strain (KS1000) wherein the mutation was created by replacing a segment of the prc gene with a fragment comprising a Kan^r marker.

The reduction of Tsp (prc) activity is desirable to reduce the proteolysis of proteins of interest. However, it was found that cells lacking protease prc show thermosensitive growth at low osmolarity. Hara et al isolated thermoresistant revertants containing extragenic suppressor (spr) mutations (Hara et al., Microbial Drug Resistance, 2: 63-72 (1996)). Spr is an 18kDa membrane bound periplasmic protease and the substrates of spr are Tsp and peptidoglycans in the outer membrane involved in cell wall hydrolysis during cell division. The spr gene is designated as UniProtKB/Swiss-Prot P0AFV4 (SPR_ECOLI).

Improved protease deficient strains comprising mutant spr gene have been described. Chen et al describes the construction of *E. coli* strains carrying different combinations of mutations in prc (Tsp) and another protease, DegP, created by amplifying the upstream and downstream regions of the gene and ligating these together on a vector comprising selection markers and a sprW174R mutation (High-level accumulation of a recombinant antibody fragment in the periplasm of Escherichia coli requires a triple-mutant (ΔDegP Δprc sprW174R) host strain (Chen C, Snedecor B, Nishihara JC, Joly JC, McFarland N, Andersen DC, Battersby JE, Champion KM. Biotechnol Bioeng. 2004 Mar 5;85(5):463-74). The combination of the ΔDegP, Δprc and sprW174R mutations were found to provide the highest levels of antibody light chain, antibody heavy chain and F(ab')2-LZ. EP1341899 discloses an E. coli strain that is deficient in chromosomal DegP and prc encoding proteases DegP and Prc, respectively, and harbors a mutant spr gene that encodes a protein that suppresses growth phenotypes exhibited by strains harboring prc mutants.

The present invention provides new bacterial strains carrying alternative spr mutants which provide advantageous means for producing recombinant proteins.

Summary ~~of the Invention~~

According to one aspect, there is provided a recombinant gram-negative bacterial cell comprising a mutant spr gene, Uniprot/ Swiss-Prot P0AFV4, encoding a spr protein having a mutation at one or more amino acids selected from N31Y, R62C, I70T, Q73R, C94A, S95F, V98E, Q99P, R100G, L108S, Y115F, D133A, V135D, V135G, L136P, G140C, R144C, H145A, G147C and H157A and wherein the cell has reduced Tsp protein activity compared to a wild-type cell.

In another aspect, there is provided a method for producing a protein of interest comprising culturing a recombinant gram-negative bacterial cell as disclosed herein in a culture medium under conditions effective to express the recombinant protein of interest and recovering the recombinant protein of interest from the periplasm of the recombinant gram-negative bacterial cell and/or the culture medium.

In a first ~~aspect of the present invention~~ embodiment, there is provided a recombinant gram-negative bacterial cell comprising a mutant spr gene encoding a spr protein having a mutation at one or more amino acids selected from N31, R62, I70, Q73, C94, S95, V98, Q99, R100, L108, Y115, D133, V135, L136, G140, R144, H145, G147 and H157 and wherein the cell has reduced Tsp protein activity compared to a wild-type cell.

~~In one embodiment, t~~The cell's genome ~~is~~may be isogenic to a wild-type bacterial cell except for the mutated spr gene and the modification required to reduce Tsp protein activity compared to a wild-type cell.

In a second ~~aspect the present invention provides~~ embodiment, there is provided a recombinant gram-negative bacterial cell having reduced Tsp protein activity compared to a wild-type cell and comprising a mutant spr gene encoding a spr protein, wherein the cell's genome is isogenic to a wild-type bacterial cell except for the modification required to reduce Tsp protein activity compared to a wild-type cell and the mutated spr gene.

The cells provided by the first and second embodiments disclosed above ~~aspects of the present invention~~ show advantageous growth and protein production phenotypes.

In a third ~~aspect, the present invention provides~~ embodiment, there is provided a method for producing a protein of interest comprising expressing the protein of interest in a recombinant gram-negative bacterial cell as defined above.

Brief Description of the Drawings

Figure 1 shows the growth profile of anti-TNFα Fab' expressing strains MXE008 and MXE009 compared to anti-TNFα Fab' expressing wild type W3110 and MXE001 strains.

Figure 2 shows the expression of anti-TNFα Fab' from MXE008 and MXE009 strains compared to the wild type W3110 and MXE001.

Figure 3 shows the growth profile of anti-TNFα Fab' expressing strains MXE008 and MXE009 compared to anti-TNFα Fab' expressing strains control W3110 and MXE001.

Figure 4 shows anti-TNFα Fab' yield from the periplasm (shaded symbols) and supernatant (open unshaded symbols) from *E. coli* strains MXE008 and MXE009 compared to control W3110 and MXE001.

Figure 5 shows the growth profile of anti-TNFα Fab' expressing strains MXE0012 and MXE017 compared to the anti-TNFα Fab' expressing strains wild-type W3110 and MXE001.

Figure 6 shows the expression of anti-TNFα Fab' in MXE0012 and MXE017 compared to the wild-type W3110 and MXE001.

Figure 7 shows the growth profile of W3110, MXE001 and MXE008 during a anti-TNFα Fab' producing fermentation.

Figure 8 shows periplasmic anti-TNFα Fab' accumulation (filled lines and symbols) and media anti-TNFα Fab' accumulation (dashed lines and open symbols) for W3110,