Free Radical Biology and Medicine 74 (2014) 245–251

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Original Contribution

Commercial Lysogeny Broth culture media and oxidative stress: A cautious tale

Benjamin Ezraty [a], Camille Henry [a], Marion Hérisse [a], Erick Denamur [b,c], Frédéric Barras [a,*]

[a] Laboratoire de Chimie Bactérienne, Aix Marseille Université–CNRS, UMR 7283, Institut de Microbiologie de la Méditerranée, CNRS, 31 Chemin Joseph Aiguier, 13009 Marseille, France
[b] INSERM, IAME, UMR 1137, F-75018 Paris, France;
[c] Université Paris Diderot, Sorbonne Paris Cité, F-75018 Paris, France

ARTICLE INFO

Article history:
Received 13 March 2014
Received in revised form
27 June 2014
Accepted 7 July 2014
Available online 15 July 2014

Keywords:
Microbiology
Oxidative stress
Enterobacteriaceae
Media brand

ABSTRACT

Lysogeny Broth (LB), most often misnamed Luria-Bertani medium, ranks among the most commonly used growth media in microbiology. Surprisingly, we observed that oxidative levels vary with the commercial origin of the LB ready to use powder. Indeed, growth on solid media of *Escherichia coli* and *Salmonella* derivatives lacking antioxidative stress defenses, such as *oxyR* mutant devoid of the H_2O_2-sensing transcriptional activator or Hpx^- strains lacking catalases and peroxidases, exhibit different phenotypes on LB-Sigma or LB-Difco. Using gene fusion and exogenously added catalase, we found that LB-Sigma contains higher levels of H_2O_2 than LB-Difco. Also we observed differences in population counts of 82 clinical and environmental isolates of *E. coli*, depending on the LB used. Further investigations revealed a significant influence of the commercial origin of agar as well. Besides being a warning to the wide population of LB users, our observations provide researchers in the oxidative stress field with a tool to appreciate the severity of mutations in antioxidative stress defenses.

Introduction

Lysogeny Broth (LB) is the bacterial culture medium most commonly used in biology. This medium was formulated in 1951 by Guiseppe Bertani in order to study P-phage plaque formation on *Shigella* [1,2]. Although anecdotal, over the years, the LB acronym was mistaken for Lennox-Broth, Luria-Broth, Luria-Bertani, or even Liquid-Broth.

The formula for LB liquid medium, as given in the Cold Spring Harbor Protocols [3], is as follow: "to 1 liter add 10 g of tryptone, 10 g of NaCl and 5 g of yeast extract, adjust the pH value to 7.0 with 5 N NaOH (≈ 0.2 ml), sterilize by autoclaving for 25 min at 120 °C" Tryptone is usually tryptic digest of casein and yeast extract is an autodigest of *Saccharomyces cerevisiae* [4]. The original formulation included 1 mg/ml glucose [1]. The various LB recipes differ in their salt concentration: LB-Miller contains 10 g/L NaCl, LB-Lennox 5 g/L NaCl, and LB-Luria 0.5 g/L NaCl [5–7]. Nevertheless, the chemical composition of LB remains ill-defined because the chemical nature of some components is impossible to control. For instance, peptides and amino acids are provided by tryptone, a protein hydrolysate, and yeast extracts contain unknown organic compounds. Altogether, this can lead to wide variations from batch to batch, and therefore the use of LB for investigating bacterial physiology has repeatedly been criticized [8]. This was discussed by D'Ari's group in 2007, who reported that "even using the same strain at the same OD_{600}, if the latter is above 0.3 the cells' physiological state will not necessarily be reproducible in different experiments since the composition of Luria-Bertani broth can vary" [9]. Despite all of these cautious warnings, the microbiology community still makes large use of LB as it offers multiple advantages to the experimenter, the first of which is that it allows bacteria to grow fast. Another advantage, directly related to the present study, is that LB powder ready to be used can nowadays be purchased from companies. As we show hereafter, an unexpected difference arises in between LBs sold by different companies.

