PublisherInfo		
PublisherName	:	BioMed Central
PublisherLocation	:	London
PublisherImprintName	:	BioMed Central

Cleaning up *E. coli*

ArticleInfo		
ArticleID	:	4353
ArticleDOI	:	10.1186/gb-2002-3-7-reports0039
ArticleCitationID	:	reports0039
ArticleSequenceNumber	:	27
ArticleCategory	:	Paper report
ArticleFirstPage	:	1
ArticleLastPage	:	4
ArticleHistory	:	RegistrationDate : 2002–5–4 Received : 2002–5–4 OnlineDate : 2002–6–24
ArticleCopyright	:	BioMed Central Ltd2002
ArticleGrants	:	

ArticleContext	:	130593377

Wim D'Haeze

Abstract

A new method of creating large genomic deletions has been used to make a 'clean', stable bacterial genome

Significance and context

Study of the many complete bacterial genomes now available has shown that they contain genes that are unnecessary for life under laboratory conditions and transposable elements that can cause random genome rearrangements and null mutations. This can have undesirable consequences when a bacterium is used for biotechnological purposes, as unnecessary gene products could affect the ease with which the material of interest can be purified, thereby increasing production costs. Metabolic waste products can also be a serious problem, even in small quantities, in the production of drugs and vaccines. Kolisnychenko *et al*. have developed a new method of deleting large stretches of DNA and tested the method on *Escherichia coli* to create a 'backbone-only' strain. This 'clean' strain can be used for biotechnological purposes, and to answer questions concerning the stability and evolution of the genome.

Key results

Kolisnychenko *et al*. deleted 12 large genomic regions from the *E. coli* MG1655 genome, to make *E. coli* MDS12. The inferred amino-acid sequence of the open reading frames (ORFs) within these deleted regions was compared with 24 bacterial proteomes, and most ORFs did not have counterparts in more than nine of these genomes. To check the precise ends of the deletions the authors sequenced across all deletion breakpoints. *E. coli* MDS12 contained 376,180 fewer base-pairs than its parental strain, giving an 8.1% reduction in genome size and a 9.3% reduction in gene content. The doubling times of *E. coli* MG1655 and MDS12 grown in rich and minimal media were nearly identical, although the final optical density of the MDS12 culture (reflecting cell numbers)was slightly, but significantly, higher than that of MG1655. There was no difference in the capacity of the two strains to be transformed by electroporation.

Methodological innovations

In the new technique of Kolisnychenko *et al*. the region to be deleted is first replaced by a PCR-generated DNA fragment. This contains the actual joint of the final deletion in its primer part, so that small direct repeats are created on the chromosome. Recombination then ensures that all vector DNA and the region to be deleted are cleanly removed from the genome. Because recombination between relatively short sequences occurs at low frequency, the process is stimulated by expression of an I-*Sce*I meganuclease, a restriction enzyme that will specifically cut the DNA at two I-*Sce*I sites present in the inserted fragment.

Links

Additional information, an explanation of the new deletion method and step-by-step protocols are available as Supplemental material from the authors' website.

Reporter's comments

The deletion method developed by Kolisnychenko *et al*. provides a powerful and efficient tool to engineer microorganisms that are used for biotechnological purposes, for example to produce pharmaceuticals or antibiotics. Using for these purposes bacterial strains that are genetically 'cleaned up' would facilitate purification and diminish production costs. The method is also an efficient way of creating deletion mutants on a large scale. The *E. coli* MDS12 strain, constructed here in order to test the new method, could be of use to produce proteins, drugs or other compounds of interest, after determining growth kinetics and behavior in industrial-sized batch cultures. A plethora of genomes of human pathogenic bacteria, plant or animal pathogens, archaea and other microorganisms are now available for use.

Table of links

References

1. Kolisnychenko V, III G Plunkett, Herring CD, Fehér T, Pósfai J, Blattner FR, Pósfai G: Engineering a reduced *Escherichia coli* genome. Genome Res. 2002, 12: 640-647. 1088-9051