Published in final edited form as:
Metab Eng. 2007 March ; 9(2): 133–141. doi:10.1016/j.ymben.2006.10.002.

Recombinant protein production in an *Escherichia coli* reduced genome strain

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Abstract

Recently, efforts have been made to improve the properties of *E. coli* as a recombinant host by 'genomic surgery' - deleting large segments of the *E. coli* K12 MG1655 genome without scars. These excised segments included K-islands, which contain a high proportion of transposons, insertion sequences, cryptic phage, damaged, and unknown-function genes. The resulting multiple-deletion strain, designated *E. coli* MDS40, has a 14% (about 700 genes) smaller genome than the parent strain, *E. coli* MG1655. The multiple-deletion and parent *E. coli* strains were cultured in fed-batch fermenters to high cell densities on minimal medium to simulate industrial conditions for evaluating growth and recombinant protein production characteristics. Recombinant protein production and by-product levels were quantified at different controlled growth rates. These results indicate that the multiple-deletion strain's growth behavior and recombinant protein productivity closely matched the parent stain. Thus, the multiple-deletion strain *E. coli* MDS40 provides a suitable foundation for further genomic reduction.

Keywords

reduced genome; deletion; growth rate; acetate; transposon; insertion sequence

Introduction

Escherichia coli is one of the most studied and well-understood microorganisms. It naturally occurs in the gut of mammals, which is an anaerobic environment with constantly changing nutrient conditions. *E. coli* is also a commonly used recombinant host for laboratory and industrial recombinant protein production. As a recombinant host, *E. coli* is exposed to only a limited and controlled set of conditions. Specifically, in an industrial fermenter, an aerobic environment is usually desired and maintained, the nutrient concentrations are maintained within narrow ranges, and attachment to the vessel is not desirable. Therefore, the genes required for survival in the gut may not be the same genes required for optimum recombinant protein production. Further, the complete genome sequence of *E. coli* has

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revealed numerous genes of unknown function and genetic material that has possibly been acquired from other organisms in the recent past (Blattner et al., 1997). In an effort to improve *E. coli* as a recombinant host, many researchers have deleted or added single genes to the genome or modified plasmids to complement the existing genome (Andersen and Krummen, 2002; Andersen et al., 2001; Baneyx and Mujacic, 2004; Bessette et al., 1999; Cebolla et al., 2002; Chen et al., 2003; Chevalet et al., 2000; Chou et al., 1996; Diaz-Ricci et al., 1991; Pecota et al., 1997). These efforts have made progress, but have not truly addressed the global issue of the numerous genes in *E. coli* with unknown function or potentially detrimental function. In this work, a multiple deletion strain – *E. coli* MDS40 – was characterized with respect to growth and recombinant protein production. *E. coli* MDS40 was derived from *E. coli* MG1655 by the excision of many large nucleotide sequences corresponding to potentially non-essential genes, while retaining the backbone *E. coli* genome.

A few research groups have created multiple deletion *E. coli* strains, although not always with the focus of improving recombinant protein production. These multiple deletion methods mainly used random techniques facilitated by transposon libraries (Yu et al., 2002), specialized transposons (Goryshin et al., 2003), and λ phage homologous recombination (Hashimoto et al., 2005). One non-random or targeted deletion method, used sequential plasmid-directed recombination events (Kolisnychenko et al., 2002). Both the random and non-random multiple deletion techniques have generated strains with 8 to 30% smaller genomes. The random method-derived strains have been observed to have significantly lower growth rates, whereas the reported targeted multiple deletion strains have been observed to have comparable growth rates to the parent strain on the most commonly used laboratory media, including a minimal medium. The targeted deletion method strains were created by excising sections of the *E. coli* MG1655 genome regarded as unnecessary or detrimental, with the aim of improving it's properties (Kolisnychenko et al., 2002; Pósfai et al., 2006). The targeted genes were selected based on a comparison of the complete genome sequences of two *E. coli* strains (MG1655 and O157:H7). This analysis revealed a 'backbone' of genetic information, which contained hundreds of strain specific 'islands'. For *E. coli* MG1655, a K12 strain, these islands were designated K-islands. The backbone genome is highly conserved and contained all essential genes. In contrast, the 'islands' contains a disproportionate number of genes with unknown function, toxin genes, transposable elements, and pseudogenes (Blattner et al., 1997; Kolisnychenko et al., 2002). In the first targeted multiple deletion strain described - *E. coli* MDS12 - twelve sequential deletions of K-islands were made to *E. coli* MG1655, resulting in a multiple deletion strain with an 8% smaller genome. Twenty-eight further sequential deletions resulted in the strain *E. coli* MDS40, which was used in this study. *E. coli* MDS40 has a 14% smaller genome than *E. coli* MG1655 or roughly 700 fewer genes. Additionally, these deletion strains have improved transformabilities and lower mutation rates (Pósfai et al., 2006).

The focus of this study was to determine if *E. coli* MDS40 was a robust and suitable host for recombinant protein production. Fed-batch fermentations were used to obtain high cell densities under controlled growth conditions in minimal medium typical of industrial processes. Cell yield, byproduct accumulation, and recombinant protein productivity were measured and compared to the parent strain, *E. coli* MG1655. Another aim was to determine the suitability of *E. coli* MDS40 as the primary multiple deletion strain for further targeted deletions. Additional deletions could, for example, target the arabinose utilization genes, such as the *E. coli* HB101 strain, to allow recombinant protein expression using the pBAD vector (Khlebnikov et al., 2002; Sorensen and Mortensen, 2005).

