Influence of shaking and viable cell numbers on microbial conjugated linoleic acid (CLA) production

Ana L. Fontes[1,2], Lígia L. Pimentel[1,2,3], Ana S. Salsinha[1], Juan M. Rodríguez[4], M. Rosário Domingues[5,6], Luis M. Rodríguez-Alcalá[1] and Ana M. Gomes[1]

[1]*Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal*
[2]*QOPNA – Unidade de Investigação de Química Orgânica, Produtos Naturais e Agroalimentares, Universidade de Aveiro, Aveiro, Portugal*
[3]*CINTESIS – Centro de Investigação em Tecnologias e Sistemas de Informação em Saúde, Faculdade de Medicina da Universidade do Porto, Porto, Portugal*
[4]Departamento de Nutrición, Bromatología y Tecnología de los Alimentos, Facultad de Veterinaria, Universidad Complutense de Madrid, Madrid, Spain
[5]Mass Spectrometry Centre, Department of Chemistry & QOPNA, University of Aveiro, Campus Universitário de Santiago Aveiro, Portugal

CATÓLICA ESCOLA SUPERIOR DE BIOTECNOLOGIA PORTO

FEMS 2019 8th Congress of European Microbiologists 7-11 July 2019 | Glasgow, Scotland

Introduction

Conjugated linoleic acid (CLA) isomers have been attracting much scientific attention due to their potential bioactive properties[1]. These compounds are naturally produced during the biohydrogenation process of dietary linoleic acid (LA) by ruminal bacteria, however, several probiotic strains isolated from human intestine and dairy products have also revealed the capacity to produce those bioactive fatty acids[2,3]. Some studies have reported that the amount of CLA produced is strain-dependent and may also be influenced by cultivation conditions[4,5]. *In vitro* studies usually test CLA production by growing strains in culture medium containing a specific LA concentration[6], but few have applied shaking during incubation. Shaking may improve access to substrate and result in better CLA yields, therefore, this work aimed to study the influence of shaking on CLA production by strains previously identified as CLA-producers.

Methods

Bifidobacterium breve DSM 20091
Lactobacillus gasseri VR-III-51
Lactobacillus plantarum DSM 20205
Lactobacillus sakei DSM 20017

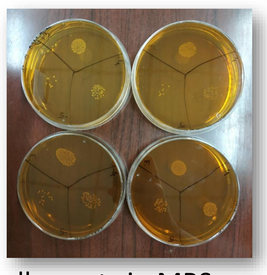
1% (v/v)

MRS + 0,5 mg/mL LA

37 ºC; 24, 48 and 72 h; 150 rpm

37 ºC; 24, 48 and 72 h; no shaking

Serial dilutions



Viable cell counts in MRS agar plates

Supernatant treatement according to Pimentel *et al.*[7]

Fatty acid analysis through GC-FID

Results

Shaking reduced LA conversion rates for all strains except for *L. gasseri* VR-III-51 (Figure 1).

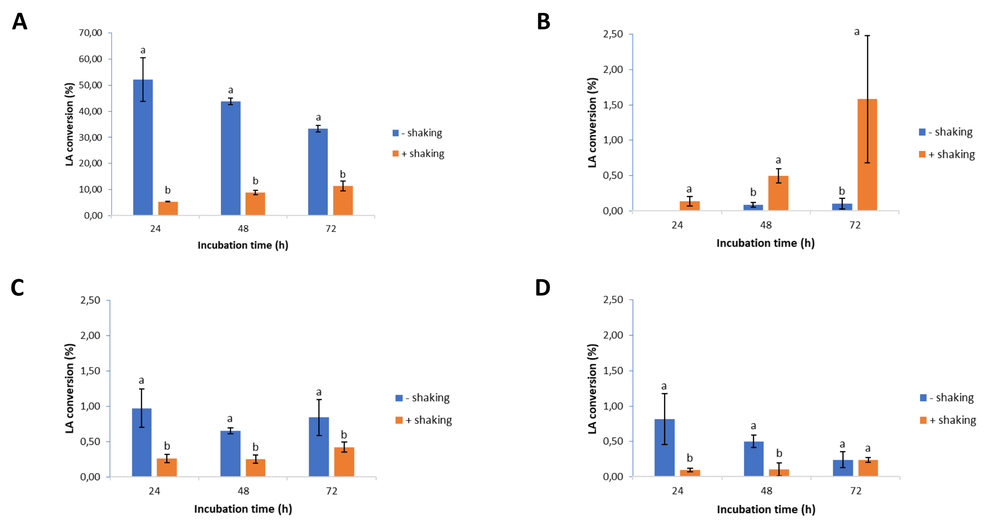


Figure 1. LA conversion rates of *B. breve* DSM 20091 (A), *L. gasseri* VR-III-51 (B), *L. plantarum* DSM 20205 (C) and *L. sakei* DSM 20017 (D) with (orange) and without (blue) shaking. Different superscript letters for significant differences (p<0,05) between shaking condition.

