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ALDEIR SABINO DOS SANTOS

INFLUÊNCIA DE QUERCETINA E RESVERATROL SOBRE PROPRIEDADES *in vitro* RELACIONADAS À FUNCIONALIDADE DE CEPAS DE *Lactobacillus* POTENCIALMENTE PROBIÓTICAS

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À minha família e amigos,

Dedico.

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RESUMO

Polifenóis são amplamente distribuídos em frutas, legumes, ervas, sementes, cereais e bebidas. Dentre os polifenóis, a quercetina (QUE) e o resveratrol (RES) têm recebido interesse, devido à associação de sua ingestão com uma variedade de benefícios para a saúde. QUE é um dos maiores representantes da classe dos flavonóides, encontrado naturalmente em maçãs e no vinho tinto, enquanto RES é o principal representante da classe dos estilbenos, naturalmente presente na casca e nas sementes de uvas, e no vinho. Porém, os benefícios da ingestão de polifenóis estão relacionados à sua biodisponibilidade, que geralmente é muito baixa. Assim, os compostos atingem o intestino grosso, onde são metabolizados por meio da ação das bactérias presentes na microbiota intestinal. Dentre as bactérias potencialmente capazes de metabolizar polifenóis, estão os probióticos. A capacidade de probióticos exercerem efeitos benéficos a saúde está relacionada a uma série de pré-requisitos *in vitro*, os quais inclui tolerância a diferentes valores de pH e a sais biliares, hidrofobicidade celular de superfície, autoagregação, coagregação com patógenos, atividade antagonista contra patógenos e capacidade de sobrevivência às condições gastrintestinais. Assim, estudos têm sugerido que a ingestão combinada de polifenóis e probióticos pode ser uma estratégia eficaz para aumentar suas funcionalidades biológicas, porém os compostos fenólicos podem exercer influências variadas sobre características específicas de microrganismos probióticos, o que revela a necessidade de estudos que avaliem essas potenciais influências que os polifenóis podem exercer sobre probióticos. O presente estudo avaliou os efeitos de QUE e RES sobre propriedades *in vitro* relacionadas à funcionalidade de cepas de *Lactobacillus* potencialmente probióticas (*L. plantarum* 49, *L. plantarum* 53, *L. paracasei* 106, *L. paracasei* 108, *L. fermentum* 263 e *L. fermentum* 296). QUE e RES mostraram baixo efeito inibitório sobre o crescimento das cepas de *Lactobacillus* testadas, com concentração inibitória mínima (CIM) de 512 - >1024 μg/mL. Na maioria dos casos, todas as concentrações testadas de QUE e RES (CIM, 1/2 CIM e 1/4 CIM) não afetaram a tolerância das cepas de *Lactobacillus* a pH ácido e sais biliares. A QUE aumentou a hidrofobicidade da superfície celular da maioria das cepas de *Lactobacillus* testadas, enquanto aumentos ou diminuições nesta propriedade variaram entre algumas destas cepas na presença de diferentes concentrações de RES. QUE e RES aumentaram a capacidade agregação e coagregação das cepas de *Lactobacillus* testadas, com *Listeria monocytogenes* e *Escherichia coli*. Estes compostos não afetaram negativamente a atividade antagonista das cepas de *Lactobacillus* contra patógenos, bem como não diminuíram sua sobrevivência quando expostos a digestão *in vitro*. Em alguns casos, a capacidade de algumas das cepas de *Lactobacillus* para antagonizar patógenos, bem como para sobreviver a etapas específicas da digestão *in vitro*, foi aumentada na presença de QUE ou RES. Esses resultados demonstram que o uso combinado de QUE ou RES com cepas probióticas de *Lactobacillus* pode melhorar as propriedades funcionais exercidas por essas bactérias no hospedeiro; no entanto, a concentração desses compostos, bem como as cepas selecionadas devem ser cuidadosamente consideradas para alcançar esses efeitos desejáveis sobre o hospedeiro.

Palavras-chave: Polifenóis, Microbiota Intestinal, Probióticos, Flavonoides, Estilbenos.

ABSTRACT

Polyphenols are extensively distributed in fruits, vegetables, herbs, seeds, cereals and beverages. Among the polyphenols present in foods, quercetin (QUE) and resveratrol (RES) have received increased interest because of the strong evidence of the association of their intake with a variety of health benefits. QUE is one of the largest representatives of the class of flavonoids found naturally in apples and red wine, while RES is the main representative of the class of stilbenes, naturally present in bark and grape seeds, and in wine. However, the benefits of ingestion of polyphenols are related to their bioavailability, which is usually very low. Thus, the compounds reach the large intestine, where they are metabolized through the action of the bacteria present in the intestinal microbiota. Among these bacteria potentially capable of metabolizing polyphenols are probiotics. The potential ability of probiotics to exert health benefits on the host has been commonly associated with the compliance of *in vitro* pre-requisites that include a set of physiological functionalities (e.g., acid and bile salts tolerance, cell surface hydrophobicity, autoaggregation, coaggregation with pathogens and antagonistic activity against pathogens) and capability of surviving during exposure to gastrointestinal conditions. Studies have suggested that the combined intake of polyphenols and probiotics may be an effective strategy to increase their biological functionalities, but phenolic compounds may exert varied influences on specific characteristics of probiotic, which reveals the need for further studies evaluating these potential influences that polyphenols may exert on probiotics. This study assessed the effects of polyphenols QUE and RES on the growth and some *in vitro* functionality-related properties of six proven potentially probiotic *Lactobacillus* strains (*L. plantarum* 49, *L. plantarum* 53, *L. paracasei* 106, *L. paracasei* 108, *L. fermentum* 263 and *L. fermentum* 296). QUE and RES showed weak inhibitory effects on the growth of the tested *Lactobacillus* strain, with minimum inhibitory concentration (MIC) as high as 512 – >1024 μg/mL. In most cases, QUE and RES at all tested concentrations (i.e., MIC, 1/2 MIC and 1/4 MIC) did not affect the tolerance of the *Lactobacillus* strains to acidic pH and bile salts. QUE increased the cell surface hydrophobicity of most of the tested *Lactobacillus* strains, while increases or decrease in this property varied among some of these strains in the presence of different RES concentrations. QUE and RES increased the ability of the tested *Lactobacillus* strains to aggregate and coaggregate with *Listeria monocytogenes* and *Escherichia coli*. These compounds did not affect negatively the antagonistic activity of the tested *Lactobacillus* strains against pathogens, as well as did not decrease their survival when exposed to an *in vitro* digestion. In few cases, the ability of some of the tested *Lactobacillus* strains to antagonize pathogens as well as to survive to specific steps of the *in vitro* digestion were increased by QUE or RES. The combined use of QUE or RES with probiotic lactobacilli could improve functional properties exerted by these bacteria on the host; however, the concentration of these compounds should be careful considered to reach these desirable effects.

