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ARTICLE

Culture media supplemented with inorganic salts improve the growth and viability of several bacterial strains

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ABSTRACT In order to improve growth and storage conditions for bacterial cultures, commonly used basic culture media, Luria-Bertani broth (LB) and glucose-yeast extract (GY) were tested along with their supplemented versions (LBS and GYS) containing a complex set of inorganic salts required for common physiological processes. The growth kinetics and viability of 15 representative strains were compared on LB/LBS or GY/GYS. Growth kinetics were examined during a 24 h period. Five out of 15 strains showed enhanced growth on LBS and GYS. Three strains showed very low viability (3 months or lower) both on the basic and salt-supplemented media. Six strains could be equally recovered after 6 or 12 months both from LB/LBS and from GY/GYS. Six of the tested 15 bacterial strains showed significantly better recovery rate on the inorganic-supplemented media LBS or GYS than on basic LB or GY. These results show that inorganic supplement of basic media may significantly improve the growth and viability of several bacterial strains.
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KEY WORDS
growth kinetics
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storage conditions
viability

Introduction

Efficient cultivation and reliable storage of bacterial cultures is a basic prerequisite in several microbiology-related fields, including human and veterinary medicine, environmental, industrial and agricultural microbiology, as well as molecular biology and biotechnological applications. For these purposes, mostly simple laboratory media are used which are based on hydrolyzed proteins (*e.g.*, meat extracts), yeast or various plant extracts. The general feature of these widely-used media (*e.g.*, Luria-Bertani broth, potato dextrose agar and glucose/yeast extract media) is that they are rich in organic compounds, but lack or contain only trace amounts of inorganic nutrients. On the other hand, plant tissue culture media always contain a complex set of inorganics (macroelements and microelements) required for growth and metabolism (George et al. 2012).

During the storage of various *Allorhizobium vitis* (formerly *Agrobacterium vitis*) strains we have observed that they lost their viability after a few months at 4 °C on glucose/yeast-extract (GY) slant agar, even if it was supplemented with $CaCO_3$ to buffer acid formation from glucose. When this medium was supplemented with AB salts (Lichtenstein and Draper 1986) the viability of *A. vitis* strains has increased up to ten months or longer. Similarly, the published 3-ketolactose medium (Bernaerts and De Ley 1963) containing only 1.0% (w/v) lactose and 0.1% (w/v) yeast extract was only weakly appropriate for this biochemical assay for *A. vitis* unless supplemented with AB salts (*our unpublished observation*). These data suggested us that the commonly used laboratory media do not contain the appropriate amount of inorganic nutrients. In an early study, Tartof and Hobbs (1987) found that phosphate supplement significantly increased the cell yield, and thereby the plasmid yield of *Escherichia coli*. A more recent study has shown that certain mono- (Na, K), and bivalent (Ca, Mg) cations had positive effects on the growth and viability of *A. vitis*, although data have not been presented on their action (Tanaka et al. 2009). They may contribute to transport or other basic physiological processes, or they are components of essential vitamins and coenzymes.

The Luria-Bertani broth (LB) and glucose/yeast extract media (GY) are widely used for bacterial cultures. In this study we compared the effects of the original LB and GY, and their modified formulae supplemented with inorganic salts on the growth and storability of various bacterial cultures. Our results show that inorganic supplements may significantly increase the viability of certain strains.

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Table 1. Bacterial strains.

