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Microorganisms and Maillard reaction products: a review of the literature and recent findings

Cynthia Helou · David Marier · Philippe Jacolot · Latifa Abdennebi-Najar · Céline Niquet-Léridon · Frédéric J. Tessier · Pascale Gadonna-Widehem

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Abstract Research on the impact of Maillard reaction products (MRPs) on microorganisms has been reported in the literature for the last 60 years. In the current study, the impact of an MRP-rich medium on the growth of three strains of *Escherichia coli* was measured by comparing two classic methods for studying the growth of bacteria (plate counting and optical density at 600 nm) and by tracing MRP utilisation. Early stage and advanced MRPs in the culture media were assessed by quantifying furosine and N^{ε}-carboxymethyllysine (CML) levels, respectively, using chromatographic methods. These measures were performed prior to and during bacterial growth to estimate the potential use of these MRPs by *Escherichia coli* CIP 54.8. Glucose and lysine, the two MRP precursors used in the MRP-rich medium, were also quantified by chromatographic means. Compared to control media, increased lag phases and decreased growth rates were observed in the MRP-rich medium for two out of the three *Escherichia coli* strains tested. In contrast, one strain isolated from the faeces of a piglet fed on a MRP-rich diet was not influenced by the presence of MRPs in the medium. Overall, CML as well as the products obtained by the thermal degradation of glucose and lysine, regardless of the Maillard reaction, did not affect the growth of the three strains tested. In addition, no degradation of fructoselysine or CML was found in the presence of *Escherichia coli* CIP 54.8.

C. Helou · D. Marier · P. Jacolot · L. Abdennebi-Najar · C. Niquet-Léridon · F. J. Tessier · P. Gadonna-Widehem (✉)
EGEAL, Institut Polytechnique LaSalle Beauvais, 19 rue Pierre Waguet, BP 30313, 60026 Beauvais, France
e-mail: pascale.gadonna@lasalle-beauvais.fr

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Introduction: Sixty years of intermittent research on the impact of Maillard reaction products (MRPs) on microorganisms

The Maillard reaction is a heat-induced non-enzymatic browning involving reducing compounds such as reducing sugars and amino groups such as free or protein-bound amino acids. Carbonyl and amino compounds react to form a labile imine that undergoes isomerization leading to the formation of early glycation products. The decomposition and subsequent reactions of these early products lead to the formation of advanced glycation end products (AGEs). Further reactions lead to the formation of brown polymers called melanoidins. The Maillard reaction is, therefore, a complex series of sequential and parallel reactions (Rabbani and Thornalley 2010) that occurs at elevated temperatures during cooking, but may take place, to some extent, at room temperature during food storage (Mastrocola and Munari 2000). Overall, the Maillard reaction leads to chemical modifications of nutrients in foods and, as a result, to the formation of flavours, colours and other neoformed compounds. The absorption and the health effects of some dietary AGEs have been studied extensively for the last 20 years (Tessier and Niquet 2007). However, the physiological consequences of exposure to AGEs are still under debate (Henle 2007).

The Maillard reaction may also affect the digestibility of glycated proteins by gastrointestinal enzymes, and various AGEs and other MRPs have been found to be excreted in the faeces (Faist and Erbersdobler 2001; Delgado-Andrade et al. 2011). This indicates a likely interaction between

these compounds and the intestinal microflora. Numerous studies have attempted to shed light on the impacts that AGEs and other MRPs have on microorganisms. The current review gives attention to the effect of various MRP-model-media on bacterial growth and highlights the fact that the findings were dependent on the strains, reactions parameters as well as the models used.

The first studies described the impact of heat treatments of culture media and certain food models on bacterial growth without really addressing the study of the Maillard reaction. Hachisuka et al. (1955) compared multiple culture media and noted a decrease of the germination time of *Bacillus subtilis* spores in the media having the nitrogen and carbon sources autoclaved together. Similarly, Field and Lichstein (1957) observed a decrease in the lag phase of four strains of propionibacteria in culture media autoclaved in the presence of a reducing sugar. In parallel, Foster (1952) observed a stimulation of homofermentative lactic acid bacteria growth in heated milk. In contrast to this finding, Viswanathan and Sarma (1957) described an inhibitory effect of skim milk powders stored at 60 °C for 6 months on the growth of *L. bulgaricus* 09.

It is only a few years later that researches started to address the Maillard reaction for its direct impact on microorganisms.

Jemmali (1969) found that the growth rates and lag phases of various lactobacilli strains were dependent on the amounts of heated glycine and dextrose present in the culture media, while only the lag phases of *E. coli* were affected. Similar observations were made by Einarsson et al. (1983) in their study on the impact of different amounts of refluxed arginine–xylose on the growth of various bacterial strains, where the effect of MRPs was found to be strain-dependent. The authors also showed that the influence on bacterial growth was dependent on the type and concentration of the MRPs used.