Keywords: Polyphenols, Gut Microbiota, Probiotics, Flavonoids, Stilbenes.

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LISTA DE ABREVIATURAS E SIGLAS

ATCC	American Type Culture Collection
BHI	Brain Heart Infusion
CFU	Colony Forming Unit
CIM	Concentração Inibitória Mínima
CLSI	Clinical and Laboratory Standards Institute
DO	Densidade Óptica
DMSO	Dimetilsulfóxido
FAO	Food and Drug Administration
GRAS	Generally Recognized as Safe
LAB	Lactic Acid Bacteria
MIC	Minimum Inhibitory Concentration
MRS	Man, Rogosa & Sharpe
OD	Optical density
PBS	Phosphate Buffered Saline
QPS	Qualified Presumedly as Segure
QUE	Quercetina
RES	Resveratrol
UFC	Unidade Formadora de Colônia
WHO	World Health Organization

SUMÁRIO

1 INTRODUÇÃO

Polifenóis são metabólitos presentes em frutas, legumes, ervas, sementes e cereais, bem como em bebidas, tais como café, chá e vinho (YI et al., 2017). Dentre esses compostos, quercetina (QUE) e resveratrol (RES) ganham destaque devido evidências da relação da sua ingestão com benefícios a saúde. A QUE é um dos principais representantes da classe dos flavonóides, naturalmente encontrada em maçãs e vinho tinto, enquanto o RES é o principal representante da classe dos estilbenos, encontrado naturalmente na casca e sementes da uva e no vinho (ALVES et al., 2016)

Os efeitos benéficos dos polifenóis (propriedades antioxidantes e anti-inflamatórias) estão relacionados principalmente à presença de uma estrutura aromática e múltiplos grupos hidroxila capazes de doar elétrons ou átomos de hidrogênio a radicais livres e espécies reativas de oxigênio (ESPINOSA et al., 2015; FARHADI et al., 2016). A presença de grupos hidroxila, também é relacionada com as propriedades antimicrobianas referidas destes compostos (SINGH et al., 2019).

Compostos fenólicos podem exercer benefícios à saúde em nível local quando agem diretamente durante a passagem pelo trato gastrointestinal, bem como em nível sistêmico após sua absorção (GELEN et al., 2017; SINHA et al., 2016). Embora possuam essa importante influência sobre a saúde, tais compostos possuem digestão e absorção limitadas (CUEVA et al., 2017). Por outro lado, sabe-se que o metabolismo de polifenóis por microrganismos específicos que compõem a microbiota intestinal está relacionado ao aumento da sua biodisponibilidade, e, consequentemente, das suas funções no organismo (LLANO et al., 2016; MORENO-INDIAS et al., 2016).

Dentre os microrganismos potencialmente capazes de metabolizar compostos fenólicos estão os probióticos, definidos como microrganismos vivos que, quando administrados em quantidades adequadas, conferem benefícios para a saúde (FAO, 2006). A ingestão desses microrganismos, também está relacionada a vários efeitos benéficos a saúde, como no tratamento da obesidade, na prevenção do desenvolvimento da aterosclerose e diminuição do estresse oxidativo (ALARD et al., 2016; BRON et al., 2017; ROBLES-VERA et al., 2018). Os probióticos são potencialmente capazes de expressar diversas enzimas como β-glucosidase, β-galactosidase e α-rhamnosidase, que estão envolvidas na metabolização de compostos fenólicos (LACEY et al., 2014a; LLANO et al., 2017).

A partir disso, tem sido proposto que a ingestão combinada de polifenóis e probióticos pode ser uma estratégia eficaz para aumentar concomitantemente as suas funcionalidades

biológicas, quando utilizadas na formulação de alimentos ou nutracêuticos (SOUZA et al., 2018; WESTFALL et al., 2017).