MATERIALS AND METHODS

Strains and expression vector

E. coli MG1655 was obtained from American Type Culture Collection (ATCC). The multiple deletion strain *E. coli* MDS40 was provided by Scarab Genomics (Madison, WI). *E. coli* MDS40 was created by 40 successive targeted deletions from the *E. coli* MG1655 genome i.e., 28 targeted successive deletions from *E. coli* MDS12 (Kolisnychenko et al., 2002; Pósfai et al., 2006). Chloramphenicol acetyltransferase (CAT) was used as the model recombinant protein encoded by the plasmid pPROEXCAT (Invitrogen). The pPROEXCAT plasmid contains a *trc* promoter that controls CAT expression via IPTG-induction, a pBR322 origin of replication, and the β-lactamase gene for ampicillin resistance. *E. coli* MDS40 was transformed with pPROEXCAT via the $CaCl_2$ method (Sambrook et al., 1989). CAT was selected as the initial model recombinant protein for a few reasons. Namely, there is an extensive body of literature available for this protein's expression in *E. coli* MG1655 and related strains. CAT levels in the cell can be easily quantified by an enzymatic assay (Rodriguez and Tait, 1983). Additionally, previous studies have observed that the unusual amino acid composition of CAT (11% phenylalanine) can trigger metabolic burden or stress responses in the host cell (Harcum and Bentley, 1993).

Shake flask cultivation

For the shake flask experiments, stock cultures stored at −80°C, were thawed and 0.5 mL was used to inoculate 10 mL of defined medium. The medium was as described in Korz et al. (1995), except the batch medium contained 8 g/L KH_2PO_4, 0.4 g/L $MgSO_4$ and 100 μg/mL ampicillin. Precultures grew overnight and were used to inoculate the shake flasks. The volume of preculture was adjusted, such that the shake flasks had equal initial cell densities. Shake flasks were induced with 5 mM IPTG, unless otherwise indicated.

Fed-batch fermentation

Fermentations were conducted in a 2 L Bioflo 110 fermenter (New Brunswick Scientific, Edison, NJ). Defined batch medium was used (Korz et al., 1995), except the batch medium contained 5 g/L glucose, 8 g/L KH_2PO_4, 0.4 g/L $MgSO_4$ and 100 μg/mL ampicillin. The feed medium contained 481 g/L glucose, 4 g/L $MgSO_4$, 40 mg/L Fe^{III}citrate, and 1.5X the trace metal concentration of the batch medium. Stock cultures stored at −80°C were thawed and used to inoculate 100 mL of defined media. Precultures were grown at 37°C to a cell density (OD_{600}) of approximately 0.8, and 75 mL was used to inoculate 1.5 L of batch medium (5% v/v) in the fermenter. The pH and temperature during the fermentations were maintained at 6.75 and 37°C, respectively. Air was sparged into the fermenter at a constant rate and the dissolved oxygen (DO) was maintained at 30% of saturation by varying the agitation rate. The batch phase of the fermentation was approximately 9.5 hours, at which time the glucose in the batch medium was completely consumed. Feeding was initiated at an exponential rate to control the growth rate (Akesson et al., 1999; DeLisa et al., 1999). Fermentations proceeded until the agitation rate reached its maximum value (1200 rpm) and the DO could no longer be maintained at 30% saturation. Fed-batch fermentations were induced with IPTG (1 mM), unless otherwise indicated.

Analytical techniques

The glucose was measured using a OneTouch Basic blood glucose meter. Samples for dry cell weight and CAT activity were cooled on ice, and then centrifuged at 3500 × *g* for 10 minutes at 4°C. The supernatant was saved (−20°C) for acetate and formate analysis. The cell pellets for the dry cell weights were resuspended in water and re-centrifuged. Washed cell pellets were resuspended in a small volume of water, placed in a pre-weighed aluminum

pan and dried at 80°C. For the CAT samples, the cell pellets were resuspended in 50 mM Tris buffer (pH 7.4) containing 30 μM dithiothreitol (TDTT buffer), re-centrifuged, and the washed pellets frozen at −20°C for analysis later. The frozen cell pellets were thawed, resuspended in TDTT buffer, sonicated on ice (Branson Sonifier 250, 3×15s pulses) and centrifuged at 17,000 × g for 20 minutes at 4°C. The cell lysate supernatant was collected and stored at −20°C. CAT activity was measured using the kinetic assay described by (Rodriguez and Tait, 1983), adapted to a 96-well plate format. Acetic acid and formate concentrations were enzymatically determined (R-Biopharm), in a 96-well plate format. Acetate and formate samples were centrifuged at 17000 × *g* for 18 minutes, to remove particulates prior to analysis.

RESULTS AND DISCUSSION

Shake flask cultivation

Growth characteristics and recombinant protein production for *E. coli* MDS40 were first investigated in parallel shake flasks in minimal media with *E. coli* MG1655 as the control. Both uninduced and induced cultures were assessed. The shake flask growth curves for uninduced and induced *E. coli* MG1655 and MDS40 are shown in Figure 1. The growth rates, as well as the final cell densities were comparable between the two strains. IPTG was added to one pair of cultures at 12 hours to induce CAT expression. Induction did not significantly impact the growth rate of either strain. The CAT expression profiles are also shown in Figure 1. The specific CAT activity increased rapidly after induction for both strains, remained level for a few hours and decreased during the stationary phase. The observed maximum specific CAT activity was comparable for *E. coli* MDS40 and MG1655. The *E. coli* MG1655 and MDS40 CAT expression profiles were similar to other reports, even with strain, plasmid and media differences (Cha et al., 2000; Gill et al., 1998; Haddadin and Harcum, 2005; Harcum and Bentley, 1999; Richins et al., 1997). Thus, *E. coli* MDS40 was capable of good growth and CAT expression under shake flask conditions.