Some authors studied the impact of MRPs from different chemical stages of the Maillard reaction. For instance, Mester et al. (1983) showed that the early intermediate glycation products stimulate bacterial growth, while Stecchini et al. (1993) showed that the more stable AGEs have an inhibitory effect on the growth. Similar results were observed by Lanciotti et al. (1999) where the early glycation products shortened the lag phase of *Bacillus stearothermophilus* and AGEs caused an extension of this lag phase and a reduction of the growth rate and final bacterial populations. Recently, Corzo-Martinez et al. (2011) who analysed various assays with different glycoconjugates and different bacterial strains found in most cases improved bacterial growth, especially for glycoconjugates at the initial stages of the Maillard reaction.

Chalova et al. (2011) noted that *Salmonella* Typhimurium LT2 was able to utilise MRPs derived from a glucose–lysine mixture as carbon sources. Their findings indicate a preference for the Amadori products (98 % disappearance) followed by AGEs (35 %) and finally melanoidins (15 %).

In other studies, MRPs have been found to cause bacterial growth inhibition. In a comparative study between melanoidins from coffee and biscuits, Rurian-Henares and Morales (2008) found that high molecular weight melanoidins had an antimicrobial activity against *Escherichia coli* due to inner and outer membrane damages. According to their findings, antimicrobial activity was bacteriostatic at low concentrations and bactericide at higher ones. Kim and Lee (2003), while studying the growth of *Aeropyrum pernix,* showed that media containing reducing sugars and tryptone inhibited the growth of this hyperthermophilic archaeon. Studies on *Aggregatibacter actinomycetemcomitans* have shown that microwaved media plates lead to larger colonies and greater cell number compared to autoclaved media, darker in colour (Bhattacharjee et al. 2009). Kostyukovsky and Marounek (1995) showed a decrease of fermentative activity of mixed rumen microorganisms in vitro in the presence of MRPs.

Other studies emphasised the effects of MRPs on other biological parameters than the growth of bacteria. For example, studies on *Listeria monocytogenes* showed that while the number of cells was not affected, the virulence expression was reduced with high concentrations of MRPs in the culture media (Sheikh-Zeinoddin et al. 2000). For their part, Moisés Laparra et al. (2011) observed an inhibition of the adhesion of *Escherichia coli* to mucin in the presence of Maillard-type glycoconjugates. Another recent example is the publication of Wiame et al. (2002) which describe the induction of fructoselysine 6-kinase and fructoselysine-6-phosphate deglycase enzymes in *Escherichia coli* grown on fructoselysine. According to the authors, these enzymes reached sufficient activity levels to account for the observed rate of fructoselysine utilisation. In another study, the same research group identified an enzyme from *Enterococcus faecium*, glucoselysine-6-phosphate deglycase, participating in the metabolism of fructation products (Wiame et al. 2005). Lastly, a recent study, based on fluorescent measurements suggests a potential secretion of AGEs by *Escherichia coli* cells (Cohen-Or et al. 2011).

In recent years, interest gradually shifted toward the study of intestinal microbiota. Ames et al. (1999) found a non-specific increase of the predominant anaerobic gut bacteria incubated in vitro with a melanoidin mixture. With regards to the AGEs, Ames et al.(2005) and Tuohy et al. (2006) showed a decrease in bifidobacteria species for ulcerative colitis patients and an increase in clostridia species for both healthy subjects and ulcerative colitis patients with high AGE levels. Swiatecka et al. (2011)

carried in vitro analyses to assess the effect of glycated pea proteins on the intestinal bacteria of a healthy human subject. Their findings suggest an increase of lactobacilli and bifidobacteria.

Comparative analysis of the results summarised in this short review is a difficult task. While the first studies aimed to answer practical laboratory issues for culture media design for example, later studies addressed microorganisms in foods or in model systems and more recent studies focused on the impact of MRPs on the intestinal microflora. This shift of focus is in parallel to the evolution of the understanding of the Maillard reaction and its potential health impacts. The variety of models used, the numerous intermediates and AGEs which can be formed by the Maillard reaction under the different preparation conditions, the diversity of protocols used for the extraction of MRPs as well as the complex mechanisms that underlie bacterial behaviour are all factors which add to the complexity of the study (Table 1). Further studies are still required to fully understand this reaction and its impacts on human health and on biotechnology. However, the use of controls should be strongly recommended for meaningful intra and inter-studies comparison.

In this study, we chose to work with individual model strains to avoid bacterial interactions. Three strains of *Escherichia coli* were selected. Two are of human origin (urine infection and faeces), while the third was isolated from piglet faeces. Considering that faecal bacteria have been in contact with non-digested food MRPs, growth differences in MRP-rich media between the two faecal strains and between these strains and the non-faecal strain would be of interest. Bacterial growth in an MRP-rich culture medium was assessed in comparison with multiple controls which are often omitted. Hence, a mixture of glucose and lysine was used under various conditions to constitute the culture media: not heated for a MRP-negative control, heated for MRP exposure and heated separately for the assessment of the impact of non-Maillard-related heat degradation products. N^{ε}-carboxymethyllysine (CML), a well-studied MRP with proven biological activity in vivo (Tessier 2009), was also used in culture media to measure the effect of this particular AGE on the growth of the model strains chosen. The evolution of some MRPs and their precursors was also monitored in the culture media to check some assumptions in the literature.