Dentre as bactérias ácido-láticas, as espécies do gênero *Lactobacillus* têm sido intensamente estudadas quanto ao seu potencial probiótico (ALBUQUERQUE et al., 2017; GHARBI et al., 2019) e mais recentemente por sua capacidade de metabolizar polifenóis (MUELLER et al., 2018). A capacidade dos probióticos exercerem benefícios à saúde do hospedeiro tem sido associada ao atendimento de alguns pré-requisitos *in vitro* que incluem um conjunto de funcionalidades fisiológicas, como tolerância a ambientes ácidos e presença de sais biliares, hidrofobicidade da superfície celular, autoagregação, coagregação com patógenos e atividade antagônica contra patógenos, bem como capacidade de sobreviver durante a exposição às condições gastrointestinais (ABUSHELAIBI et al., 2017; ALBUQUERQUE et al., 2017). Isso revela que impactos no crescimento e/ou nas funcionalidades biológicas *in vitro* de probióticos resultantes das interações desses microrganismos com um ou mais compostos no mesmo ambiente, podem indicar um potencial fator de influência para alcançar os efeitos benéficos desejados à saúde do hospedeiro.

Pesquisas sobre a interferência dos polifenóis sobre bactérias probióticas são escassas, de modo que apenas poucos estudos disponíveis na literatura avaliaram a influência de alimentos ou bebidas ricas em polifenóis, ou polifenóis individuais sobre o crescimento e/ou algumas funcionalidades relacionadas de bactérias ácido-láticas probióticas, incluindo espécies de *Lactobacillus*. Ainda assim, esses estudos têm encontrado resultados variados (CHAN et al., 2018; LLANO et al., 2017; PACHECO-ORDAZ et al., 2018). No entanto, não há na literatura pesquisas que investigaram os efeitos de QUE e RES sobre diferentes propriedades relacionadas às funcionalidades de cepas de *Lactobacillus* potencialmente probióticas.

A partir desses aspectos, o presente estudo avaliou a influência de diferentes concentrações de QUE e RES sobre o crescimento e capacidade de tolerar diferentes valores de pH e sais biliares de seis cepas de *Lactobacillus* potencialmente probióticas. Os efeitos de diferentes concentrações de QUE e RES sobre as propriedades de autoagregação e coagregação destas cepas, bem como sobre sua capacidade de exercer atividade antagônica contra patógenos e tolerar a exposição às condições gastrointestinais simuladas também foram avaliados.

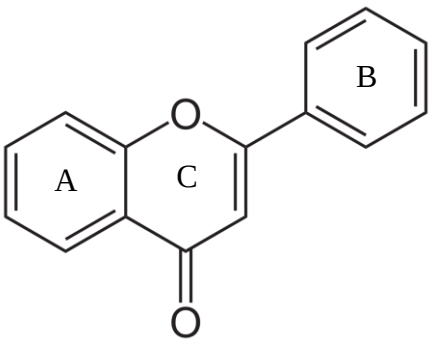
2 REVISÃO DA LITERATURA

2.1 EFEITOS BENÉFICOS DOS POLIFENÓIS

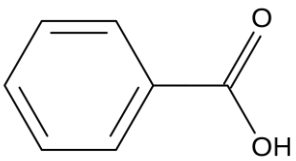
Cerca de 8000 compostos que ocorrem naturalmente em plantas são fenóis, os quais são encontrados, principalmente, em suas formas ligadas como glicosídeos ou ésteres, e uma parte menor na forma livre (SILVA; BARREIRA; OLIVEIRA, 2016). Os polifenóis são constituídos quimicamente por um grupo hidroxila ligado a um anel aromático (HARNLY; BHAGWAT; LIN, 2007). Apesar de apresentar o mesmo esqueleto básico, a posição e o número de grupos hidroxila do fenol apresentam forte influência nas suas propriedades biológicas (GRANATO et al., 2016; SILVA; BARREIRA; OLIVEIRA, 2016).

O *Phenol-Explorer*, um banco de dados de polifenóis, classifica 501 polifenóis em seis classes (flavonóides, lignanas, metabólitos não fenólicos, outros polifenóis, ácidos fenólicos e stilbenos) e 31 sub-classes diferentes, de acordo com sua estrutura química, sendo, geralmente, agrupados em flavonóides e não flavonoides (NEVEU et al., 2010). Os polifenóis do tipo flavonóide têm uma estrutura primária de dois anéis de benzeno (A e B), conectados por um anel C de pirona heterogêneo (Figura 1). Os fenólicos do tipo não-flavonóides formam um grupo mais diversificado, que inclui desde compostos dos ácidos benzoicos mais simples aos mais complexos, tais como estilbenos, lignanas e galotaninas, taninos hidrolisáveis e elagitaninas (OZDAL et al., 2016).

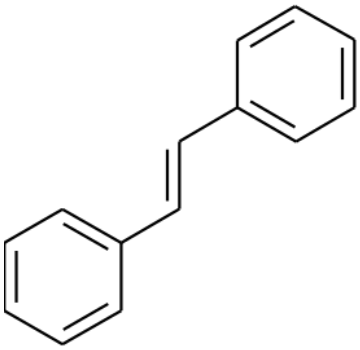
Figura 1. Estrutura básica de compostos fenólicos flavonoides e não-flavonóides.



