

Effects of Roundup and glyphosate on three food microorganisms: *Geotrichum candidum*, *Lactococcus lactis* subsp. *cremoris* and *Lactobacillus delbrueckii* subsp. *bulgaricus*.

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Abstract

Use of many pesticides poses the problem of their effects on environment and health. Among them, the effects of glyphosate (G) with its adjuvants and its by-products are regularly discussed. The aim of the present study was to shed light on the real impact on biodiversity and ecosystems of Roundup® (R), a major herbicide used worldwide, and the G it contains, by the study of their effects on growth and viability of microbial models, namely on three food microorganisms, *Geotrichum candidum* (Gc), *Lactococcus lactis* subsp. *cremoris* (Ll) and *Lactobacillus delbrueckii* subsp. *Bulgaricus* (Ld), widely used as starters in traditional and industrial dairy technologies.

Presented results evidence that R has an inhibitory effect on microbial growth and a microbicide effect at lower concentrations than those recommended in agriculture. Interestingly, G at these levels has no significant effect on the three studied microorganisms.

Our work is consistent with previous studies which demonstrated that the toxic effect of G was amplified by its formulation adjuvants on different human cells and other eukaryotic models. Moreover, these results should be considered in the understanding of the loss of microbial diversity and microbial concentration observed in raw milk for many years.

Results and discussion

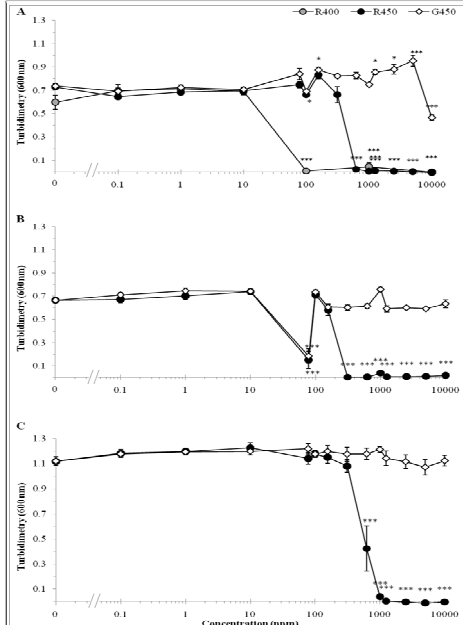
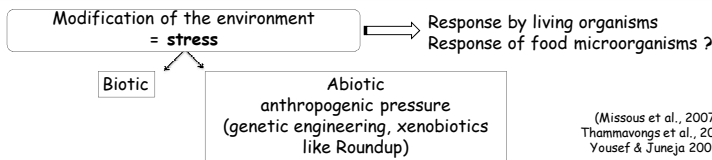


Figure 1: Growth inhibition of three microorganisms (A : Gc, B : Ll and C : Ld) after 24 hours of incubation in growth media supplemented with R (400 and 450) or equivalent amount of G evaluated by turbidimetry (600 nm).

Introduction



(Missous et al., 2007; Thammavongs et al., 2008; Yousef & Juneja 2003)

Uncontrolled microbial diversity => lack of interest in the endangered bacteria hypothesis (Panoff et al., 2002)

R formulations = G (active component) + adjuvants (Cox 2004):

- > Major herbicide used worldwide
- > have negative effects on fauna + flora and are cytotoxic to human cell lines (Relyea 2005; Richard et al., 2005; Benachour et al., 2007; Benachour & Seralini 2009; Gasnier et al., 2009)

Glyphosate :

- > blocks EPSPS (5-enolpyruvylshikimate-3-phosphate synthase), key enzyme involved in aromatic amino acids biosynthesis via shikimic acid pathway in plants (Barry et al., 1992)
- > affects growth + metabolism of *Saccharomyces cerevisiae* (Braconi et al., 2006)

The work described here was carried out on three microorganisms of food interest, to highlight the impact of the R herbicide and of G.

Relatively comparable toxicity profiles on three essential food microorganisms, in a 24 h period (Fig 1A, B and C) while the fungus is phylogenetically far from the two others (Pace 2001). R is always more potent than G, and in all cases, toxic from levels 10-100 times below the lowest agricultural uses (10 000 ppm). R effect was not proportional to G concentration in the R formulation, confirming adjuvants may not be inert (Richard et al., 2005; Benachour & Seralini 2009). Other inhibition pathways must be involved since G is weakly responsible of the cytotoxicity on eukaryotic cells (Richard et al., 2005; Benachour et al., 2007; Benachour & Seralini 2009) and cellular endocrine disruption (Gasnier et al., 2009).

A specific biphasic inhibitory effect was transiently observed at low doses (around 100 ppm) for Ll, and possibly for Gc. At non toxic concentrations, some endocrine biphasic disrupting effects have already been observed in human cells with R (Gasnier et al., 2009).

Materials and methods

Strains and culture conditions

Gc ATCC 204307 in MSF (pH 5.6)
Ld subsp. *bulgaricus* CFL1 in MRS, (pH 6.4)

R and G treatments



diluted in autoclaved culture media adjusted to each medium pH

Ll subsp. *cremoris* ATCC 19257 in M17 containing lactose (pH 7.1)

Turbidimetry and survival measurements

Evaluation of growth/turbidimetry: 600 nm - microplate reader (Metertech S960)



Evaluation of viability: colony counting



Statistical analysis: Data were expressed as mean ± SEM for three independent determinations (n=9). Significant differences were determined by Student t-test with p<0.05 *, p<0.01 ** and p<0.001 ***.

| Strain | R | MIC (ppm) | MMC (ppm) |
|--|-----|-----------|-----------|
| <i>Geotrichum candidum</i> ATCC204307 | 400 | 100 | 1 000 |
| | 450 | 625 | 1 000 |
| <i>Lactococcus lactis</i> subsp. <i>cremoris</i> ATCC19257 | 450 | 312 | 625 |
| <i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i> CFL1 | 450 | 1 000 | 1 250 |

Table 1 : Minimal inhibitory concentration (MIC) and minimal microbicide concentrations (MMC) for three microorganisms (Gc, Ll and Ld) after 24 hours of incubation in growth media supplemented with R400 or R450.

Microbicide effect of R was obtained at concentrations 1.2, 1.6 and 2 times higher than the measured minimal inhibitory concentrations for Ld, Gc and Ll, respectively.

Conclusion

The pesticide Roundup sprayed on Roundup tolerant GMOs and on non agricultural soils could thus impact specific microbial diversity including food interest microorganisms. This is illustrated by the fact that actual food processing requires industrial food starters for milk fermentation. Furthermore, unpredictable consequences of Roundup on soil microorganisms have to be considered.

Acknowledgments

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