Materials and methods

Reagents

Chemical products and solvents were of the highest grade and acquired from Sigma-Aldrich and Fisher Scientific. Standards of furosine dihydrochloride, CML and (D_2)-CML were purchased from PolyPeptide Laboratories France SAS and ($^{15}N_2$)-lysine was provided by CortecNet.

Bacto™ Trypton, Bacto™ Yeast Extract and Plate Count Agar (PCA) were purchased from BD (Becton, Dickinson and Company). Maximum recovery diluent was acquired from Scharlau Microbiology.

Bacterial strains

Escherichia coli strains (CIP numbers 54.8 and 52.167) were obtained from the Collection de l'Institut Pasteur, France. The remaining strain of *Escherichia coli* was isolated from piglet faeces fed on a MRP-rich diet and identified using Gram staining, oxydase and API 20E tests (Biomérieux, France).

Culture media design

D-glucose and L-lysine were mixed in equivalent proportions (20 g/L) in a 200-mM phosphate buffer pH 7.5. This preparation was either autoclaved at 121 °C for 15 min to obtain a MRP-rich supplement (GL-A) or filtered using a sterile 0.22 µm membrane to get the corresponding control (GL-F). Another supplement consisting of glucose and lysine autoclaved separately and mixed afterwards was prepared (20 g/L each) (GL-AS).

A mixture of yeast extract (20 g/L) and tryptone (20 g/L) containing no reducing sugar and, therefore, having a limited risk of potential MRPs formation was autoclaved (YET). In order to obtain the different bacterial culture media, 50 mL of either GL-A, GL-F and GL-AS were added to 50 mL of the YET mixture. Thus, the YET+GL-A medium was defined as the MRP-rich medium, the YET+GL-F and the YET+GL-AS as the two negative controls. All these media had a final concentration of glucose and lysine of 10 g/L each. Two other media named YET+40 %GL-F and YET+40 %GL-A were prepared to get a final concentration of glucose and lysine of 4 g/L each.

The YET mixture was diluted twice and used as the basic culture medium (d-YET). And one last medium was obtained by adding CML to the basic culture medium at a final concentration of 30 µg/mL (YET+CML).

Growth studies

Bacterial cells were collected from an 18-h culture on PCA at 37 °C and suspended in physiological water (NaCl 0.9 %) to obtain a final concentration around 4×10^9 CFU/mL. From the obtained inoculum, 1 mL was introduced into 100 mL of the different experimental media. The inoculated media were incubated at 37 °C in an orbital shaker at 70 rpm.

Table 1 Summary of the major studies on the impact of MRPs on microorganisms: selected microorganisms, MRP-models, growth assessment techniques and major findings

Study	Microorganisms	Growth measurements	MRPs	Impacts on growth
Jemmali (1969)	*Lactobacillus arabinosus, L. casei, L. acidophilus* *Escherichia coli*	Transmittance percentage at 490 nm	Heated dextrose and glycine	Decrease of lag phases and improvement of growth rates of *Lactobacilli* for certain levels of MRPs Increase of *Escherichia coli* lag phases
Einarsson et al. (1983)	*Escherichia coli* NCTC 9001 *Escherichia coli* NCTC 10418 *Staphylococcus aureus* *Salmonella senftenberg* *Lactobacillus plantarum* *Pseudomonas fragi* *Bacillus subtilis* *Pseudomonas fluoerescens*	Plate enumeration Minimum inhibitory concentration (MIC)	Heated D-glucose and L-histidine or D-xylose and L-arginine	Increase of lag phases with increased concentrations of MRPs Increase of inhibitory effect with increased molecular weight
Stecchini et al. (1993)	*Staphylococcus aureus* *Listeria monocytogenes* *Salmonella* Typhimurium *Salmonella enteritidis* *Aeromonas hydrophila*	Plate enumeration	Heated D-glucose and L-glycine	Strain-dependent Inhibitory effects
Kostyukovsky and Marounek (1995)	Mixed rumen microorganisms	Volatile fatty acids accumulation	Heated glucose with either urea, glycine, acidic and enzymic hydrolysates of casein, peptone or casein	No fermentation
Ames et al. (1999)	Faecal samples from one healthy female and 2 healthy males. (Batch fermentation)	Fluorescence in situ hybridisation (FISH) Plate enumeration	Melanoidins > 3 kDa from a heated glucose lysine mixture	Non-specific increase of the anaerobic bacteria
Lanciotti et al. (1999)	*Bacillus stearothermophilus*	Plate enumeration Headspace CO_2 determination	Heated D-glucose or D-fructose and L-glutamic acid	Early stage MRPs: decrease of lag phase; AGEs: increase of lag phase, decrease of maximal growth and final numbers
Sheikh-Zeinoddin et al. (2000)	*Listeria monocytogenes*	Optical density (OD) 600 nm	Glucose autoclaved with the culture media	Reduction of virulence gene expression No impact on bacterial growth
Kim and Lee 2003	*Aeropyrum pernix*	OD 660 nm	D-glucose supplementation of the amino-rich culture medium (incubation at 90 °C)	Inhibition of growth
Tuohy et al. (2006)	Faecal bacteria from one healthy subject and 1 ulcerative colitis patient. (in vitro gut model)	FISH	Glycated bovine serum albumin	Increase of clostridia numbers for both subjects Decrease of bifidobacteria numbers for ulcerative colitis patients