Flavonóide



Ácido benzóico



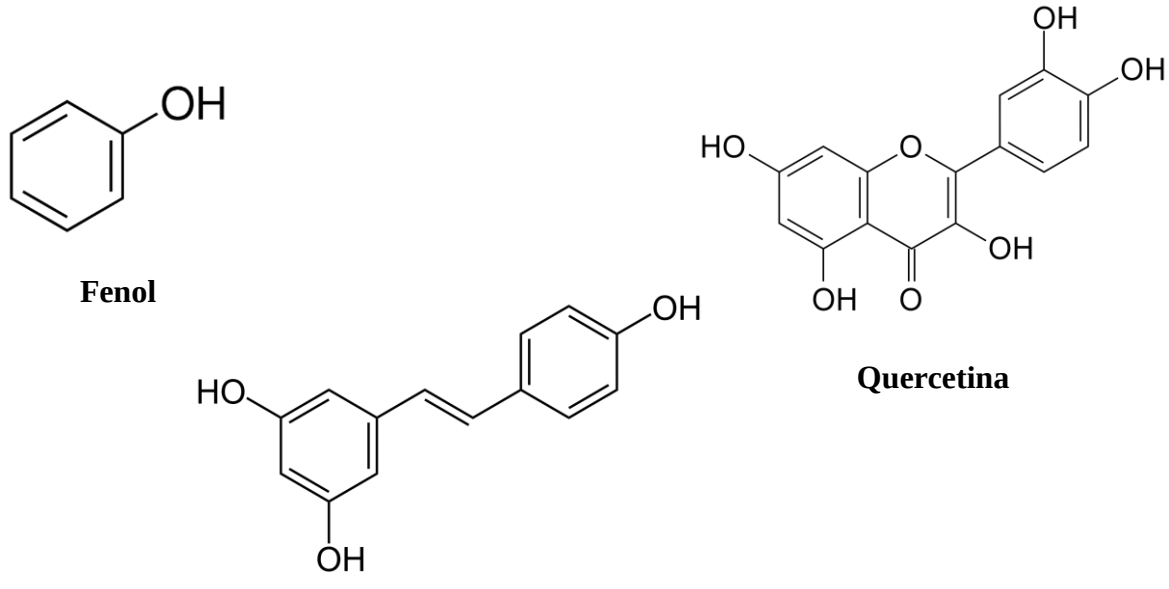
Estilbeno

Fonte: Banerjee; Dhar (2018).

Os polifenóis são constituintes naturais da dieta humana, pois estão presentes em quantidades abundantes em frutas, legumes, ervas, sementes, cereais e em bebidas, como café, chá e vinho. Seus efeitos benéficos à saúde dos consumidores são primariamente relacionados à sua ação antioxidante, não somente pela sua habilidade em doar hidrogênio ou elétrons, mas também em virtude de seus radicais intermediários estáveis. Esses radicais impedem a oxidação de várias moléculas, particularmente, de lipídeos, bem como podem inibir diferentes moléculas pró-inflamatórias, tais como glutathione S-transferases, citocinas TNF-alfa e quimiocina IL-8 (FARHADI et al., 2016; CRASCÌ et al., 2018). A partir disso, estudos têm demonstrado que os benefícios da ingestão de polifenóis podem ocorrer de forma local e sistêmica, atuando na modulação da função imunológica, na prevenção de alguns tipos de câncer, diabetes, doenças inflamatórias e distúrbios metabólicos (DOLINSKY et al., 2013; CUEVA et al., 2017; GELEN et al., 2017).

Dentre a grande variedade de polifenóis presente nos alimentos, QUE e RES (Figura 2) ganham destaque devido à associação da ingestão de alimentos ricos nesses polifenóis com benefícios à saúde. A QUE [2- (3,4-di-hidroxifenil) -3,5,7-tri-hidroxi-4H-cromen-4ona] é um dos principais representantes da classe dos flavonóides (ALVES et al., 2016). Sua ingestão está relacionada com a prevenção e tratamento de doenças crônicas não-transmissíveis, como obesidade, hipertensão (GELEN et al., 2017) e câncer (WU et al., 2018).

Figura 2. Estrutura química de compostos fenólicos.



Fonte: Souza et al. (2018).

O RES (3,5,4'-tri-hidroxi-trans-estilbeno) é o principal representante da classe dos estilbenos. Estudos apontam que a ingestão de RES está associada com a prevenção e tratamento de doenças como hipertensão (DOLINSKY et al., 2013), doença de Alzheimer (PASINETTI et al., 2015) e câncer (SINHA et al., 2016). Propriedades antiviral, antibacteriana e anti-inflamatória desses compostos também são amplamente citadas na literatura (RODRÍGUEZ-PÉREZ et al., 2016; KUMAR; VIJAYALAKSHMI; NADANASABAPATHI, 2017; SINGH et al., 2019).