Fed-batch fermentation

Since *E. coli* MDS40 was capable of growth rates and recombinant protein production comparable to the parent strain in shake flasks, the next step was to assess growth characteristics and recombinant protein expression under industrial fermentation conditions. Fed-batch fermentations were used to assess *E. coli* MDS40 at three different the fed-batch growth rates: 0.15 h^{-1} (low), 0.25 h^{-1} (intermediate), and 0.5 hr^{-1} (high), where the batch phase growth rates were not different. The parent strain, *E. coli* MG1655, was only assessed at 0.25 h^{-1}, as the behavior of *E. coli* MG1655 and other related strains has been well investigated with respect to recombinant protein productivity at these controlled growth rates (Curless et al., 1990; Hellmuth et al., 1994; Sanden et al., 2003; Turner et al., 1994). The growth curves for *E. coli* MG1655 and MDS40 are shown in Figure 2. The growth rates in the batch phase were approximately 0.45 h^{-1} for both strains. All batch phases lasted between 9.5 and 10 hours, at which point the initial glucose was completely consumed. The dry cell weights obtained for *E. coli* MG1655 and *E. coli* MDS40 at the end of the batch phase were not significantly different (2.72 ± 0.71 and 2.16 ± 0.25 g/L, respectively). Thus, the strains have equal substrate yield coefficients.

The specific CAT activity profiles for both strains are shown in Figure 2. For all fermentations the specific CAT activity increased rapidly due to induction, leveled off, then decreased for later fermentation times. The intermediate fed-batch growth rate fermentations for *E. coli* MDS40 and MG1655 were conducted in duplicate and show the biological variability in CAT expression for the well-controlled fed-batch fermentations. The multiple deletion strain produced a comparable quantity of the recombinant protein at intermediate

fed-batch growth rate, although slightly less CAT when normalized to total protein. As expected, the specific CAT activity was lower for the high fed-batch growth rate culture. At the lower fed-batch growth rate, the specific CAT activity remained elevated for a longer time period. Both of these expression characteristics are similar to what has been observed for *E. coli* MG1655 under similar conditions (Hoffman et al., 2002; Koo and Park, 1999; Levisauskas et al., 2003; Riesenberg et al., 1990).

Since the genome of the *E. coli* MDS40 strain is smaller, it was hypothesized that the controlled glucose feed rate might result in higher biomass levels due to improved substrate yield coefficients for *E. coli* MDS40 compared to *E. coli* MG1655. Instead, there were no measurable or significant difference in the biomass levels or substrate yields from glucose; however, we did observe slightly higher acetate levels during the batch phase for *E. coli* MDS40. For the low and intermediate fed-batch growth rates, the accumulated acetate was consumed by *E. coli* MDS40, and both strains, respectively, after the feed started. Additionally, acetate did not accumulate in the fed batch phase for the low and intermediate fed-batch fermentations. However, for the high growth rate (0.5 h^{-1}) fed-batch fermentations, acetate accumulated in the batch phase and continued to accumulate in the fed-batch phase. Acetate accumulation is often observed in cultures where the growth rate is greater than 0.3 h^{-1} for *E. coli* MG1655 (Lee, 1996; Swartz, 1996). Induction did not significantly affect acetate accumulation for either strain under the well-controlled growth conditions.

Acetate accumulation is considered an indicator of overflow metabolism or anaerobic growth conditions (termed mixed acid metabolism). Formate also accumulates during mixed acid conditions (Han et al., 1992; Lee, 1996), thus the formate levels were measured for the fed-batch fermentations. The formate concentrations for all three fed-batch growth rates are shown in Figure 4. Unlike acetate, which accumulated in the batch phase, and then was consumed in the fed batch phase, formate was nearly undetectable in the batch phase. Formate accumulated in the fed-batch phase for all three fed-batch growth rates. At the intermediate fed-batch growth rate the two strains had nearly identical formate profiles. The induced cultures accumulated more formate than the uninduced cultures for both strains. For the low and high fed-batch growth rates, the formate profiles were independent of the induction strength. These formate profiles indicate that mixed acid metabolism was not the source of the accumulated acetate (Bylund et al., 2000; Castan et al., 2002; Xu et al., 1999). Thus, the slightly higher acetate levels observed for *E. coli* MDS40 in the batch phase, where growth rates were higher, is likely due to overflow metabolism. Very precise metabolism studies are needed to determine if *E. coli* MDS40 reach overflow metabolism more readily than *E. coli* MG1655. If *E. coli* MDS40 reaches overflow metabolism conditions more readily, it might be possible to redirect the excess carbon into recombinant protein by either fermenter control or genetic modifications.