Code*	Strain	Reference**	Growth conditions (medium/ temperature)
SZMC 21395	*Agrobacterium tumefaciens* C58 (= *Agrobacterium fabrum*)	Sciaky et al. 1978	GY/25 °C
SZMC 21396	*Allorhizobium vitis* Tm4 (= *Agrobacterium vitis*)	Szegedi et al. 1988	GY/25 °C
SZMC 21397	*Allorhizobium vitis* AT1	Szegedi et al. 1988	GY/25 °C
SZMC 21398	*Allorhizobium vitis* S4	Szegedi et al. 1988	GY/25 °C
SZMC 0209	*Bacillus subtilis*	n.a.	LB/30 °C
SZMC 21399	*Escherichia coli* DH5α	Hanahan et al. 1991	LB/37 °C
SZMC 21400	*Escherichia coli* LE392	Zyskind and Bernstein 1992	LB/37 °C
SZMC 21401	*Burkholderia phytofirmans* PsJN	Toklikishvili et al. 2010	GY/25 °C
SZMC 6269	*Chromobacterium violaceum* wt85	n.a.	LB/25 °C
SZMC 21402	*Erwinia amylovora* K21	Hevesi, M., personal communication	GY/25 °C
SZMC 21403	*Novosphingobium* sp. Rr 2-17	Gan et al. 2012	GY/25 °C
SZMC 21404	*Pseudomonas fluorescens*	n.a.	LB/25 °C
SZMC 0567	*Serratia marcescens*	n.a.	LB/30 °C
SZMC 21405	*Serratia plymuthica* IC1270	Dandurishvili et al. 2010	LB/30 °C
SZMC 0227	*Streptococcus faecalis*	n.a.	LB/30 °C

*SZMC: Szeged Microbiology Collection, Szeged, Hungary, **n.a.: not available

Materials and Methods

Bacterial strains

Strains used in this work (Table 1) were stored in glycerol stocks at -80 °C. For daily use they were cultured on GY or LB media at 25, 30 or 37 °C (Table 1) for two days, and then stored at 4 °C.

Media

Culture media were based on LB or GY (Table 2) and prepared in deionized water. The $CaCl_2 \times 2\ H_2O$ stock solution (3% calcium-chloride, w/v) was autoclaved in aliquots and stored at 4 °C. For FeEDTA stock solution, 1.12 g $FeSO_4 \times 7\ H_2O$ and 1.5 g Na_2EDTA were completely dissolved separately in 100-100 ml water, then mixed and stored in aliquots at -20 °C. The microelement stock solution contained 1.0 g $MnSO_4 \times 7\ H_2O$, 0.5 g $ZnSO_4 \times 7\ H_2O$, 25 mg $Na_2MoO_4 \times 2\ H_2O$ and 2.5 mg $CoCl_2 \times 6\ H_2O$ dissolved separately, mixed and made up to 100 ml. Aliquots were kept at -20 °C. All solutions were prepared in deionized water. Since both tryptone and yeast extract contain the sufficient amount of amino acids, nitrogen-source (ammonium or nitrate salt) was not added. Media supplemented with the inorganics (Table 2) were designated as LBS or GYS. The pH was adjusted to 7.0-7.2 with 3% NaOH when it was necessary. Solid media were prepared with 1.2% (w/v) agar. Bacterial suspensions were made in phosphate-buffered saline (PBS), containing 1.236 g/l Na_2HPO_4, 0.18 g/l NaH_2PO_4 and 8.5 g/l NaCl.

Growth on agar plates

To get preliminary data about the suitability of LB and GY for a given bacterium, the growth of the tested strains were compared on solid GY and LB media as well as on their salt-supplemented derivatives LBS and GYS (Table 2) prepared with 1.2% (w/v) agar. Plates were inoculated with a loopful bacterial suspension (OD_{600} = 0.2 for the two *E. coli* strains and OD_{600} = 0.8 for the others) and then incubated at 25, 30 or 37 °C depending on the strain (Table 1). Bacterial growth was recorded after 24 and 48 hrs.

Table 2. Culture media used for bacterial growth and storage studies.

Medium	Components	Concentration
Luria-Broth medium (LB)	triptone	10.0 g/l
	yeast extract	5.0 g/l
	NaCl	5.0 g/l
Glucose-yeast extract medium (GY)	glucose	10.0 g/l
	yeast extract	5.0 g/l
	$CaCO_3$ (for storage only)	2.0 g/l
Inorganic supplement medium	K_2HPO_4	2.0 g/l
	KH_2PO_4	1.0 g/l
	$MgSO_4$ x 7 H_2O	0.5 g/l
	$CaCl_2$ x 2 H_2O stock*	10.0 ml/l
	FeEDTA stock*	5.0 ml/l
	Microelement stock*	1.0 ml/l

*see Materials and Methods

Table 3. Viability of tested microorganisms on different storage media at 4 °C.