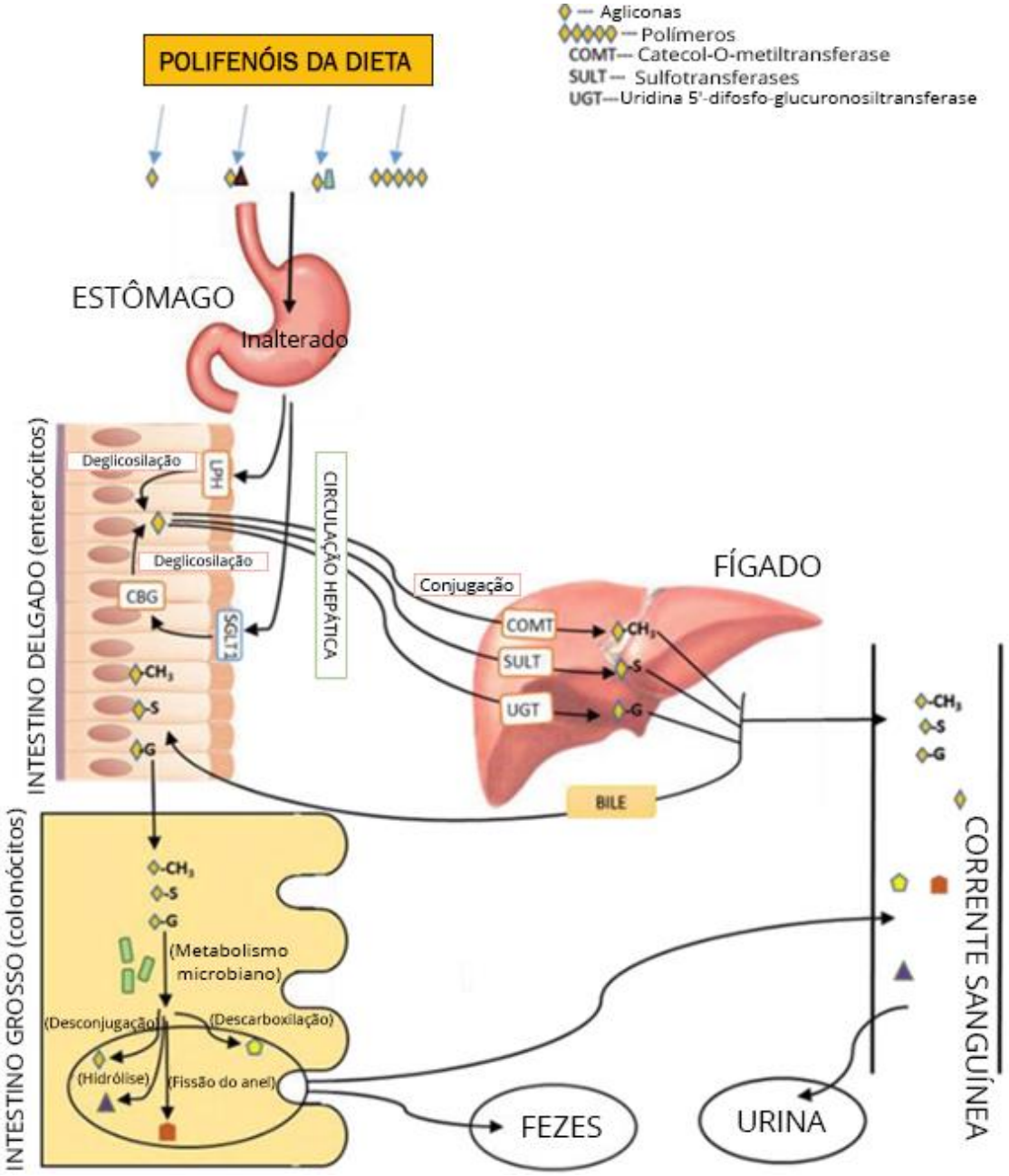
Porém, os benefícios da ingestão de polifenóis na saúde humana dependem de sua biodisponibilidade, absorção e metabolismo, visto que esses compostos, geralmente, são altamente polares para serem absorvidos através da difusão passiva por enterócitos no intestino delgado (ROSSI et al., 2013). Estima-se, que apenas 5 a 10% da ingestão total de polifenóis podem ser absorvidos no intestino delgado, enquanto que 90 a 95% atingem o cólon (PEREIRA-CARO et al., 2015; LLANO et al., 2016). Os compostos que atingem o intestino grosso são metabolizados por meio da ação das bactérias presentes na microbiota intestinal (Figura 3), sendo que essa capacidade de metabolização é específica da espécie e das cepas microbianas presentes nesse ambiente, e está relacionada à sua capacidade de produzir diferentes enzimas, tais como β-glucuronidases, sulfatases e glucosidases (POSSEMIERS et al., 2011; YUKSEKDAG et al., 2017). De forma geral, essa metabolização ocorre com clivagem do anel A e C, clivagem do anel C e desidroxilações e hidrogenações de porções de alceno, em polifenóis como RES (STEVENS; MAIER, 2016).

Flavonóis, como a QUE, são hidrolisados extensivamente em seus produtos derivados pela microbiota intestinal nos anéis A e B como resultado da clivagem do anel C. Esta clivagem resulta na formação de ácido 2- (3,4-dihidroxifenil) acético, ácido 2- (3-hidroxifenil) acético e ácido 3,4-di-hidroxibenzóico a partir do anel B, enquanto que o floroglucinol, 3- (3, 4-di-hidroxifenil) propiónico e ácido 3- (3-hidroxifenil) propiônico são formados a partir do anel A. A reação é mediada por quercetina-dioxigenases produzidas pela microbiota intestinal, também referidas como quercetinases. A partir disso, os metabólitos entram na via catabólica de ácidos fenilo e benzóico para gerar ácido protocatequico e ácido 2- (3,4-di-hidroxi) –fenilacético como os principais metabolitos. (STEVENS; MAIER, 2016; CUEVA et al., 2017).

As informações sobre o metabolismo do RES são mais escassas, porém um estudo observou que, além de dehidroresveratrol, dois outros metabolitos são provenientes do metabolismo microbiano do *trans*-resveratrol: 3,4-dehidroxi-trans-estilbeno e 3,4-dehidroxibibenzil (lunularina) (BODE et al., 2013). Após metabolização do polifenois, os

produtos derivados, gerados a partir da ação microbiana, podem ser absorvidos no intestino grosso e depois metabolizados no fígado pelas enzimas da fase II, como glutationa S-transferases, em metabolitos conjugados (glucurônidos e sulfatos), os quais podem ser distribuídos aos tecidos (CUEVA et al., 2017; BANERJEE; DHAR, 2018).

Figura 3. Rotas metabólicas para polifenóis e seus metabólitos em humanos



Fonte: Banerjee; Dhar (2018).

