While acetate resistance is well known from vinegar-producing strains, essentially nothing is known about the molecular mechanisms that confer such a high level of resistance. Moreover, the direct molecular targets of anion accumulation or pH-related effects are far from being completely resolved. To identify targets of weak organic acids and transferable mechanisms of their resistance, we selected *E. coli* clones from genomic libraries of *A. aceti* and *S. capitis* with improved resistance.

Material and methods

Bacteria and media

The bacteria we used are listed in Table 1. *A. aceti* and *S. capitis* were routinely grown on YPG medium containing 30 g/l glucose, 2 g/l bacto peptone, and 5 g/l yeast extract (pH 6.5) at 30°C. *E. coli* was grown at 37°C in M9 minimal medium (Emmerling et al. 2002) or in Luria Broth (LB) containing 10 g/l NaCl, 10 g/l bacto tryptone, and 5 g/l yeast extract. Both media were supplemented with 5 g/l glucose to prevent consumption of the organic acids, which may reduce toxicity.

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Table 1 Bacterial strains

Strain or plasmid	Relevant phenotype	Source
A. aceti	DSMZ 2002	DSMZ[a]
S. capitis	DSMZ 6180	DSMZ
E. coli DH5α	F$^-$ *endA1, hsdR17*(rk$^-$mk$^+$) *supE44 thi$-$* λ^- *recA1 gyrA96 relA1* $\varphi80\Delta lacAm15$	DSMZ
E. coli MG1655	F$^-$ λ^- *rph$-$l*	DSMZ

[a] Deutsche Sammlung von Mikroorganismen und Zellkulturen

Recombinant DNA techniques and reagents

All genetic experiments were done according to standard protocols (Sambrook et al. 1989). DNA sequences were obtained with the Global Edition IR2 System (LI-COR, Lincoln, Neb.) or by primer walking (Microsynth, Balgach, Switzerland). PCR fragments were purified from agarose gels with the QIAEX II Gel Extraction Kit (Qiagen) or directly from the PCR mix with the Wizard PCR Preps Kit (Promega). The *E. coli* MG1655 *recG* was amplified using the primers TCGTCATGAA AGGTCGCCTG TTAG (5′) and GAT-CTGCAGG AAGGTAGGGT AAC (3′). The *E. coli* O157:H7 *recG* was amplified using the primers TCGTCATGAA CAGGTCGCCT GTTA (5′) and GATCTGCAGC GGCTGAAATT CTG (3′). The *E. coli* MG1655 *ruvAB* operon was amplified using the primers ATCGAATTCA TGTGATAGGC AGACTCAG (5′) and CGAT-CTGCAG CGTCGCATCA GGCATAT (3′). The *E. coli* MG1655 *ruvABC* operon was amplified using the primers GTACGAATTC ATGGTTTCTC ACGATCTGCA TC (5′) and CGATCTGCAG CGTCGCATCA GGCATAT (3′).

Construction of genomic libraries

Genomic DNA of *A. aceti* and *E. coli* was isolated using the SDS lysis method (Sambrook et al. 1989). For isolation of *S. capitis* genomic DNA, resuspended cells were passed five passes through a French pressure cell and incubated at 37°C for 30 min in the presence of 10 μg/ml RNase and 10 mg/ml lysozyme. 100 μl of 30% (w/v) *N*-laurylsarcosine was added and the incubation was continued until the mixture became transparent. Further isolation was done as with *A. aceti*.

Plasmid-based genomic libraries with >99% genome coverage of *A. aceti* and *S. capitis* were constructed from gel-separated, partially *Sau*3A-digested DNA fragments of 2.5–8 kb. These fragments were used for library construction of about 10,000 pBK-CMV-based clones using the ZAP Express Predigested Gigapack Cloning Kit (Stratagene). Average insert sizes were verified by restriction digestion analysis of 20 randomly picked clones that confirmed the expected fragment distribution (data not shown).