Components such as riboflavin, reducing sugars, transition metal ions, thiols, and flavonoids can generate reactive oxygen species (ROS), as a result of exposure to heat during autoclaving, or light during storage [10]. All of these components are present in LB and the level of "LB-released ROS" is an additional hidden variable. Adding catalase, pyruvate, or alpha-ketoglutaric acid peroxide, which all degrade ROS, onto LB plates was found to enhance the survival rate of stressed bacteria [11]. Similarly, adding catalase onto LB plates suppressed the sensitivity of an *E. coli recA* mutant [12]. Light excited riboflavin was also found to cause ROS production in eukaryotic cell culture media [13,14].

Abbreviations: LB, Lysogeny Broth; ROS, reactive oxygen species

* Corresponding author.
E-mail address: barras@imm.cnrs.fr (F. Barras).

http://dx.doi.org/10.1016/j.freeradbiomed.2014.07.010
0891-5849/

Also, different levels of ROS were found to depend on the commercial brand of tryptone [15].

Our laboratory is pursuing studies on oxidative stress and metal toxicity in *E. coli* and *Salmonella* [16–19]. Over the years, we observed that mutations altering genetic systems involved in resistance to oxidative stress or metal toxicity often exhibited unstable phenotypes and it occurred to us that the basis of phenotypic instability could be associated with LB. This prompted us to carry out a systematic comparison of LB and we realized that the commercial brand, rather than the way it had been handled or stored, was the cause of the apparent lack of reproducibility.

Materials and methods

Growth media and chemicals

LB-Sigma, LB-Difco, LB-Fisher, and LB-Invitrogen were sterilized simultaneously to avoid differences in autoclave conditions. To prevent the photochemical formation of hydrogen peroxide, media were shielded from light and used within 2 days of preparation. Agar used throughout this study was from Bio-Rad, except in experiments reported in Figs. 8 and S4, in which agar was from Difco, Bio-Rad, and Sigma as indicated.

Studies on LB agar plates

Strains were grown anaerobically (Coy Chamber) in LB at 37 °C. When the OD600 reached 0.4–0.5, 100 μl of serial dilutions of cell suspensions in phosphate buffer (0.05 M, pH 7.4) were spread onto different LB agar plates. Colony forming units (CFU) were counted after incubation for 16 h at 37 °C.

β-Galactosidase assay after plating

The strains are cultured to an OD600 of 0.1, and 1 ml was plated onto LB plate. After 30 min of incubation at 37 °C, the bacteria were resuspended with 1 ml of phosphate buffer (pH 7). The β-galactosidase levels were measured as described by Miller [20].

Table 1
Bacterial strains.

Strains	Relevant genotype	Source or reference
E. coli strains		
MG1655	Parental strain	Lab collection
Hpx$^-$	Δ*katE* Δ*katG ahpC*::kan^r	[22]
Hpx$^-$ *dps*	Δ*katE* Δ*katG* Δ*dps ahpC*::kan^r	[22]
Hpx$^-$ *recA*	Δ*katE* Δ*katG recA::cat ahpC*::kan^r	This study
Hpx$^-$ *suf*	Δ*katE* Δ*katG* Δ*suf ahpC*::kan^r	This study
QC2413	Δ*oxyR*::Kan^r	Lab collection
BEFB01	Δ*sodA*::Cm^r Δ*sodB*::Kan^r	[23]
ahpc::lacZ	MG1655 *ahpC::lacZ*	This study
soxS::lacZ	MG1655 *soxS::lacZ*	This study
IAI strains		[24]*
S. typhimurium strains		
12023	Parental strain	Lab collection
HpxF$^-$	Δ*katE* Δ*katG* Δ*katN* Δ*ahpCF* Δ*tsaA*	[25]
ST10	Δ*oxyR*::Tn10 (Tet^r)	[26]