Table 1 continued

Study	Microorganisms	Growth measurements	MRPs	Impacts on growth
Rurian-Henares and Morales (2008)	*Escherichia coli* ATCC 11775	OD 600 nm MIC	Melanoidins from coffee and biscuits	Inhibition related to molecular weight and to concentration Cell membrane disruption
Bhattacharjee et al. (2009)	*Aggregatibacter actinomycetemcomitans*	OD 600 nm Plate enumeration	Sterilisation by autoclaving or by microwaving	Larger colonies and greater numbers for the microwaved medium
Swiatecka et al. (2011)	Faecal samples from one healthy female. (Batch fermentation)	FISH Short chain fatty acids analysis	Glycated pea protein	Increase of *Bacteroides, Lactobacillus* and *Bifidobacterium* numbers
Corzo-Martinez et al. (2011)	*Streptococcus salivarius, S. thermophilus* *Lactobacillus reuteri, L. plantarum, L. delbrueckii, L. brevis, L. gasseri, L. acidophilus, L. casei, L. plantarum* *Bifidobacterium breve, B. lactis*	OD 600 nm	Heated β lactoglobulin-galactose; β lactoglobulin-lactose; Sodium caseinate-galactose; Sodium caseinate-lactose	*Different impacts depending on* strain MRPs and Maillard reaction stage
Chalova et al. (2011)	*Salmonella* Typhimurium	OD 630 nm	Heated glucose and Nα-acetyllysine	Utilisation of MRPs as carbon sources Implication of the glyoxalate cycle in this assimilation

At 2, 4, 6, 8, 10 and 24 h, 2 mL samples of each culture media were withdrawn. The first milliliter was used for optical density (OD) measures at 600 nm using a Beckman DU 530 UV/Vis Spectrophotometer in triplicate. The second milliliter was diluted to appropriate concentrations in total recovery diluent tubes and 1 mL was plated in PCA in duplicate. Colonies were counted after 48 h at 37 °C and expressed as colony forming units per milliliter (CFU/mL). Maximum growth rates μ (h^{-1}) and lag parameters (h) were calculated by fitting the natural logarithmic curves to a sigmoid model using the Microsoft Excel add-in DMfit v. 2.1 (Baranyi and Roberts 1994).

Quantification of glucose, furosine, lysine and CML

Glucose analysis was achieved using a Thermo Finnigan Surveyor HPLC system coupled to an evaporative light-scattering detector (Alltech). Culture media were diluted in 80 % methanol in water, filtered using a 0.45 μm membrane and injected into a Prevail Carbohydrate ES column (250 × 4.6 mm, 5 μm). An isocratic elution was employed using a mixture of acetonitrile and water (75/25 v/v). The quantitative analysis of glucose was performed by means of an external standard calibration curve.

Fructoselysine was measured indirectly via the quantification of furosine after acid hydrolysis. Furosine was analysed according to Schleicher and Wieland (1981) with minor modifications. In brief, 0.5 mL of culture media was incubated with 6 M HCl for 20 h at 110 °C. The acid hydrolysates were dried in a Speed-Vac concentrator, dissolved in deionized water and filtered through a 0.45 μm membrane. Samples were injected on a SpectraSystem HPLC-UV system using a 1 mL min^{-1} flow of 5.6 mM orthophosphoric acid. Detection and quantification were made at 280 nm using an external standard of furosine. Approximate retention time was about 2.55 min.

Lysine and CML analysis were done according to Niquet-Léridon and Tessier (2011) with minor modifications. In brief, 0.5 mL of culture media was incubated with 1 mL of borate buffer (200 mM, pH 9.5) and 1 mL of sodium borohydride (1 M in NaOH 0.1 M) at room temperature for 4 h in triplicate. Afterwards, samples were incubated at 110 °C for 20 h with 6 M HCl and 300 μL of each acid hydrolysate were vacuum-dried. Dry residues were reconstituted with NFPA 20 mM containing adequate amounts of (D_2)-CML and ($^{15}N_2$)-lysine and filtered using a 0.45 μm membrane. Analyses were done by HPLC-ESI-MS/MS using a Hypercarb column (100 × 2.1 mm, 5 μm). The separation was performed using a 0.2 mL min^{-1} gradient of 20 mM NFPA in water and acetonitrile. The ESI interface operated in positive mode and tandem MS analyses were carried out in multiple reactions monitoring mode. The specific transitions m/z 205.0 → m/z 130.0 and

m/z 147.0 → m/z 130.0 were used for the detection and quantification of CML and lysine, respectively. The specific transitions m/z 207.0 → m/z 130.0 and m/z 149.0 → m/z 131.0 were used for the detection and quantification of the two internal standards, (D_2)-CML and ($^{15}N_2$)-lysine. Quantification of lysine and CML was achieved using stable-isotope dilution and standard curves.