Selection of weak organic acid resistant E. coli
from genomic libraries

Selection for weak organic acid resistance was done in 15 ml polypropylene tubes filled with 5 ml LB medium at 37°C and 200 rpm. The medium was supplemented with weak organic acids or uncouplers and 5 g/l glucose, and the final pH was adjusted to 6.5. Inoculation was done with 100 μl *E. coli* libraries from exponentially growing cultures. Growth was defined as the ability to attain an optical density at 600 nm (OD$_{600}$) of at least 0.1 overnight. Upon growth of cultures at a given concentration of selective agents, 100–200 μl aliquots were used to inoculate a fresh tube with a higher concentration of the selective agent. Inoculation at higher concentrations was continued until no further growth was apparent. Plasmid pools of populations that were harvested at the highest concentration of each selective agent were plated, clones isolated, and isolated plasmids were analyzed by restriction enzyme analysis.

For selection on LB-agar plates, both genomic libraries were plated at different concentrations of K-acetate. Clones were isolated from the plates at the highest concentration of acetate and their plasmids were analyzed by restriction enzyme analysis.

Growth experiments

Final OD$_{600}$ and maximum specific growth rates of *E. coli* cultures with different chemicals were determined in batch cultures grown in 96-well format. For this purpose, 1 ml cultures were grown in 96-deep-well plates (Kuehner, Basel, Switzerland) at 37°C and 300 rpm. Growth was monitored by following OD$_{600}$ in 100 μl aliquots on a 96-well microplate reader (SpectraMax Plus; Molecular Devices, Sunnyville, Calif.). Inocula were prepared from clones grown to an OD$_{600}$ of 0.5 in 5 ml medium, supplemented with 50 μg/ml kanamycin. The maximum specific growth rate was determined by log-linear regression analysis of OD$_{600}$ versus time, with the growth rate (μ) as the regression coefficient. The inhibition constant K_i for acetate was calculated from the maximum specific growth rate at different concentrations of acetate (Aiba et al. 1973). To avoid pH-related growth effects, supplementation with organic acid was done such that the final pH of the medium was 6.5 in all cases.

Nucleotide sequence accession numbers

The nucleotide sequences of *recG* of *A. aceti* and *S. capitis*, and of pST6 have been deposited in the GenBank sequence database and were assigned the accession numbers AF548636, AF548637, and AY158078, respectively.

Results

Genomic library selection in liquid cultures containing weak organic acids or uncouplers

E. coli-based genomic libraries of the acetate-resistant species *A. aceti* and *S. capitis* were selected at successively increasing concentrations of weak organic acids in LB medium batch cultures. Specifically, we used the saturated straight-chain monocarboxylic acids formate, acetate, propionate, and butyrate, as well as two other weak organic acids and food preservatives, benzoate and lactate. Additionally, the synthetic uncouplers CCCP (carbonylcyanide *m*-chloromethoxy phenylhydrazone) and DNP (2,4-dinitrophenol) were used. Descendants of the *A. aceti* library that grew at 15 and 17.5 g/l K-acetate were plated onto LB agar plates containing 12 g/l K-acetate. Restriction enzyme analysis of plasmids isolated from the eight largest colonies on these plates revealed two types of clones. The predominant plasmid species (pST1) contained an insert of approximately 6,000 bp (see Table 2). The less frequent plasmid pST18 contained a 3,000 bp insert. Selection of the *S. capitis* genomic library at the highest K-acetate concentration of 17.5 g/l yielded a single plasmid species (pST14) containing a 2,900 bp insert (Table 2).

While selection with benzoate at 3 g/l did not yield any resistant clones, all other compounds selected reproducibly one or a few plasmids, almost all of which were

Table 2 Identified plasmids with genomic DNA of *A. aceti* or *S. capitis* that conferred resistance to various weak organic acid and synthetic uncouplers in batch cultures of *E. coli* MG1655

Selective agent	*A. aceti* library		*S. capitis* library	
	Isolated clone(s)	Abundance[a] (%)	Isolated clone(s)	Abundance (%)
Na–formate (14 g/l)[b]	n.d.	–	pST14	>90
K–acetate (17.5 g/l)[c]	pST1, pST18	75/25	pST14	>80
K–acetate (12.5 g/l) (on plate)	pST1, pST6	31/7	pST7, pST16	23 / 38
Na–propionate (15 g/l)	pST1	>80	pST14	>80
Na–butyrate (17.5 g/l)	pST6	>80	pST14	>80
Na–lactate (50 g/l)	pST6	40	pST14	30
CCCP (0.1 mM)	pST1	>80	n.d.	–
DNP (1 mM)	pST1	>90	n.d.	–