It has been reported that acetate accumulation lowers cell yields and recombinant protein productivity (Akesson et al., 1999; Diaz-Ricci et al., 1991; Farmer and Liao, 1997; Lee, 1996; Takahashi et al., 1999; Turner et al., 1994). Thus, the poorer recombinant protein productivity at the high fed-batch growth rate was hypothesized to be due to the slightly higher acetate concentration. To test this hypothesis, acetate was added to duplicate *E. coli* MDS40 shake flasks. Duplicate *E. coli* MDS40 shake flasks, without acetate addition, were also assessed to provide a baseline for this strain. Sodium acetate was added to the shake flasks at just under 1 OD. All four flasks were induced to produce CAT at approximately 7 hours. The growth curves for all four cultures are shown in Figure 5. As expected, the growth rate of the cultures exposed to additional acetate was lower. Unexpectedly, the specific CAT activities for the acetate stressed cultures were higher than the control (unstressed) cultures as shown in Figure 5. The shake flask experiment demonstrated that

the presence of acetate alone does not cause reduced recombinant protein productivity; however, it demonstrates that the conditions that favor the production of acetate by the cells does lower recombinant protein productivity. Thus, it is possible that the lower observed specific CAT activity in the high fed-batch growth rate fermentation cultures could have been due to redirection of the carbon resources from the recombinant protein to acetate, due to overflow metabolism, as seen with *E. coli* MG1655. The effect of growth rate on recombinant protein expression has been addressed in literature for strains related to *E. coli* MG1655, and the *E. coli* MDS40 strain behavior was consistent with respect to productivity and growth rates (Hoffman et al., 2002; Koo and Park, 1999; Levisauskas et al., 2003; Riesenberg et al., 1990).

Many expression systems are available to produce recombinant proteins in *E. coli*. Some expression systems have extremely powerful promoters which have been observed to stress the cells or cause a metabolic burden (Glick, 1995; Haddadin and Harcum, 2005; Hoffman et al., 2002; Sanden et al., 2003; Wood and Peretti, 1990). To assess the effect of strong promoters on *E. coli* MDS40, the effect of changing the induction strength was investigated. It has been previously observed that very high induction strengths (> 5 mM) can overwhelm *E. coli* JM105, a MG1655 derivative (Harcum et al., 1992). Since the low and high fed-batch growth rate cultures provided a broader range of expression, these cultures were examined at both 1 mM (normal) and 5 mM (high) induction strengths. As expected, the high induction strength resulted in higher specific CAT activity (Figure 2) for both the low and high fed-batch growth rate fermentations, although not proportionally higher. A non-proportional increase in recombinant protein expression with respect to induction strength has been observed in other *E coli* strains (Bentley et al., 1991; Ramirez and Bentley, 1995). Interestingly, the induction strength had less effect on recombinant protein production for the low fed-batch growth rate culture.

Although *E. coli* is a well studied and a commonly used recombinant host, the behavior of *E. coli* can still be unpredictable at times, especially while expressing recombinant protein (Andersson et al., 1996; Baneyx, 1999; Baneyx and Mujacic, 2004; Gill et al., 2000; Oh and Liao, 2000; Sanden et al., 2003; Swartz, 1996). One of the overall goals of the multiple deletion strain project is to improve the growth/yield properties and robustness of *E. coli* as a recombinant host by eliminating potentially nonessential genes. A short-term goal of this project and the focus of this study was to assess the capability of one multiple deletion strain (*E. coli* MDS40) under industrial conditions. Since conditions in a fermenter, especially high cell densities, are significantly different from those in shake flasks, both of these conditions were examined (Andersson et al., 1996; Gill et al., 2001; Humphrey, 1998; Lee, 1996; Yoon et al., 2003). Under the conditions assessed *E. coli* MDS40 and its parent strain *E. coli* MG1655 had similar performance characteristics. The similar behavior between the two strains is encouraging as *E. coli* MDS40 was derived from *E. coli* MG1655 by 40 large sequential deletions encompassing nearly 700 genes. These deletions included all K-islands, numerous hypothetical operons, all transposable genetic elements, many genes related to the synthesis of flagella and fimbrae, and some lippopolysaccharide (LPS) genes. A disproportionate number of genes of unknown function were located within K-islands, thus the consequences of removing these genes on growth and recombinant protein production were unknown. An underlying hypothesis of this project has been that if the transcription and translation of these unknown genes does not occur, the number of endogenous proteins in *E. coli* MDS40 should be less than *E. coli* MG1655. This lower number of endogenous proteins should have two positive effects on recombinant protein production: 1) Reduced energy requirements due to fewer proteins being synthesized, and 2) More efficient protein purification due to fewer endogenous/native proteins to remove. For example, in the case of flagella, the energy saving may be two-fold: 1) Reduced energy needs due to eliminated synthesis of the flagella proteins, and 2) Reduced energy needs associated with the flagella

operation. An additional benefit of the multiple deletion strain includes removal of mobile genetic elements, such as insertion sequences and transposons. Deletion of these mobile elements provides some significant genome and plasmid stabilization (Pósfai et al., 2006), which could improve strain stability during long fermentation runs with transient stresses (Cavin et al., 1999; Faure et al., 2004; Glick, 1995). The slightly higher acetate accumulation in the batch phase observed for *E. coli* MDS40 may indicate that overflow metabolism can occur more easily in the deletion strain; however, more precise metabolic studies are necessary to clarify this point. Mathematical models of *E. coli* have demonstrated higher yields of certain metabolites as a result of gene deletions (Burgard et al., 2003); however, these models did not account for the specific genes deleted to create *E. coli* MDS40, due to lack of information regarding these genes. In all, the multiple deletion strain performs comparably to its parent strain under industrial conditions. This deletion strain provides a sound basis for further deletions that target the specific needs of a particular industrial fermentation. Additionally, further analysis is required to quantify the energy and nutrient efficiencies due to reduction of endogenous proteins.