Statistical analyses

Bacterial growth data are expressed as the average of three independent experiments. Error bars represent standard deviation from the mean. Log_{10} (CFU/mL) differences were considered significant if superior to 0.3 (AFSSA 2007).

One-way ANOVA and Tukey's tests were performed with the GraphPad Prism (version 3.0) to determine if there were significant differences in the levels of precursors and MRPs during incubation compared to initial values. A P value <0.05 was considered as significant.

Results and discussion

Growth of *Escherichia coli* CIP54.8 strain on a MRP-rich medium and various other control media

The growth of *E. coli* CIP54.8 on the different culture media is reported in Fig. 1. All the results discussed below are compared to the growth of this strain in the basic d-YET medium. As expected, the addition of a filtered glucose lysine mixture (GL-F) to the YET medium had a stimulating effect on the growth of *E. coli*. However, no significant difference was observed between the two concentrations of GL-F studied (4 and 10 g/L). A very short lag phase was observed indicating that this strain did not need to adapt to this culture medium. The final *E. coli* population was significantly higher compared to that of the d-YET.

Similar results were obtained from assays carried out with filtered glucose added to the YET medium, whereas no variation of growth was observed with added filtered lysine (data not shown). These observations indicate that the addition of easily assimilated carbon sources such as glucose stimulates bacterial growth, especially in media deficient in such nutrients (Prescott et al. 1995). The lack of effect regarding the addition of filtered lysine is explained by the fact that nitrogen sources are present enough in the YET mixture.

The growth curve of bacteria assayed with MRPs at the highest concentration (YET+GL-A) shows a different profile (Fig. 1). As presented in Table 2, there was almost a fourfold increase of the lag phase of growth when bacteria

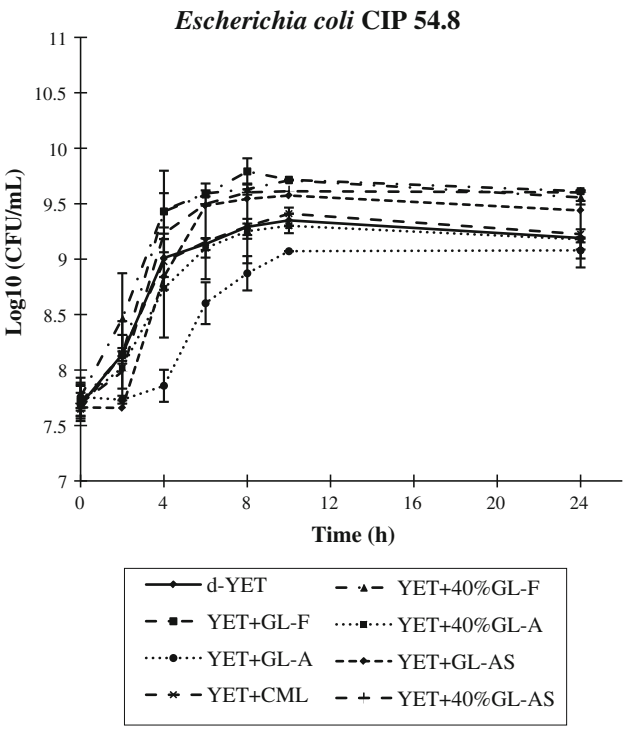

Fig. 1 Growth of *Escherichia coli* CIP 54.8 on various control and MRP-rich media incubated at 37 °C for 24 h

were exposed to the highest level of MRPs compared to those assayed in the d-YET medium. In addition, cell numbers were lower than those of the d-YET medium for the first 10 h of incubation. These results, consistent with those obtained in previous studies (Jemmali 1969; Einarsson et al. 1983; Lanciotti et al. 1999), were not observed when the level of MRPs was lower in the medium (YET + 40 %GL-A).

N^{ϵ}-Carboxymethyllysine (CML) was found to be present in the YET+GL-A medium. For that reason, another culture medium was designed to shed light on the role played by this particular MRP in the inhibition of *E. coli* CIP54.8 growth and division. Free CML was added to the d-YET at the concentration found in the YET+GL-A medium (30 µg/mL). The growth of the strain on the YET + CML medium showed no significant difference from that observed on the basic d-YET medium. It can be concluded, therefore, that while MRPs are responsible for the modifications of bacterial growth, CML does not have its own effect on growth when present as the unique MRP in the medium. The same conclusion was drawn with respect to hydroxymethylfurfural and high molecular weight fractions (MW > 10 kDa) extracted from the GL-A mixture (data not shown).