[a] Estimated abundance of clones in the final selection culture
[b] Highest concentration at which clones were isolated
[c] K-acetate was used rather than Na-acetate because it exerted stronger selective pressure

Fig. 1 Alignment of partial RecG sequences from *A. aceti*, *S. capitis*, and *E. coli* (**A**). Amino acids of the *E. coli* helicase motifs (Lloyd and Sharples 1991) and identical residues in the *A. aceti* and *S. capitis* sequences are shown *in bold*. Putative promoter and ribosome binding sites (*RBS*) of *A. aceti* and *S. capitis recG* (**B**). The upstream region of *recG* from *A. aceti* is compared to the *E. coli* σ^S (sigma 38) consensus promoter and RBS. The putative promoter and RBS of *S. capitis recG* are identified by homology to the sequences of *S. aureus recG* (Kuroda et al. 2001). Bases identical to the consensus or reference sequence are shown *in bold*

A

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                      Motif I                                  Motif Ia
              * * * * * * * * * * * * * * * *          * * * * * * * * * * * * * * * * * *
E. coli       RDMALDV PMMRLVQGDVGSGKTLV AALAALRAIAHGK QVALMAPTELLAEQHANNFR NWFAPLG 345
A. aceti      ADLSAST PMMRLLQGDVG AGKT FVAMNAMLQTVESGA QAALMAPTE ILARQHFETLSRLCPT.. 366
S. capitis    RDLKAPIR MHRLLQGDVGSGKT VVAAICMYALKTAGY QSALMVPTE ILAEQHAESLMELFGDT. 336

                                                             Motif II
                                                     * * * * * * * * * * * * * *
E. coli       IEVGWLAGKQKGKARLAQQEAIASGQVQMIVGTHAIFQEQVQ FNGLALVIIDEQHRF GVHQRLA 409
A. aceti      .ECVYLSGTIKGAARRKTLAAIADGTAKIVVGTHALFQDGVT FHDLGLAVIDQQHRF GVRQRMN 429
S. capitis    MNVALLTGSVKGKKRRILLEQLENGSIDCLIGTHALIQDDVV FENVGLVITDEQHRF GVNQRQM 400

                       Motif III
              * * * * * * * * * * * * *
E. coli       LWEKGQQQGFHPHQ LIMTATPIPRTLA MTAYADLDTSVIDELPPGRTPVTTVAIPDTRRTDIID 473
A. aceti      LSAKGEA....TDI LVMTATPIPRTL QLMEWGEMSVSRLDSKPPGRQPIRTTLHSMDSLDSVLA 489
S. capitis    LREKGAM....TNV LFMTATPIPRTLA ISVFGEMDVSSIKQLPKGRKPIITSWAKHEQYEQVLA 460

                                 Motif IV
                       * * * * * * * * * * * * * * * * * * * * *
E. coli       RVHHACITEGRQAYWVCTLI EESELL.EAQAAEATWEELKLA LPELNVGLVHGRMKPAEKQAVM 536
A. aceti      GISRA.LRDGVQVFWVCPLI ENSETQ.AAAAAEERWASLEQRFEGL.VGLAHGKQDITVRQEAL 556
S. capitis    QMTSE.LRKGRQAYVICPLI ESSEHLEDVQNVVALYESLQQYYGADKVGLLHGKLTPDEKDDVM 523

                       Motif V                          Motif VI
              * * * * * * * * * * * * * * * * * *       * * * * * * * * * * * * * * * * *
E. coli       AS FKQGELHLLVATTVIEVGVDVP NASLMIIENP ERLGLAQLHQLRGRVGRGA VASHCVLLYKT 600
A. aceti      DR FRLGKTR LLVATTVIEVGVD IPAASVMVIEQA ERFGLAQLHQLRGRVGRG SKQSFCLLLHDR 614
S. capitis    QR FSDKE IDI LVSTTV VEVGVNVP NATFMMIYDAD RFGLST LHQLRGRVGR SEHQSYCVLIASP 587
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B