CONCLUSION

This study was conducted to assess the ability of the multiple deletion strain – *E. coli* MDS40 – to produce recombinant protein under industrial conditions. *E. coli* MDS40 was generated by the targeted sequential deletion of 40 regions of the *E. coli* MG1655 genome. The *E. coli* MDS40 genome is 14% smaller than MG1655 with approximately 700 fewer genes. The targeted genes included all K-islands, flagella and fimbrae genes, and some LPS synthesis genes. K-islands contain a disproportionate number of genes with unknown function, hypothetical genes, transposons and insertion sequences. The multiple deletions did not affect the ability of *E. coli* MDS40 to grow in defined minimal medium to high cell densities at controlled growth rates. The growth characteristics of the parental strain (*E. coli* MG1655) and *E. coli* MDS40 were not significantly different. Recombinant protein productivity for the *E. coli* MDS40 strain was also comparable to the parent strain. A complete analysis of the cell composition will provide a better understanding as to how the multiple deletions affect protein synthesis and cell energetics.

Acknowledgments

We would like to thank Dr. György Pósfai for constructing the *E. coli* MDS40 strain, and Dr. John Campbell for reviewing the manuscript. Financial support was provided to Clemson University by Scarab Genomics, which was funded by NIH grant GM35682 (FRB).

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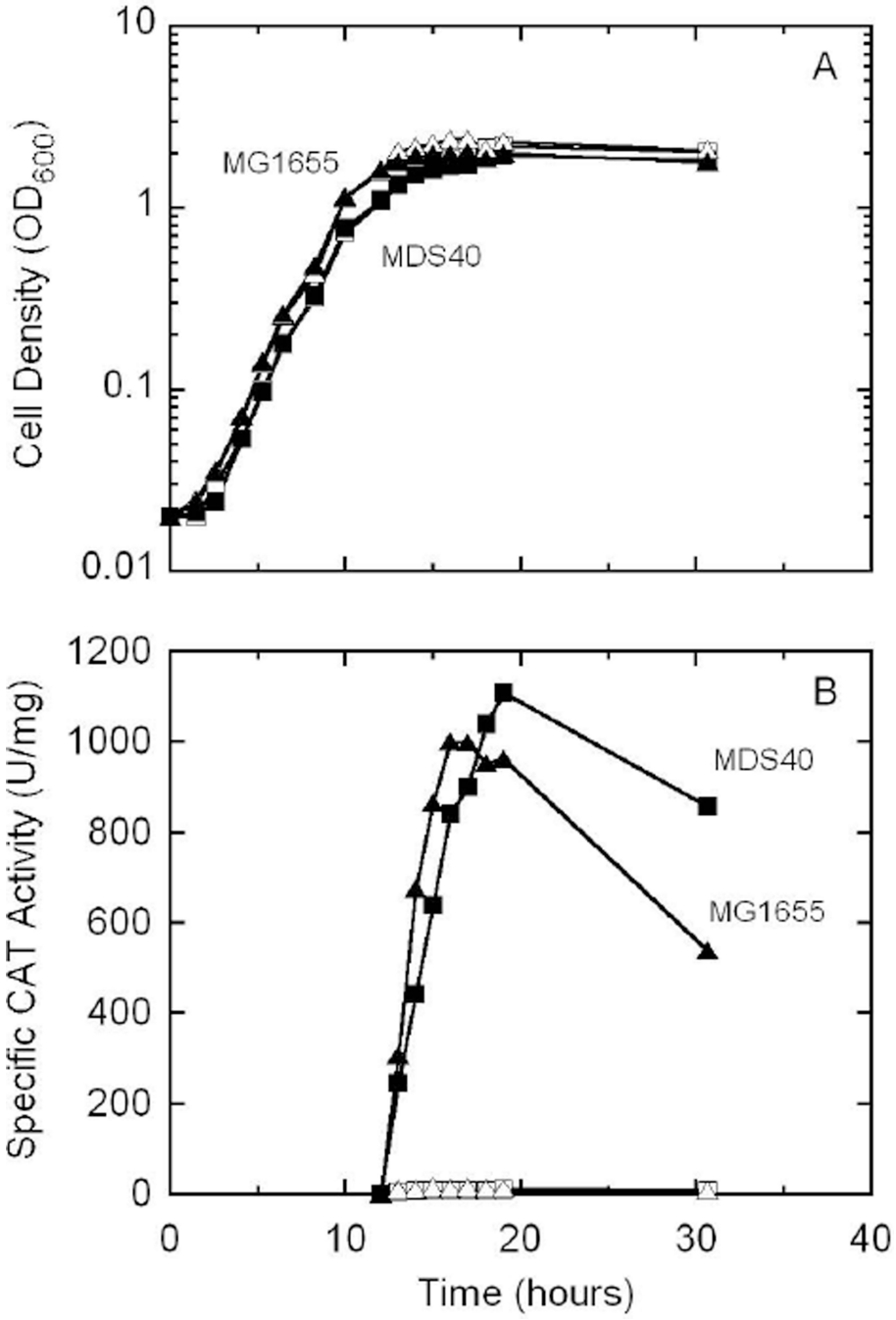

Figure 1.