Code	Strain	Medium*	3	6	9 months	12	Viability months
SZMC 21395	*Agrobacterium tumefaciens* C58	GY	+	+	+	+	12
		GYS	+	+	+	+	12
SZMC 21396	*Allorhizobium vitis* Tm4	GY	+	+	+	(+)	12
		GYS	+	+	+	+	12
SZMC 21397	*Allorhizobium vitis* AT1	GY	+	(+)	-	n.t.	6
		GYS	+	+	+	+	12
SZMC 21398	*Allorhizobium vitis* S4	GY	+	(+)	-	n.t.	6
		GYS	+	+	+	+	12
SZMC 0209	*Bacillus subtilis*	LB	+	+	+	+	12
		LBS	+	+	+	+	12
SZMC 21399	*Escherichia coli* DH5α	LB	+	(+)	-	n.t.	6
		LBS	+	+	(+)	-	9
SZMC 21400	*Escherichia coli* LE392	LB	+	(+)	-	n.t.	6
		LBS	+	+	(+)	-	9
SZMC 21401	*Burkholderia phytofirmans* PsJN	GY	+	(+)	-	n.t.	6
		GYS	+	+	+	+	12
SZMC 6269	*Chromobacterium violaceum* wt85	LB	-	n.t.	n.t.	n.t.	0
		LBS	-	n.t.	n.t.	n.t.	0
SZMC 21402	*Erwinia amylovora* K21	GY	+	(+)	-	n.t.	6
		GYS	+	+	+	+	12
SZMC 21403	*Novosphingobium* sp. Rr 2-17	GY	+	+	+	+	12
		GYS	+	+	+	+	12
SZMC 21404	*Pseudomonas fluorescens*	LB	+	-	n.t.	n.t.	3
		LBS	+	-	n.t.	n.t.	3
SZMC 0567	*Serratia marcescens*	LB	+	-	n.t.	n.t.	3
		LBS	+	-	n.t.	n.t.	3
SZMC 21405	*Serratia plymuthica* IC1270	LB	+	+	-	n.t.	6
		LBS	+	+	-	n.t.	6
SZMC 0227	*Streptococcus faecalis*	LB	+	(+)	-	n.t.	6
		LBS	+	+	-	n.t.	6

*see Materials and methods. Strains were recovered on LBS (*Pseudomonas fluorescens*, *Serratia plymuthica* IC1270, *Escherichia coli* LE 392 and *E. coli* DH5α), or on GYS (other strains).

Growth kinetics in liquid media

Strains were cultured for 24 h in 48-well microtiter plates using the basic media (LB and GY) and media supplemented with inorganics (LBS or GYS). All strains were precultured in LB or GY liquid media with shaking for 24 h at appropriate temperatures. Then 1 ml aliquots of cultures were centrifuged and the pelleted cells were washed three times with 0.2% glycerol. These stocks were used during the subsequent experiments. Each well contained a cell concentration of 10^5 cells/ml. The OD_{600} value was measured in every hour during the 24 h growth period with a SPECTROstar Nano microplate reader (BMG Labtech, Ortenberg, Germany).

Storage experiments

Strains were inoculated parallely in three replicates onto LB and LBS slant agar media, or GY and GYS slant agar media supplemented with 0.2% $CaCO_3$ (Table 3) as it was found appropriate for a particular strain. Then cultures were incubated for 2 days at 37 °C (for *E. coli* LE 392 and DH5α) or at 25 or 30 °C (for the other strains, Table 1), and then stored at 4 °C. Viability of the strains was checked after three, six, nine and 12 months. To this end, loopful samples were suspended in 1.2 ml PBS and one loop was inoculated onto LBS (for strains kept on LB and LBS) or onto GYS (for strains kept on GY or GYS) plates. Recovery of strains was scored after three days of incubation at 37 °C (for *E. coli* LE 392 and DH5α) or at 25, or 30 °C (for the other strains).