A. aceti

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                                   -10                            RBS
E. coli σS consensus sequence    CTATACT                       TAAGGAGGTG                    START
                                 | | | | | |                   | | | | |                    CODON
                TTTTGTTCCATCCTGCTAATGCCCTATACCTGCCGTTCCATCGCCTGTCAGCCCGAGGAGCACGGGAAGCGCCCGTTGTCACGATG
                                                                                             MET
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S. capitis

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                    -35      -  15 bp  -   -10                     RBS
S. capitis     AACATGATAGAATATATTAT.GACT.ATTGAAGAAAAACATTA.TTAAGTTTTAGGTGATAGATCATGACAAAAGTA
               | | | | | |  -  17 bp  -    |  | | |                      | | | | | | |    M   T   K   V
S. aureus      GTCATGATAAAATAAATAACATACTAAATGATACGTAAAATCAAATAAAACATAGGTGATTTATTTTGGCTAAAGTA
                                                                                     M   A   K   V
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identical to those identified in the acetate-resistant clones (Table 2). The only exceptions were selections with butyrate and lactate as well as with acetate on solid media.

Identification of the *recG* gene on resistance-conferring plasmids

At least one representative of each of the six identified plasmids was sequenced. With the exception of the *A.*

aceti plasmid pST6, all other selected plasmids contained only one open reading frame in common. The deduced protein of this open reading frame from *A. aceti* and *S. capitis* had 37 and 36% identity with the ATP-dependent helicase RecG of *E. coli*. Moreover, all seven helicase motives were conserved in the deduced putative RecG proteins (Lloyd and Sharples 1991), including the ATP binding motif I (Mahdi et al. 1997) and the highly conserved DExH residues (motif II) of the DNA-RNA helicase subfamily (Lloyd and Sharples 1993) (Fig. 1). In contrast to *S. capitis* and *E. coli*, the putative *A. aceti* RecG protein had an extended N-terminus, which is about 20 amino acids longer than that of most RecG proteins.

Based on spacing and orientation of the neighboring open reading frames, we conclude that both *recG* genes are monocistronic (data not shown). Upstream of *S. capitis recG*, we identified regions with significant homology to the promoter and RBS sequences of *S. aureus recG* (data not shown). A potential ribosome-binding site for *recG* of *A. aceti* was found by alignment with the *E. coli* consensus sequence. By sequence alignments to various promoter sequences, we also identified a potential –10 promoter region with a significant homology to the *E. coli* σ^S consensus sequence (Lee and Gralla 2001). As may be expected, no corresponding –35 region was found, since σ^S-dependent promoters appear to lack a conserved –35 sequence (Wise et al. 1996). While expression of *recG* is not responsive to σ^S in *E. coli* (Lloyd and Sharples 1991), this promoter homology suggests that *recG* may be under the control of an alternative sigma factor in *A. aceti*. Unfortunately, previous proteome analyses of *A. aceti* cannot verify whether RecG is induced in the presence of acetate, since this protein was not present in the resolved size- and pI-range (Lasko et al. 1997; Steiner and Sauer 2001).

The three identified open reading frames on pST6 exhibited significant homologies to nodulin-related proteins, cytochrome b_{561}, and chromate transport proteins. However, the gene responsible for selection of pST6-harboring clones was not further elucidated, although the chromate transport protein is the most promising candidate that might confer increased resistance of *E. coli* to butyrate or lactate.