Growth characteristics for *E. coli* MDS40 and MG1655 cultured in shake flasks. (A) Cell densities for MDS40 - induced (■) and uninduced (□); *E. coli* MG1655 - induced (▲) and uninduced (△). (B) Specific CAT activity for *E. coli* MDS40 - induced (■) and uninduced (□); *E. coli* MG1655 - induced (▲) and uninduced (△).

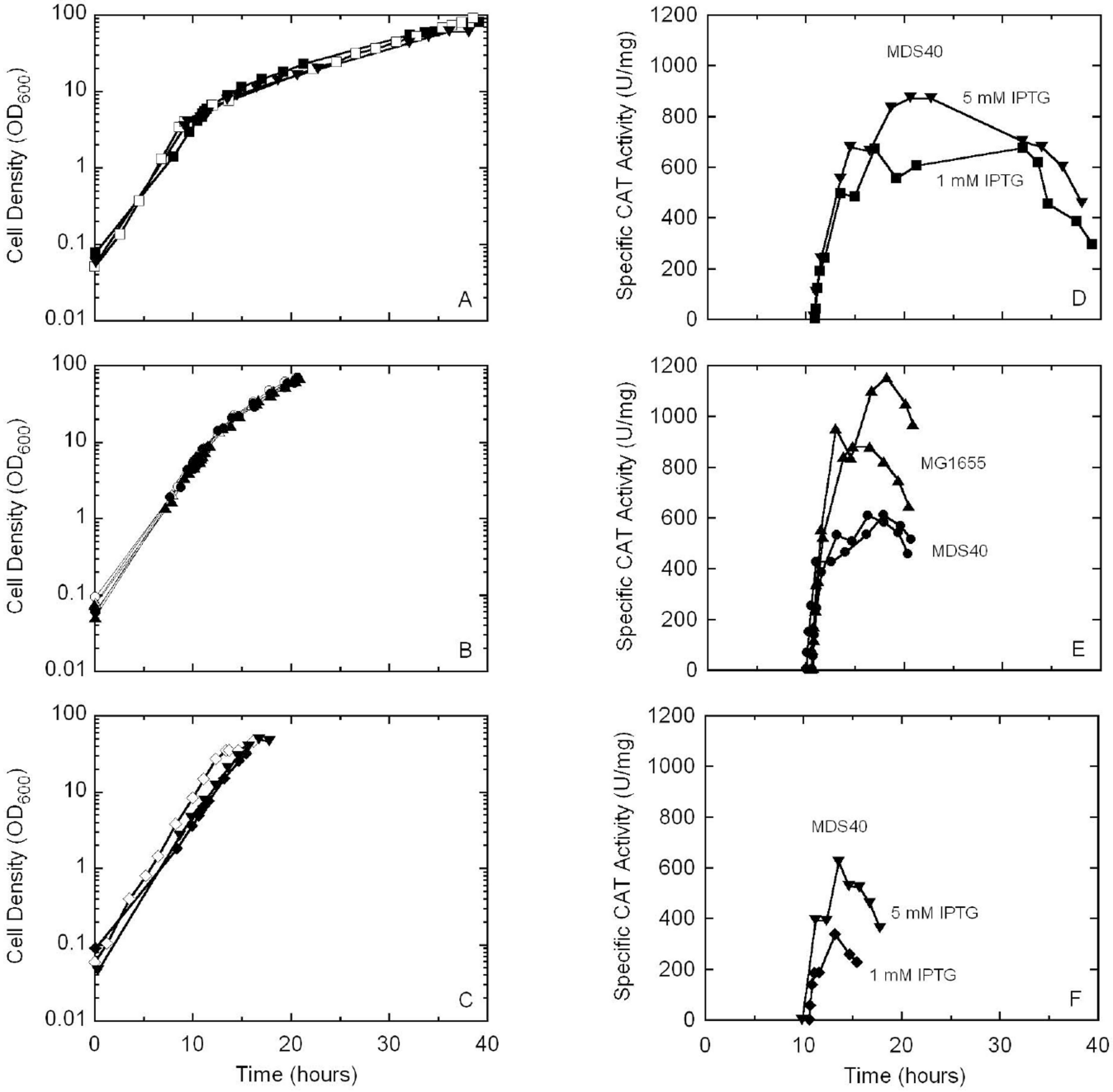

Figure 2.

Growth characteristics for *E. coli* MDS40 and MG1655 at controlled growth rates. (A) Cell densities for *E. coli* MDS40 – induced with 1 mM IPTG (■), 5 mM IPTG (▼), and uninduced (□) at the low growth rate (0.15 hr^{-1}). (B) Cell densities for *E. coli* MDS40 - induced (●) and uninduced (○) at the intermediate growth rate (0.25 hr^{-1}). Cell densities for *E. coli* MG1655 - induced (▲) and uninduced (△) at the intermediate growth rate are also shown. (C) Cell densities for *E. coli* MDS40 – induced with 1 mM IPTG (◆), 5 mM IPTG (▼), and uninduced (◊) at the high growth rate (0.5 hr^{-1}). (D) Specific CAT activity for *E. coli* MDS40 induced with 1 mM IPTG (■) and 5 mM IPTG (▼) at the low growth rate. (E) Specific CAT activity for *E. coli* MDS40 (●) and *E. coli* MG1655 (▲) at the

intermediate growth rate. (F) Specific CAT activity for *E. coli* MDS40 induced with 1 mM IPTG (◆) and 5 mM IPTG (▼) at the high growth rate.