Results

Growth of bacterial strains on solid media

During a preliminary survey, all strains were inoculated from suspensions onto LB and GY as well as their salt-supplement-

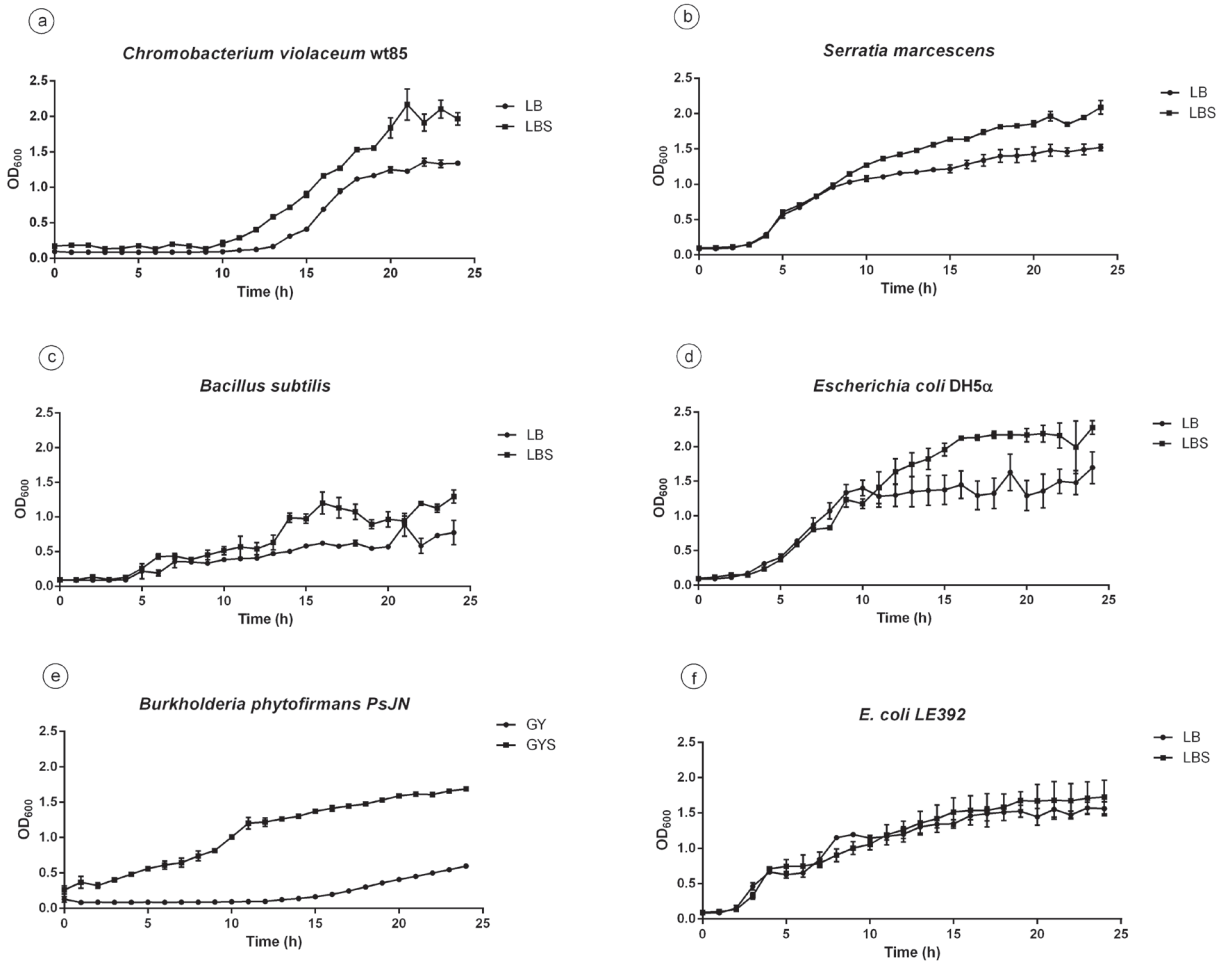

Figure 1. Growth kinetics of *C. violaceum* wt85 (a), *S. marcescens* (b), *B. subtilis* (c), *E. coli* DH5α (d), *Burkholderia* sp. PsJN (e), and *E. coli* LE392 (f) in liquid culture media. Some of these strains (a, b, c, d) showed more intensive growth in LBS medium than in LB, and one of them (e) showed more intensive growth in GYS medium than in GY. In case of *E. coli* LE392 (f) no growth difference was detected in the two tested media. Results are presented as mean ± SEM.

ed derivatives LBS and GYS in order to find an appropriate medium for each strain. After 24 h and 48 h growth, visual scorings of the plant associated bacteria *Agrobacterium tumefaciens* C58, *Allorhizobium* spp., *Burkholderia* sp., *Erwinia* sp. and *Novosphingobium* sp. showed better growth on GY-based media than on LB. The other strains preferred LB-based media to GY. Thus, for storage studies the appropriate media were prepared based on these observations. After 24 h of growth, *A. vitis* strains Tm4, AT1 and S4 as well as *E. amylovora* K21 and *Bacillus subtilis* showed more intensive growth on GYS than on GY medium. The two *E. coli* strains LE9392 and DH5α also grew better on LBS than on LB. These differences in growth on the basic and salt-supplemented media were still clear after two days for the three *A. vitis* and two *E. coli* strains. The other bacteria did not exhibit seemingly higher growth rates (data not shown).

Growth kinetics of bacterial strains in liquid media

The investigations made in liquid media were performed after the preliminary selection of the most appropriate medium for each strain (see above). *Chromobacterium violaceum, Serratia marcescens, B. subtilis* and *E. coli* DH5 showed more intensive growth in LBS media than in LB (Fig. 1 a, b, c, d). In the case of *E. coli* LE392 we did not detect any differences in growth (Fig. 1f). *Burkholderia* sp. PsJN grew better in GYS than in GY (Fig. 1e). The other strains did not show significant differences. We compared the results obtained on solid and in liquid media. The growth of *C. violaceum, S. marcescens, B. subtilis* and *E. coli* DH5α were enhanced on both solid and liquid media in the presence of inorganic elements. In the case of *E. coli* LE392, growth enhancement was