Phenotypes of RecG and RuvAB helicase-overexpressing *E. coli* strains

The highly reproducible isolation of *recG*-containing plasmids from clones that were resistant to a relatively broad range of selective agents suggests a role of RecG in this resistance phenotype. To verify that RecG is indeed the resistance-conferring factor, overexpression plasmids were constructed that contained exclusively the *recG* genes of *A. aceti*, *S. capitis*, wild-type *E. coli* MG1655, or the acetate resistant *E. coli* O157:H7 (Diez-Gonzalez and Russell 1997) (Fig. 2). Additionally, we constructed overexpression plasmids for the multi-subunit helicase RuvAB and the resolvase RuvC of *E. coli* MG1655,
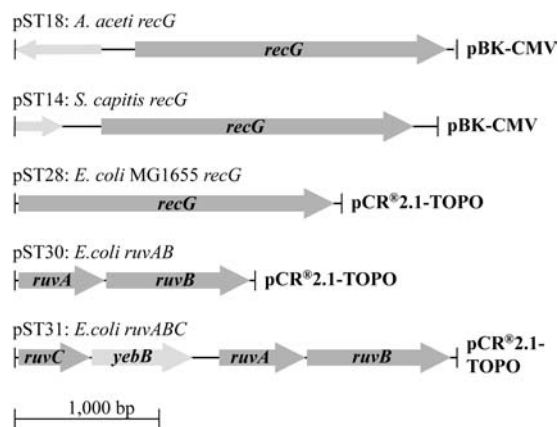

Fig. 2 Constructed helicase overexpression plasmids. Vector backbones are indicated *on the right*. *Light gray arrows* indicate incomplete or irrelevant open reading frames

which has a largely overlapping function with RecG in the processing of damaged replication forks (McGlynn and Lloyd 2002; Sharples et al. 1999).

Since acetate challenge reduces final cell densities in a variety of bacteria (Lasko et al. 2000; Steiner and Sauer 2003), we first determined final optical densities of helicase-overexpressing strains in batch cultures that contained weak organic acids, synthetic uncouplers, and ATPase inhibitors. For this purpose, triplicate experiments were performed in 96-deep-well plates and OD_{600} values were determined over a 5-day period to assure sufficient growth of all cultures (Fig. 3). Expression of RuvAB and all RecG proteins increased the final OD_{600} up to 35% in formate-challenged cultures, when compared to the control in the presence of formate (Fig. 3A). In acetate-challenged cultures, only RecG from *A. aceti* and *E. coli* exerted a significant positive effect. These trends were also confirmed in additional experiments with 4 and 8 g/l Na-acetate and with 5 and 10 g/l K-acetate (data not shown). In propionate-challenged cultures, all helicase-overexpressing strains attained 20–40% higher final OD_{600} values than the control, but RecG of *A. aceti* was particularly effective with a more than doubled OD_{600} at 5 g/l. No improvements were apparent in butyrate-challenged cultures. Final OD_{600} values of cultures overexpressing RecG from *E. coli* O157:H7 or RuvABC from *E. coli* MG1655 were indistinguishable from those cultures overexpressing RecG from *E. coli* MG1655 or RuvAB, respectively (data not shown).

To assess whether the helicase relieved primarily toxic effects of anion accumulation, pH-related effects, or ATP-availability, we grew cultures in the presence of uncouplers and ATPase inhibitors, which mimic certain aspects of weak acid toxicity (Russell and Diez-Gonzalez 1998). Specifically, we tested the proton-gradient uncoupling food preservative benzoate, the synthetic uncouplers CCCP and DNP, and the ATPase inhibitor azide (Fig. 3B). In benzoate-challenged cultures, all recombinant strains attained approximately doubled final OD_{600}, when compared with the control. In DNP-challenged

Fig. 3 Final OD$_{600}$ values of *E. coli* batch cultures overexpressing RecG or RuvAB in media containing different short-chain fatty acids (**A**), benzoate and the synthetic uncouplers DNP and CCCP (**B**), or the ATPase and cytochrome *c* inhibitor azide (**C**). The maximum final OD$_{600}$ of unchallenged *E. coli* is 2.8 under these conditions