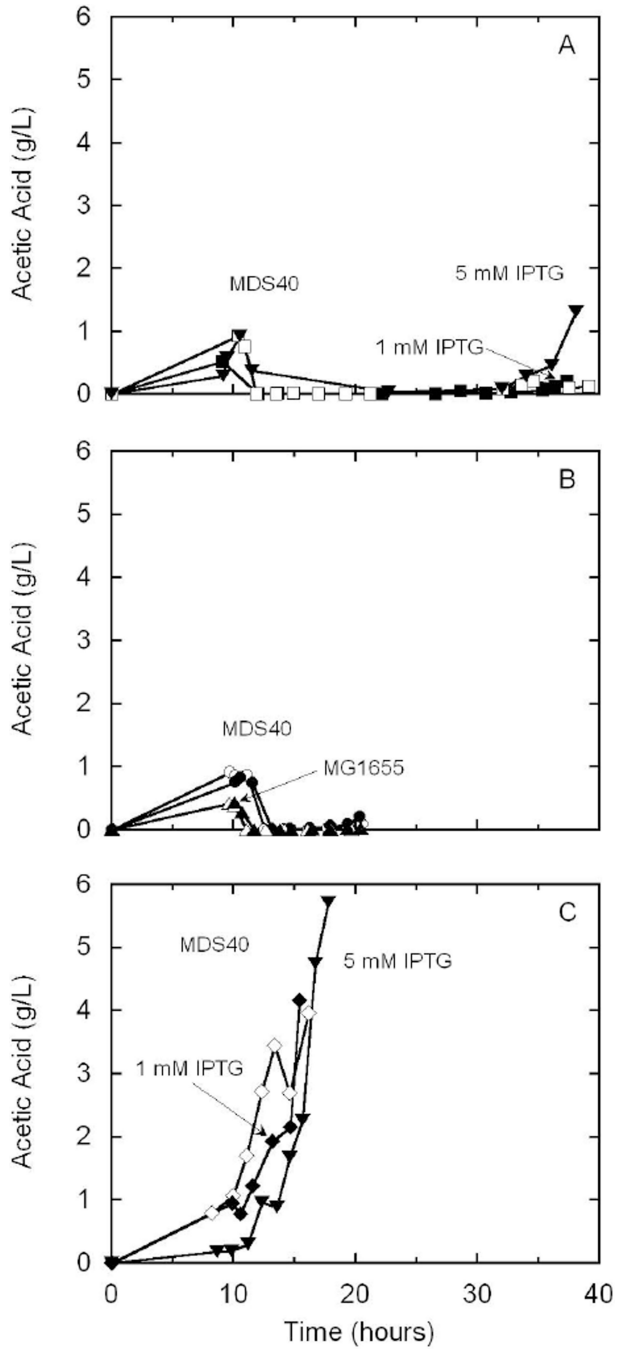

Figure 3.

Acetate concentrations in the fermentation broth. (A) *E. coli* MDS40 – induced with 1 mM IPTG (■), 5 mM IPTG (▼), and uninduced (□) at the low growth rate (0.15 h^{-1}). (B) *E. coli* MDS40 induced (●) and uninduced (○) at the intermediate growth rate (0.25 h^{-1}). *E. coli* MG1655 – induced (▲) and uninduced (△). (C) *E. coli* MDS40 – induced with 1 mM IPTG (◆), 5 mM IPTG (▼), and uninduced (◊) at the high growth rate (0.5 h^{-1}).

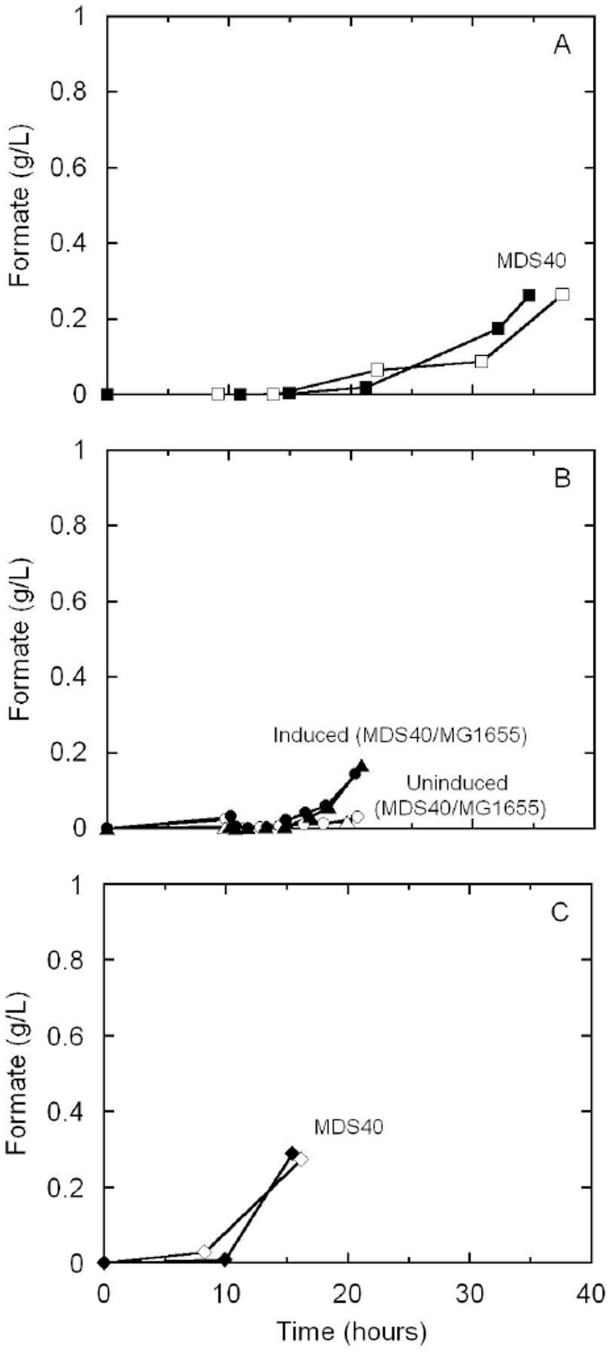

Figure 4.