A further bacterial growth was carried out using a medium containing glucose and lysine autoclaved separately (GL-AS) under the same conditions as those for the GL-A mixture. The main aim of this assay was to observe

Table 2 Growth rate (μ) and lag phase of the 3 strains of *Escherichia coli* on the various culture media tested

	Escherichia coli CIP 54.8		*Escherichia coli* CIP 52.167		*Escherichia coli* from piglet faeces	
	μ (h^{-1})	Lag time (h)	μ (h^{-1})	Lag time (h)	μ (h^{-1})	Lag time (h)
d-YET	1.00	0.96	1.16	2.02	1.33	2.62
YET+GL-F	1.49	1.35	1.31	1.90	1.62	1.82
YET+GL-A	0.86	3.73	1.14	5.08	1.54	3.21
YET+CML	0.96	0.79	1.46	1.89	1.30	2.39

the effects particular to heated glucose and lysine, regardless of the MRPs effects. As seen in Fig. 1, the bacteria grown on the YET+GL-AS medium had a higher growth rate (1.3 h^{-1}), a higher final population, but a longer lag phase compared to those grown on the d-YET medium. When compared to the YET+GL-A medium, the growth rate of bacteria assayed with GL-AS and the final population were higher and the lag phase was shorter. Consequently, it can be deduced that non-Maillard degradation products of glucose and lysine contribute partly to the extension of the lag phase observed in the YET+GL-A medium. The reductions of the growth rate and of the size of the final microbial population, however, appear to be due only to the presence of MRPs. The increase of the lag phase of growth in the YET+GL-AS medium can be attributed to the partial chemical modification of glucose noticeable by the browning of the glucose solution (caramelization) after heating (Manley-Harris and Richards 1996; Arribas et al. 2010).

The impacts of MRPs on bacterial growth: comparison between three *E. coli* strains

The impacts observed on *E. coli* CIP54.8 were compared to those of two other *E. coli* strains. Figure 2 summarises the results obtained for the growth of the three strains on the selected culture media. All three *E. coli* strains grown on the YET+GL-F medium showed improved bacterial cell numbers, boosted growth rates and reduced lag phases when compared to those grown on the d-YET medium.

However, the MRP-rich medium did not cause identical effects on the three studied strains. On the one hand, *Escherichia coli* isolated from piglet faeces was minimally affected by this medium, and its growth was similar to the

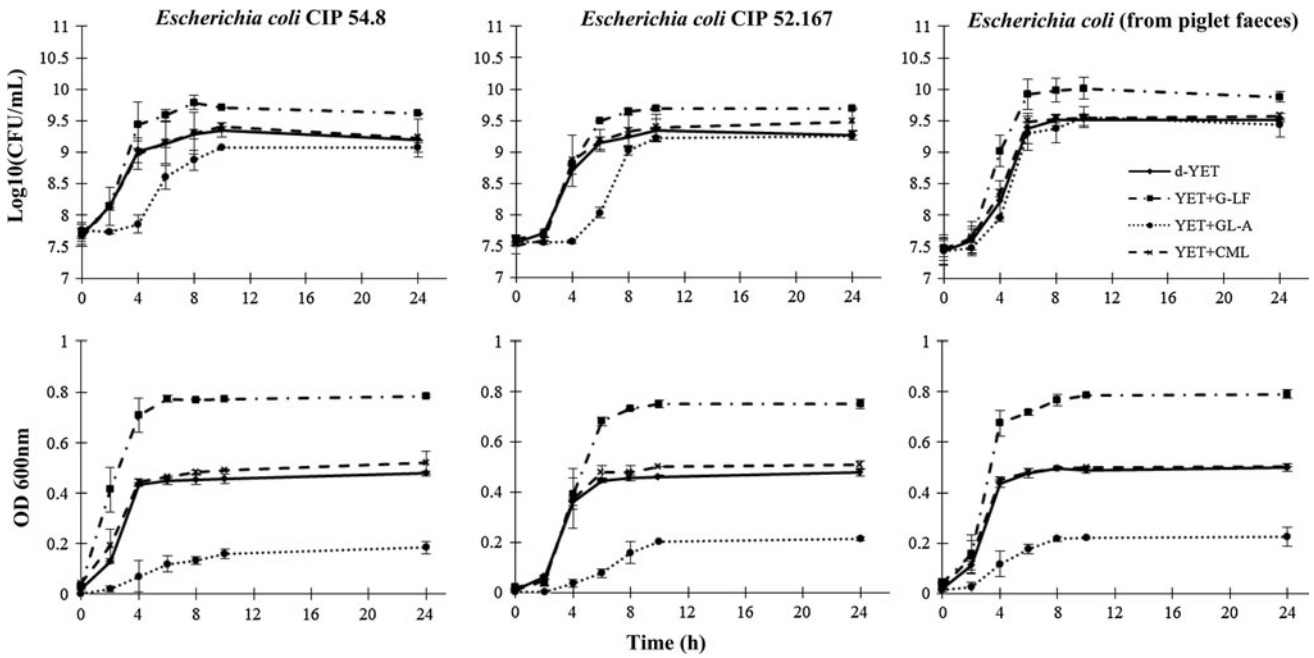

Fig. 2 Growth of *Escherichia coli* CIP 54.8, CIP 52.167 and the strain isolated from piglet faeces on the different culture media. Comparison of two techniques: plate counting and optical density at 600 nm