2.2 PROPRIEDADES DE BACTÉRIAS PROBIÓTICAS

Bactérias láticas são componentes naturais da microbiota intestinal e, em sua maioria, reconhecidas como seguras (*generally recognized as safe* - GRAS) ou presumidamente qualificadas como seguras (*qualified presumption of safe* – QPS) (ZHANG et al., 2013; GARCIA et al., 2016). Essas bactérias estão amplamente distribuídas na natureza e constituem um grupo composto por 13 gêneros distintos (ONGOL, 2012). Podem ser classificadas, com relação ao seu metabolismo em homofermentativas, quando produzem ácido lático como o principal ou único produto (*Lactococcus, Pediococccus, Enterococccus*, *Streptococcus* e alguns *Lactobacillus*); ou heterofermetativas, quando produzem quantidades similares de ácido lático, dióxido de carbono e etanol, além de compostos de sabor e aroma, como acetaldeído e diacetileno (*Leuconostoc, Weissela* e alguns *Lactobacillus*) (JAY; LOESSNER; GOLDEN, 2005).

Lactobacillus é um gênero de bactérias anaeróbicas ou microaerófilas, Gram-positivas, em forma de bastonete, no qual está inserida uma considerável parcela dos probióticos conhecidos atualmente (AOUDIA et al., 2016). Os probióticos têm sido amplamente reconhecidos devido aos seus benefícios a saúde, existindo um número cada vez maior de produtos probióticos disponíveis para os consumidores, os quais incluem, principalmente, iogurte e outros tipos de produtos lácteos fermentados, como suplementos alimentares (ALVES et al., 2016; GARCIA et al., 2016; ROBLES-VERA et al., 2017).

Inicialmente, o aumento do conhecimento sobre a ação dos probióticos resultou no surgimento de um importante segmento no mercado de alimentos e suplementos probióticos (ZOUMPOPOULOU et al., 2017). A ingestão de probióticos é considerada como parte de uma alimentação saudável, pois seu consumo tem sido associado a uma variedade de benefícios para a saúde humana, tais como auxílio na prevenção e tratamento da obesidade (MAZLOOM; SIDDIQI; COVASA, 2019); câncer (SHARMA, 2019); diabetes (RAZMPOOSH et al., 2016); síndrome metabólica (WANG et al., 2015) e doenças ósseas (RIZZOLI, 2019).

Os probióticos apresentam ainda propriedades imunoprotetoras (VITETTA; VITETTA; HALL, 2018), neuroprotetoras (WESTFALL et al., 2017) e anti-inflamatórias (PLAZA-DÍAZ et al., 2017), além de auxiliar na melhoria da intolerância à lactose (OAK; JHA, 2018), diminuição dos níveis de colesterol (MAJEED et al, 2019), bem como repercutem em benefícios na saúde oral (TESTER; AL-GHAZZEWI, 2018) e intestinal (BRON et al., 2017).

Os benefícios atribuídos à ingestão de probióticos são relacionados, principalmente, a sua capacidade de modular a composição da microbiota intestinal, bem como a exclusão competitiva de patógenos; modulação de atividades enzimáticas relacionadas à metabolização de carcinógenos e outras substâncias tóxicas; e produção de ácidos graxos voláteis, os quais desempenham importante papel na manutenção da homeostase energética e na regulação da funcionalidade em tecidos periféricos (PLAZA-DIAZ et al., 2019).

Para ser considerado um probiótico, os microrganismos devem atender alguns pré-requisitos relacionados à aspectos de funcionalidade, segurança e propriedades tecnológicas (CASAROTTI et al., 2017; YERLIKAYA, 2019). Os aspectos de funcionalidade das cepas probióticas estão relacionados ao papel que irão desempenhar no trato gastrointestinal humano após a sua ingestão, processo que se inicia na boca, continua no estômago, intestino delgado, sendo finalizado no cólon (FAO/WHO, 2006; MAGALHÃES et al., 2018.). A acidez gástrica é considerada o principal mecanismo de defesa contra microrganismos, pois estes agentes precisam suportar o efeito de aproximadamente 2,5 litros de suco gástrico secretados diariamente no trato digestivo humano. Os sais biliares secretados no intestino delgado também representam um desafio para a sobrevivência bacteriana. Para exercer seus efeitos no organismo hospedeiro, é interessante que cepas probióticas possuam a habilidade de sobreviver em ambientes ácidos e quando expostos a sais biliares (SHEHATA et al., 2016). Além de sobreviver, algumas cepas probióticas possuem a capacidade para desconjugar os sais biliares, que é relacionada a redução dos níveis séricos de colesterol no hospedeiro (CHOI; CHANG, 2015).

As propriedades de hidrofobicidade da superfície celular e capacidade de agregação estão relacionadas às habilidades de cepas probióticas de colonizar a mucosa intestinal, porém não é um pré-requisito obrigatório para uma forte capacidade de adesão. Dessa forma, cepas de *Lactobacillus* com baixa hidorfobicidade celular podem, ainda assim, apresentar altos níveis de adesão às células intestinais (RAMOS et al., 2013; SOUZA et al., 2018, MOHANTY et al., 2019). O potencial hidrofóbico também pode estar relacionado com a promoção de proteção contra patógenos. As propriedades hidrofóbicas e a carga superficial das bactérias podem diferir entre as linhagens devido à variação no estado fisiológico das células e expressão de proteínas associadas à superfície ou à composição do meio (ALBUQUERQUE et al., 2017; HERNÁNDEZ-ALCÁNTARA et al., 2018).