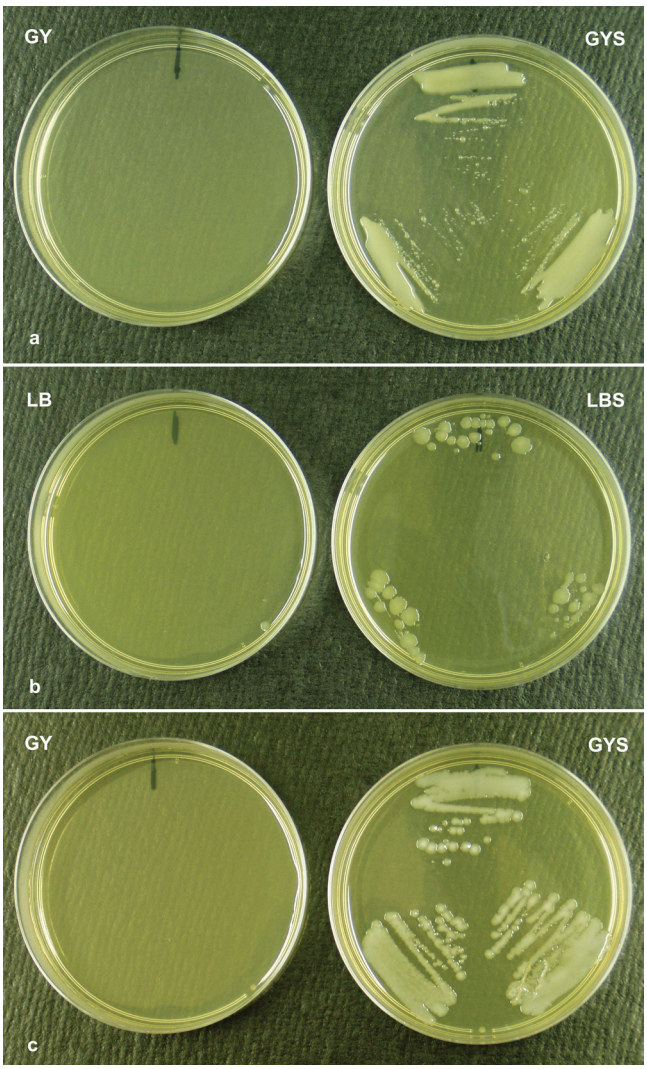

Figure 2. Recovery of *A. vitis* AT1 (a), *E. coli* LE392 (b) and *E. amylovora* K21 (c) strains on basic GY or LB (left) and salt-supplemented GYS or LBS media (right) after nine months of incubation at 4 °C. Each pair of plates shows the results of three replicates.

observed only on solid media. We observed the same results for the other tested strains; enhancement was achieved only on solid media (data not shown).

Viability of strains on basic and supplemented media

The tested strains highly varied in their viability during storage at 4 °C on basic LB/LBS or GY/GYS media solidified with agar (Table 3). The first group showed a very weak recovery or complete loss of viability after three to six months of storage in active cultures regardless of the media (LB and LBS) compared. The second group including six of the tested

Table 4. Summarized data on the viability of the tested bacterial strains stored at 4 °C on basic and inorganic-supplemented media.

Group I. Strains with low viability (≤3 months) on both media*	Group II. Strains with equal viability (6-12 months) on both media*	Group III. Strains with better viability on LBS or GYS medium than on the basic medium*
C. violaceum wt85	*A. tumefaciens* C58	*A. vitis* AT1
P. fluorescens	*A. vitis* Tm4	*A. vitis* S4
S. marcescens	*B. subtilis*	*E. coli* DH5α
	Novosphingobium sp. Rr2-17	*E. coli* LE392
	S. plymuthica IC1270	*B. phytofirmans* PsJN
	S. faecalis	*E. amylovora* K21

*LB or LBS, and GY or GYS, see also Table 3.

15 strains remained viable up to 6-12 months, but differences in their recovery from the basic and salt-supplemented media were not observed. Six strains, namely *A. vitis* AT1 and S4, *E. coli* LE392 and DH5α as well as *E. amylovora* K21 and *Burkholderia phytofirmans* PsJN could be recovered only until six month of storage from the basal medium (LB or GY), but they remained viable up to 9-12 months on the salt-supplemented LBS or GYS media (Fig. 2). These data are summarized in Table 4.

Discussion

Basic bacterial media like LB and GY are usually rich in organic compounds, but they contain low amounts of basic inorganics. Therefore we supplemented these media with various anions and cations, which are components of nucleoside triphosphates, nucleic acids and enzymes or contribute to various physiological processes. Growth kinetics of five strains were enhanced after using supplementation with inorganics in both solid and liquid media. No difference was detected in liquid cultures in the case of *E. coli* LE392, although its growth was slightly enhanced on solid media. The inorganic supplement also improved the viability, thus increased the recovery rate of several bacteria (six of the 15 tested strains).

Our data confirm the previous observations on the salt requirements of *A. vitis* (Tanaka et al. 2009) and extend these observations to other bacterial genera. Thus, the storage of bacterial cultures in small laboratories, where ultra-freezer is not available may become much safer with less frequent culture transfer. It also reduces the risks of contamination and failures due to missing strain designation. Considering these results, for daily laboratory work we propose the application of the inorganic-supplemented versions of the commonly used bacterial media, including LB, GY and others, containing

simple organic extracts. Our results may be adapted to other natural bacterial or fungal media (*e.g*., PDA) to improve the efficiency of isolation from bacterial populations of various environmental, plant, food or medical samples.

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