one observed on the d-YET medium. On the other hand, the two other strains showed reduced growth rates and prolonged lag phases when cultured in the MRP-rich medium (Table 2). In addition, the level of alteration is not identical for both strains. The lack of adverse effect of MRPs on the growth of *E coli* isolated from piglet faeces could be explained by the origin of this strain. In fact, it was isolated from the intestinal microflora of a piglet fed a diet high in MRPs (under publication). In this way, we make the assumption that *E. coli* confronted to MRPs for a few weeks might have adapted to utilise these compounds. In vitro assays with *Salmonella* Typhimurium LT. 2 showed similar adaptation phenomena (Chalova et al. 2011). When subjected to MRPs as a unique carbon source instead of glucose, *Salmonella* Typhimurium showed modifications of transcriptional responses, and MRPs levels decreased in the medium indicating their degradation by the bacteria. The discovery of certain deglycating enzymes secreted by bacteria further sustains this hypothesis (Wiame et al. 2002; Wiame et al. 2005; Monnier 2005). These results support the conclusion that bacterial growth alterations induced by MRPs and measured by the cultivability on agar media are strain-dependent.

The CML enriched medium had no effect on the bacterial growth of all three *E. coli* strains tested when compared to the one measured on the basic d-YET medium. This confirms the previous observation that CML at the concentration found in the YET+GL-A medium is not directly implicated in the MRP-related growth alterations.

OD measurements applied to MRP-rich media

Bacterial growth is frequently determined only by measuring the optical density of the culture. However, in the literature, there are several pieces of evidence suggesting that MRPs affect the cultivability of bacteria, a parameter that cannot be measured by OD. In the current study, two standard bacteriological techniques (i.e. plate counting and optical density measurement at 600 nm) have been used to provide complementary information (Barer 1997). As shown in Fig. 2, the bacterial growth curves vary according to whether the measurement was done by the one or the other method.

For instance, the numbers of bacteria expressed in CFU/mL of the YET+GL-A assays compared to those of the d-YET assays indicate that MRPs influence the lag and exponential growth phases, but not the stationary phase of the growth. However, when the growth is measured by OD, all the growth phases seem to be affected by the presence of MRPs, including the stationary phase. In addition, when the OD values are used to calculate the lag phase duration, its difference increases between the strains cultivated on the YET+GL-A medium and those cultivated on the d-YET medium. Unless careful attention is paid to its meaning, the analysis of growth curves based on OD data might lead to an erroneous conclusion. In fact the difference between the growth curves obtained by the two techniques can be explained by the presence of a dark brown colour in the YET+GL-A medium. The OD of a liquid culture of microorganisms is the combination of light scattering and light absorption (Lin et al. 2010). The dark brown colour of the YET+GL-A medium contributes significantly to the absorbance at 600 nm and interferes with the measurement of the bacterial density. Hence, while OD measurements were always corrected for the background colour of the medium, the values measured were underestimated due to the dark colour. Therefore, despite the fact that OD measurements are time and cost effective, they are not suitable for comparing media of different compositions and colours. In other words, comparing the bacterial growth in MRP-rich media to the growth in other media requires a combination of complementary analyses unaffected by the characteristics of the culture media.

Culture media evolution during incubation

The evolution of the amount of the two substrates of the MRPs, glucose and lysine, and of two MRPs, fructoselysine and CML, was traced in the YET+GL-A medium in the presence or absence of *E. coli* CIP 54.8 (Fig. 3).

In the absence of bacteria, glucose level decreased slightly while lysine and fructoselysine levels remained relatively constant over the duration of the assay. CML level gradually increased during the 24 h incubation. The evolutions of the MRPs and their precursors indicate that, as expected, the Maillard reaction occurs in culture media at 37 °C. The ten-fold difference in concentration between glucose and lysine explains why the level of the latter was apparently stable. As an intermediate of the Maillard reaction, it is not surprising that fructoselysine (measured as furosine) was stable. In addition, CML, a stable end-product of the Maillard reaction, was accumulating during the incubation.

The YET+GL-A medium has changed differently when inoculated with *E. coli* CIP 54.8. The growth profile of *E. coli* on the YET+GL-A medium (Fig. 1) and the evolution of the amount of glucose in this medium (Fig. 3) were inversely proportional. During the lag phase, the amount of glucose remained constant. It started to decrease with the exponential growth phase and was totally consumed after 10 h of incubation. Lysine amount decreased following a similar profile, but traces remained in the medium even after 24 h of incubation. Fructoselysine level decreased slightly and CML level remained relatively stable during incubation, neither increasing nor decreasing.