Além disso, a coagregação exercida por bactérias láticas, particularmente *Lactobacillus*, pode ser considerada uma característica positiva, uma vez que pode promover efeitos antagonistas contra patógenos, como *Listeria monocytogenes*, *Esherichia coli*,

Salmonella Enteritidis, *Salmonella* Typhimurium e *Staphyococcus aureus* (TUO et al., 2013; ANGMO et al., 2016). Esses efeitos antagonistas ocorrem por meio de vários mecanismos que envolvem a produção de compostos antimicrobianos, como ácidos orgânicos (ácido lático, ácido acético e ácido propiônico), peróxido de hidrogênio e bacteriocinas, bem como pela competição por sítios de ligação na mucosa intestinal (DOBSON et al., 2012; SCHIRRU et al., 2012).

Por meio das propriedades de adesão, formação de barreira por autoagregação ou pela ação direta contra patógenos por meio de coagregação, bactérias láticas podem atuar na prevenção da colonização intestinal por patógenos, agindo de forma benéfica no hospedeiro (TODOROV et al., 2008; VIJAYAKUMAR et al., 2015).

Outra característica importante é a segurança de uso dos microrganismos probióticos, que engloba resistência a antibióticos, atividade hemolítica e degradação da mucina (DAS; KHOWALA; BISWAS, 2016). Estudos mostram que há uma incidência generalizada de resistência a antibióticos adquirida por isolados de bactérias láticas, o que desperta preocupação, pois há o risco desses microrganismos transferirem os genes resistentes para outras bactérias, inclusive patógenos (PESAVENTO et al., 2014, SHARMA et al., 2016). A atividade hemolítica deve ser ausente em cepas probióticas, pois a hemólise consiste no rompimento da membrana plasmática da hemácia, resultando na diminuição da circulação dessas células, o que pode levar a uma redução da oxigenação dos tecidos (KAUSHANSKY et al., 2010). Para que sejam consideradas seguras, ainda é importante atentar para a atividade de degradação da mucina em probióticos, pois a produção de enzimas degradantes da mucina tem sido sugerida como um determinante de virulência para alguns enteropatógenos, e é considerada uma característica não desejável para os probióticos (ZHOU et al., 2001; MONTEAGUDO-MERA et al., 2012).

Além de cumprir os aspectos relacionados à segurança e propriedades biológicas de probióticos, características relacionadas à produção e ao processamento de alimentos também são de grande importância nesses microrganismos, como a capacidade de sobrevivência durante a produção e armazenamento dos produtos e do fornecimento de propriedades nutricionais, físico-químicas e sensoriais ao produto final (DOMINGOS-LOPES et al., 2017; CORBO et al., 2017).

2.3 POTENCIAIS IMPACTOS DE POLIFENÓIS SOBRE A MICROBIOTA INTESTINAL E MICRORGANISMOS PROBIÓTICOS

Nas últimas décadas, a compreensão da composição e das funções da microbiota intestinal humana tem aumentado de forma significativa. Os microrganismos que compõe o intestino são essenciais para muitos aspectos da saúde humana. O hospedeiro e as bactérias comensais estabelecem uma relação que tem grande impacto na nutrição e no estado geral de saúde do hospedeiro (VALDES et al., 2018). A microbiota intestinal pode oferecer proteção contra infecções, desempenhar papel na modulação do sistema imunológico e fornecer carbono e energia para células epiteliais intestinais. Ainda, pode exercer função anti-inflamatória, inibindo histona desacetilases em células T reguladoras, sintetizar vitaminas e aminoácidos essenciais; regular o metabolismo da gordura; e produzir ácidos orgânicos (ácido lático, acético, propiônico e butírico), ácidos graxos de cadeia ramificada (ácidos isobutírico, isovalérico e 2-metilbutírico), amônia, aminas e vários outros produtos finais, os quais influenciam, de forma geral, no estado de saúde do hospedeiro (VALDES et al., 2018; CLEMENTE; MANASSON; SCHER, 2018).

Um estado disbiótico pode causar a desregulação das várias funções listadas acima, o que, por sua vez, tem sido citado como condição com potencial de contribuir para o desenvolvimento de doença inflamatória intestinal (ZUO; NG, 2018), lúpus eritematoso sistêmico (LUO et al., 2018), eczema (ABRAHAMSSON et al., 2012), asma (DAVIS, 2015), esclerose múltipla (CHEN et al., 2016), artrite (SCHER et al., 2015), diabetes tipo II (HOUGHTON et al., 2018.), obesidade (GÉRARD, 2016) e autismo (LUKENS et al., 2018).

Sabe-se, entretanto, que não só a microbiota intestinal está envolvida no metabolismo dos polifenóis, mas que esses compostos podem também afetar a composição da microbiota intestinal, revelando uma relação bidirecional entre polifenóis e microrganismos que compõe esse sistema (CUEVA et al., 2017). Assim, estudos têm sugerido que a ingestão combinada de polifenóis e probióticos pode ser uma estratégia eficaz para aumentar suas funcionalidades biológicas (Quadro 1).