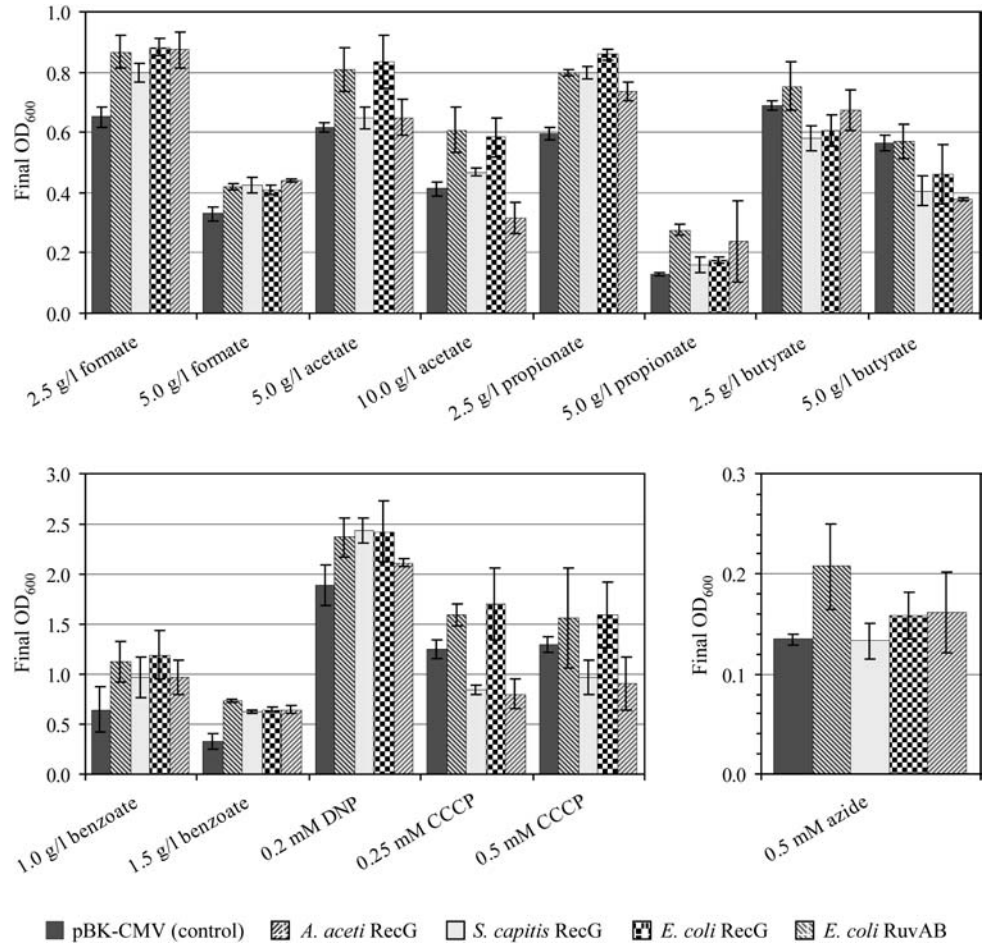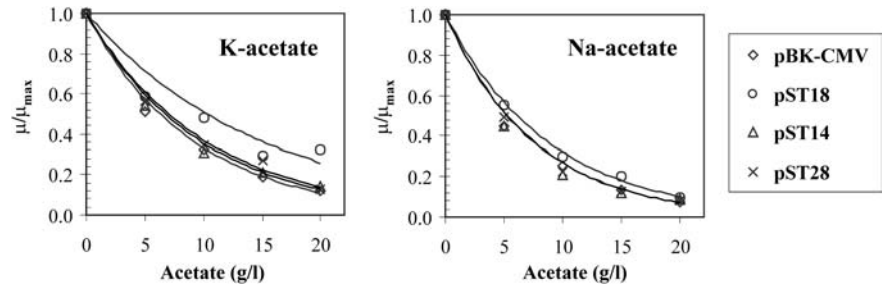**Fig. 4** Inhibition of the normalized maximum specific growth rate of *E. coli*, overexpressing RecG from *A. aceti* (pST18), *S. capitis* (pST14), or *E. coli* MG1655 (pST28) at increasing concentrations of acetate

cultures, the lower concentration was insufficient to exert a negative effect, while overexpression of RecG, but not RuvAB, increased the final OD$_{600}$ by 25–30% at the higher concentration. In CCCP-challenged cultures, only RecG from *A. aceti* and *E. coli* increased the final OD$_{600}$ by about 20%. The addition of 0.5 mM of the ATPase inhibitor azide reduced the final OD$_{600}$ to 0.1–0.2 (Fig. 3C), while 1 mM completely inhibited growth (data not shown). Again RecG of *A. aceti* increased the final OD$_{600}$ by 55%, while all other recombinant strains were indistinguishable from the control.