* The natural *E. coli* isolates correspond to the IAI collection [24] encompassing 15 commensal strains and 67 extraintestinal pathogenic strains [from blood ($n=6$), urine ($n=53$) and miscellaneous samples ($n=8$)] of human origin. They belong to various phylogenetic groups and have been characterized for many phenotypes as the intrinsic virulence in a mouse model of sepsis, the growth in various media, and the resistance to H_2O_2 [24,27].

lacZ fusion construction

The *ahpC::lacZ* fusion was constructed using the method described by Mandin and Gottesman [21]. Briefly, the *ahpC* or *soxS* promoters were amplified by PCR with the appropriate oligonucleotides. Using mini-lambda mediated recombineering, the PCR product was then directly recombined with the chromosome of a modified *E. coli* wild-type strain (PM1205), which carries a P_{BAD}-*cat-sacB* cassette inserted in front of *lacZ* at the 9th codon. Recombinants were selected for loss of the *cat-sacB* genes, resulting in the translational fusion of *ahpC* or *soxS* to *lacZ* (Table 1).

Results and discussion

Different types of commercial LB provide different oxidative environments

In the course of our studies on oxidative stress response in *E. coli* K-12 we experienced difficulties in stabilizing the phenotype of

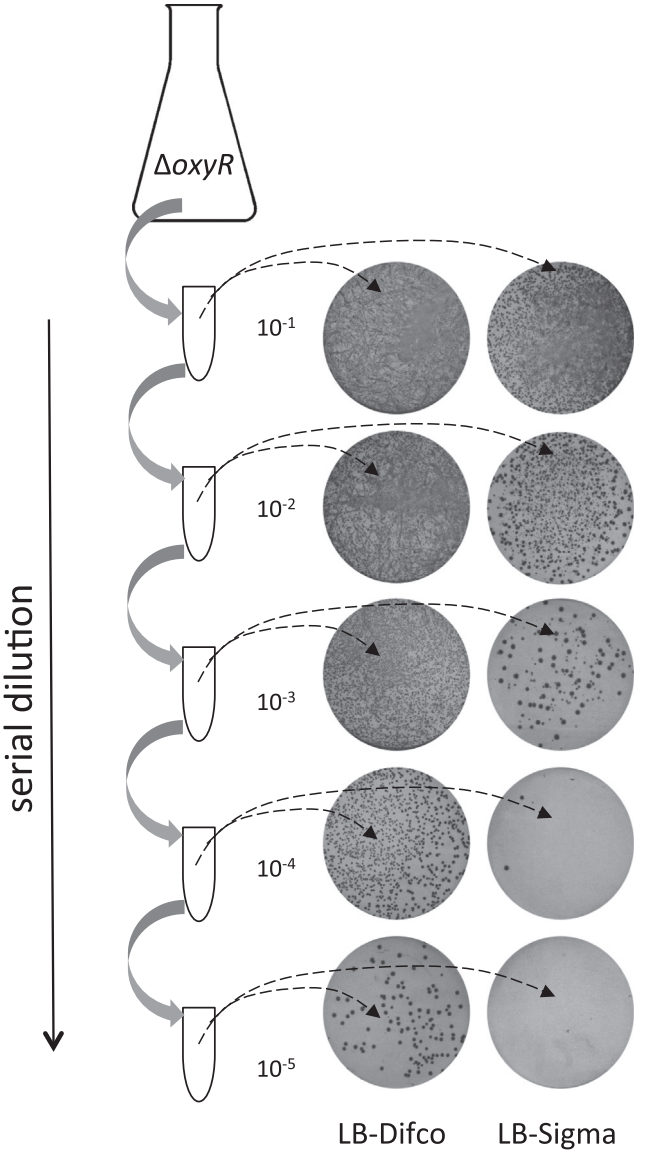

Fig. 1. Evaluation of the population size of an *oxyR* mutant depends on the commercial LB used. The *E. coli* K-12 *oxyR* mutant was cultured overnight at 37 °C in LB medium under anaerobic conditions. Cultures were diluted, and 100 μl of each dilution was spread onto LB-Sigma and LB-Difco medium plates. The plates were incubated at 37 °C for 16 h and then scanned.