In what concerns correlation between viable cell numbers (data not shown) at the end of each incubation period and LA conversion rates, it was observed a negative correlation for *L. gasseri* VR-III-51 with shaking, a positive correlation for *B. breve* DSM 20091 without shaking (Figure 2) and no correlation for *L. plantarum* 20205 and *L. sakei* DSM 20017, either with or without shaking.

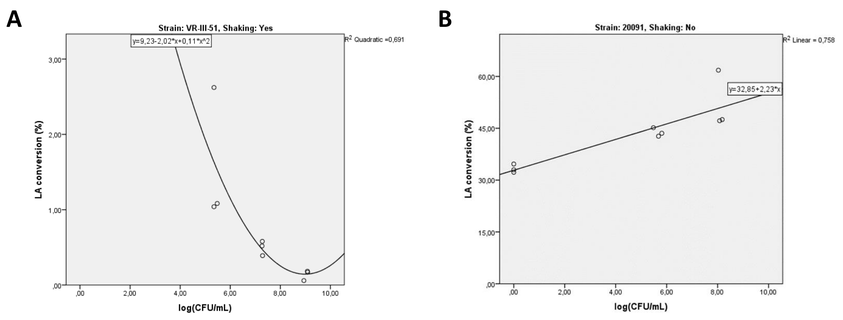


Figure 2. Correlation between LA conversion rate and viable cell numbers of *L. gasseri* VR-III-51 with shaking (A) and *B. breve* DSM 20091 without shaking (B). P<0,05 according to Kendall correlation test.

Conclusions

In conclusion, shaking and viable cell numbers (in agreement of incubation time) affect microbial CLA production in a strain-dependent manner.

References

1. Yang B, Chen H, Stanton C, et al. Review of the roles of conjugated linoleic acid in health and disease. *J Funct Foods*. 2015;15:314-325.
2. Shokryzadan P, Rajion MA, Meng GY, et al. Conjugated linoleic acid: a potent fatty acid linked to animal and human health. *Crit Rev Food Sci Nutr*. 2017;57(13):2737-2748.
3. Fontes AL, Pimentel LL, Simões CD, Gomes AMP, Rodríguez-Alcalá LM. Evidences and perspectives in the utilization of CLNA isomers as bioactive compounds in foods. *Crit Rev Food Sci Nutr*. 2017;57(12):2611-2622.
4. Renes E, Linares DM, González L, Fresno JM, Tornadijo ME, Stanton C. Production of conjugated linoleic acid and gamma-aminobutyric acid by autochthonous lactic acid bacteria and detection of the genes involved. *J Funct Foods*. 2017;34:340-346.
5. Terán V, Pizarro PL, Zacarías MF, Vinderola G, Medina R, Van Nieuwenhove C. Production of conjugated dienoic and trienoic fatty acids by lactic acid bacteria and bifidobacteria. *J Funct Foods*. 2015;19:417-425.
6. Yang B, Gao H, Stanton C, et al. Bacterial conjugated linoleic acid production and their applications. *Prog Lipid Res*. 2017;68:26-36.
7. Pimentel LL, Fontes AL, Gomes AM, Rodríguez-Alcalá LM. Considerations about the in situ derivatization and fractionation of EFA and NEFA in biological and food samples. *MethodsX*. 2015;2:475-484.

Acknowledgements

This work was supported by National Funds from FCT – Fundação para a Ciência e Tecnologia through project **"Pro-TECh-CLnA - Microbial Production of Bioactive Conjugated Linolenic Acid Isomers to Obtain Functional Ingredients and Foods"** reference PTDC/AGR-TEC/2125/2014. We would also like to thank the scientific collaboration under the FCT project UID/Multi/50016/2019. Financial support for the authors A.L. Fontes and L.L. Pimentel was provided by fellowships SFRH/BD/117721/2016 and SFRH/BPD/119785/2016, respectively, granted by the Portuguese government through FCT.

FCT Fundação para a Ciência e a Tecnologia

REPÚBLICA PORTUGUESA