To elucidate whether RecG also affected maximum specific growth rates under weak organic acid challenge, RecG-overexpressing strains were grown in LB medium in 96-deep-well microtiter plates with different concentrations of K- and Na-acetate (Fig. 4) and 10 g/l of Na-formate, Na-propionate, and Na-butyrate (data not shown). In the presence of formate and butyrate, all strains overexpressing RecG of *A. aceti*, *S. capitis*, and *E. coli* grew slightly faster than the control strains that were transformed with pBK-CMV or pCR 2.1-TOPO (data not shown). On acetate, however, only those strains overexpressing RecG of *A. aceti* grew at significantly higher rates (Fig. 4). Using the data shown in Fig. 4, we calculated the inhibition constant (K_i) that specifies the concentration of an inhibiting agent at half the maximal growth rate (Aiba et al. 1973) (Table 3). While the K_i values of strains overexpressing RecG from *E. coli* or *S.*

Table 3 Inhibition constants of *E. coli* overexpressing RecG of *A. aceti*, *S. capitis*, and *E. coli* upon challenge with K- or Na-acetate. Values were calculated from an exponential fit to the maximum specific growth rates in batch cultures that were supplemented with 0, 5, 10, 15, and 20 g/l acetate

Plasmid	Origin of RecG	Inhibition constants	
		K-acetate	Na-acetate
pBK-CMV	None	9.5 g/l	7.9 g/l
pST18	*A. aceti*	17.1 g/l	8.9 g/l
pST14	*S. capitis*	10.2 g/l	8.2 g/l
pST28	*E. coli*	10.3 g/l	7.9 g/l

capitis were indistinguishable from that of the control, the strain overexpressing RecG of *A. aceti* had a significantly higher K_i value, which was most evident with the K-acetate that was also used during the selection.

In *Pseudomonas aeruginosa*, RecG was described to be important for defense against oxidative stress (Ochsner et al. 2000). To examine whether acetate stress-induced damage may be counteracted by overexpression of RecG, we determined the viability of *E. coli* strains with the different RecG constructs upon incubation in up to 100 g/l acetate (pH 6.5) for up to 2 h. Generally, viability decreased, at most, one order of magnitude after 2 h (data not shown), and overexpression of RecG had no detectable influence on this viability decrease.

Discussion

Overexpression of the ATP-dependent helicases RecG and RuvAB was shown to improve the resistance of *E. coli* to weak organic acids. While RecG proteins from three tested species exerted this beneficial effect, RecG from *A. aceti* was the most effective. This property of the helicases was identified by the highly reproducible selection of *recG*-containing plasmids from weak organic acid-challenged genomic libraries of *A. aceti* and *S. capitis*. Construction of overexpression plasmids that encode only RuvAB or RecG, including the native *E. coli* protein(s), verified that other potential plasmid-based factors are not responsible for the described phenotype.

Overexpression of ATP-dependent helicases reduced not only the toxic effects of weak organic acids but also the effects of the synthetic uncouplers CCCP and DNP and of the ATPase and cytochrome *c* inhibitor azide. This indicates that the weak organic acid-induced effect is not caused primarily by anion accumulation under the conditions studied, but rather by the decrease of the intracellular pH or possibly ATP availability, since these are the common characteristic of weak organic acids, uncouplers, and azide. Further evidence for this hypothesis comes from the firmly established low-pH-induced DNA damage in bacteria (Audia et al. 2001; Foster 1995; Hanna et al. 2001). Since the homologous recombination protein RecG also promotes rescue of replication forks that are stalled at damaged DNA (McGlynn and Lloyd

2002; Sharples et al. 1999; Singleton et al. 2001), our results provide indirect evidence that the mechanisms of helicase-mediated resistance may be via DNA replication-repair. This view is also in agreement with the previously reported higher sensitivity of DNA repair mutants to weak organic acid and low pH challenges (Cherrington et al. 1991; Hanna et al. 2001; Sinha 1986). Lastly, the results presented here suggest that the proposed RecG effect may be of physiological relevance in acidic environments, since acid-habituated *E. coli* exhibit a higher DNA repair activity (Raja et al. 1991).

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