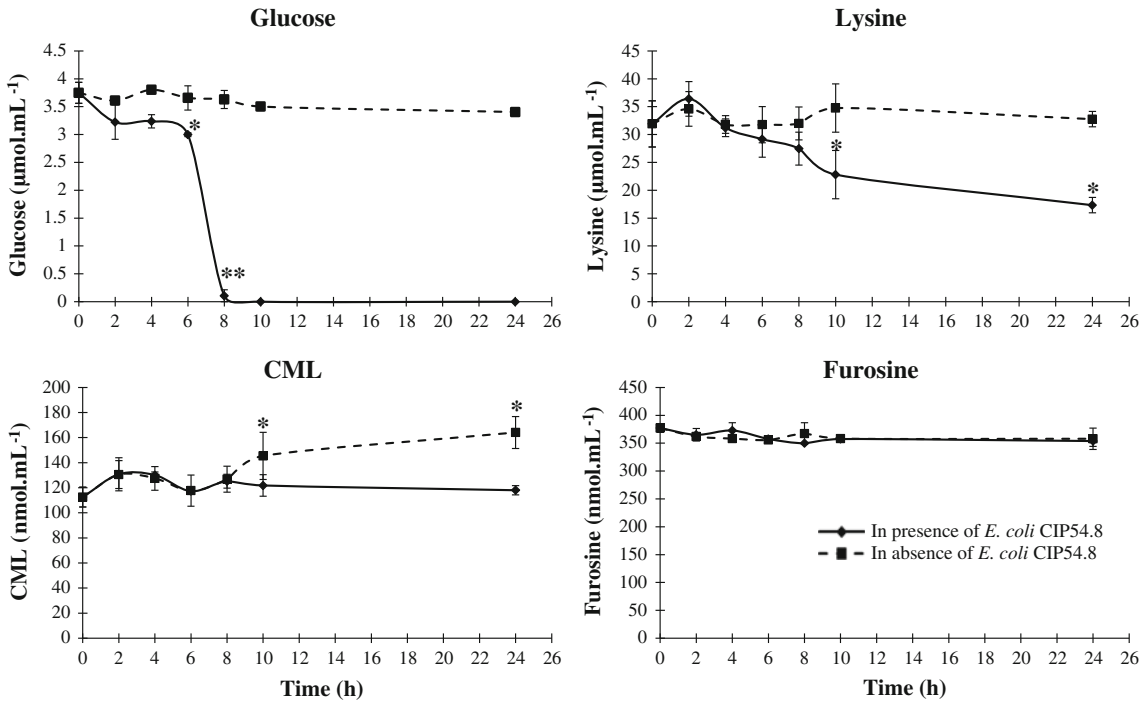

Fig. 3 Evolution of the amounts of glucose, lysine, fructoselysine (measured as furosine) and CML in the YET+GL-A culture medium incubated at 37 °C for 24 h in the presence or in the absence of *Escherichia coli* CIP 54.8. (Values with different *asterisks* in a series indicate a significant difference at the 5 % probability level)

The presence of *E. coli* modified the reaction parameters. Precursors are consumed by the bacteria and, thus, are no longer available in sufficient amounts for the Maillard reaction to take place. Constant amount of CML all through the incubation period indicates that this MRP is not consumed by *E. coli*, thereby reinforcing the observations that CML had no influence on bacterial growth (Fig. 1).

Protein glycation was found to take place within bacterial cells (Mironova et al. 2001). This observation was the motivation for other studies on the potential glycation induced by microorganisms. A recent study on the subject had suggested that an *E. coli* strain was capable of secreting AGEs (Cohen-Or et al. 2011). However, these findings should be handled with care due to the lack of control samples. The monitoring and the subtraction of the fluorescence coming from the culture medium over time should have been performed in this study for a better interpretation of the data.

The metabolic activity of bacteria may contribute to the liberation or modification of precursors that lead to the formation of MRPs in the extracellular environment. However, as revealed by our findings, culture media go through composition changes during incubation, partly due to Maillard reaction between nutrients at 37 °C and should be taken into account before drawing any conclusions.

Conclusion

Maillard reaction products (MRPs) were found to have a strain-dependent influence on bacterial growth. They induced an increase of the lag phase and a decrease of growth rates most likely associated with bacterial adaptation mechanisms. CML, hydroxymethylfurfural and high molecular weight MRPs extracted from the GL-A mixture were not involved in the observed growth alterations. Further studies are needed to identify the MRPs responsible for these growth modifications.

These observations made by plate counting techniques on the lag phase and the growth rate could indicate other effects of the MRPs on the cultivability. OD measures compared to plate counting were supposed to shed light on this issue. However, heat-induced browning interferes with the measurement of absorbance at 600 nm and affects the bacterial count measured by OD in MRP-rich media. Consequently, to produce results truly reliable and interpretable, it is essential to combine complementary methods of growth assessments and analytical methods for the measurements of substrates and reaction products in the culture media. Growth assessment techniques are not alternative, but complementary; they each contribute to a part of the information that should not be interpreted without the remaining parts.

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Conflict of interest The authors declare that they have no conflict of interest.

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