Formate concentrations in the fermentation broth. (A) *E. coli* MDS40 – induced (■) and uninduced (□) at the low growth rate (0.15 h^{-1}). (B) *E. coli* MDS40 induced (●) and uninduced (○) at the intermediate growth rate (0.25 h^{-1}). *E. coli* MG1655 – induced (▲) and uninduced (△). (C) *E. coli* MDS40 – induced (◆) and uninduced (◊) at the high growth rate (0.5 h^{-1}).

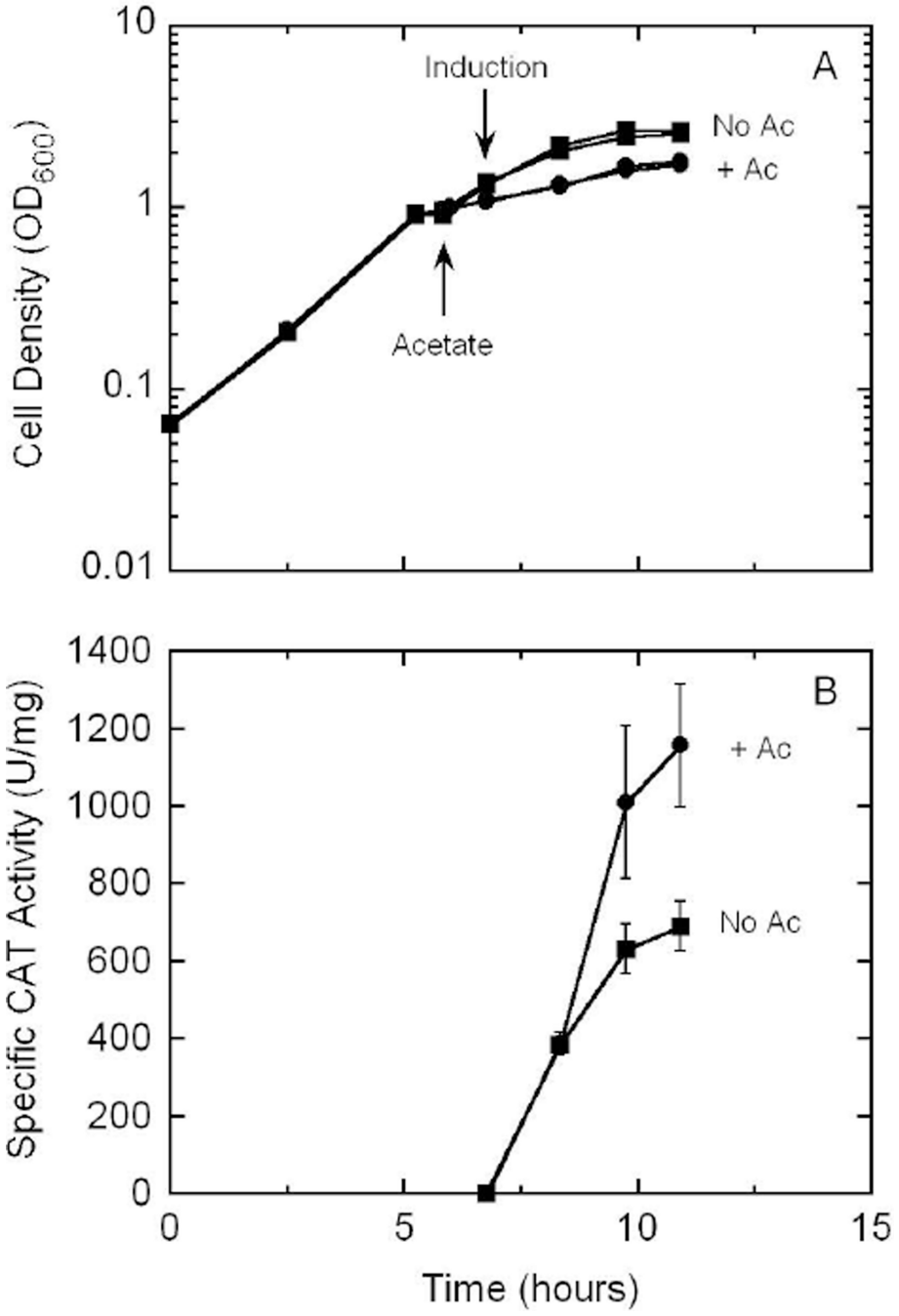

Figure 5.

Growth characteristics for *E. coli* MDS40 stressed by acetate in shake flasks. (A) Cell densities for cultures with the acetate addition (●) and no acetate addition (■). (B) Specific CAT activity for cultures with the acetate addition (●) and no acetate addition (■). Error bars indicate 95% confidence intervals.