A administração diária de extratos de oxicoco e uva, ricos em pro-antocianidinas tipo A e B, respectivamente, aumentou a população da bactéria *Akkermansia muciniphila* em amostras fecais de camundongos com alto teor de gordura e alto teor de açúcar (ANHÊ et al., 2015; ROOPCHAND et al., 2015). A suplementação com extrato de casca de romã aumentou a população fecal de *Bifidobacterium* spp. em ratos alimentados com um alto teor de gordura na dieta (NEYRINCK et al., 2013). Em outro estudo, a suplementação de RES e ácido

sinápico impactou a microbiota intestinal em diferentes níveis taxonômicos, melhorando a proporção de bactérias produtoras de butirato e inibindo o crescimento de espécies bacterianas associadas a doenças inflamatórias, como *Bacteroides* e *Desulfovibrionaceae* (YANG et al., 2018). Além da capacidade de modular a microbiota, estudos *in vitro* têm demonstrado que a presença de polifenóis pode favorecer o crescimento de bactérias benéficas (MORENO-INDIAS et al., 2016; CAMPANELLA et al., 2017).

Quadro 1. Estudos que avaliaram os efeitos *in vitro* de compostos fenólicos sobre probióticos.

Referência	Composto fenólico ou extrato	Probióticos	Principais resultados
Tabasco et al. (2011)	Extrato de semente de uva enriquecido com e a fração rica em monômeros e oligômeros de Flavan-3-ol 0.25 - 1 mg/mL)	*L. plantarum, L. casei* e *L. bulgaricus*	Os probióticos atingiram o crescimento máximo em meios contendo fração rica em monômeros e oligômeros de Flavan-3-ol
Lacey et al. (2014b)	(-)-epigalocatequina, (+)-catequina, (-)-epicatequina, (-)-epicatequina-3-galato, quercetina - 3-*O*-galactosídeo, quercetina-3-*O*-glicosídeo, Kaempferol-3-O-rutinosídeo e kaempferol-3-O-glicosídeo	*B. animalis* B94	Os flavonóides glicosilados permitiram uma maior taxa de sobrevivência das bactérias probióticas com percentagens de sobrevivência superiores a 50% às 48 h de incubação
Shinde; Sun-Waterhouse (2015)	Extrato aquoso de casca de maçã e extrato alcoólico de casca de maçã, ricos em polifenóis (rutina, epicatequina, florizina, ácido colorogênico, quercetina e ácido p-cumárico).	*L. acidophilus* ATCC 1643	Aumento da adesão bacteriana, induzido principalmente pelo extrato aquoso de casca de maçã (1% e 2%).
Volstatova et al. (2017)	Extratos de maçã (polpa e casca)	*L. gasseri* R e *L. casei* FMP	Extratos de polpa de maçã (10 e 20 mg/mL) diminuíram a adesão de *L. gasseri* e a adesão de de *L. casei* FMP. A casca de maçã (10 mg/mL) aumentou a adesão de *L. gasseri* R e *L. casei* FMP .A casca de maçã a 20 mg/mL aumentou a adesão de ambas as cepas.
Campanella et al. (2017)	Subproduto da uva	*L. plantarum* 12A, *L. plantarum* PU1, *L. paracasei* 14A e *Bifidobacterium breve* 15A	Exerceu efeito protetor, a 0,4%, sobre probióticos durante a passagem pelas condições estomacais
Llano et al. (2017)	Extrato fenólico de vinho	*P. pentosaceus* CIAL-86 e *L. plantarum* CIAL-121	O Extrato fenólico de vinho (0.6 mg/mL) exerceu aumento da inibição da adesão de *E. coli* CIAL-153 às células Caco-2.
Coman et al. (2018)	Extratos das cascas de ameixa, uvas vermelhas italianas, e	*Lactobacillus rhamnosus* IMC 501, *Lactobacillus*	Os extratos (0.24 – 250 mg/ml) não apresentaram atividade

	diferentes partes de sabugueiro	*paracasei* IMC 502 e *Lactobacillus plantarum* IMC 509	inibitória sobre as cepas probióticas. A combinação de extratos de sabugueiro com cada um dos probióticos mostrou maior atividade antioxidante.
Chan et al. (2018)	extratos ricos em polifenóis de seis temperos e ervas medicinais (canela-da-indonésia, canela chinesa, orégano, knotweed japonês, casca de romã e cravo)	*L. acidophilus, L. delbrueckii* subsp. *bulgaricus, L. casei*, L. *plantarum* e *L. rhamnosus*	Os valores de CIM dos extratos, sobre bactérias do ácido-lácticas, foram superiores a 2500 μg/mL, na maioria dos casos

Estudo com bagaço da uva, rico em ácido gálico e (-) epicatequina, demonstrou que esses polifenóis podem atuar de forma protetora sobre *L. plantarum* 12A e PU1, *L. paracasei* 14A e *Bifidobacterium breve* 15A durante a passagem pelo estômago. No mesmo estudo, uma diminuição no teor de ácido gálico foi observada após a fermentação, indicando que os probióticos testados foram capazes de metabolizar alguns compostos fenólicos presentes no bagaço de uva (CAMPANELLA et al., 2017). Outro estudo avaliou o efeito de um extrato de semente de uva enriquecido com frações monoméricas ou oligoméricas de flavan-3-ol sobre o crescimento de cepas probióticas. A taxa máxima de crescimento para *L. plantarum*, *L. casei* e *L. bulgaricus* foi encontrada no meio com extrato e frações oligoméricas. No entanto, a exposição a 1 mg/mL de extrato de semente de uva causou efeitos inibitórios leves em todas as cepas de *Lactobacillus* testadas, sendo atribuídos às elevadas quantidades de compostos derivados de galato no material utilizado (TABASCO et al., 2011).

Estudo com extratos etanólicos de frutos vermelhos (casca de ameixa, e diferentes partes de sabugueiro), caracterizados como fontes ricas em polifenois e antocianinas, observou efeitos positivos desses extratos sobre o crescimento de *L. rhamnosus* IMC 501 e *L. paracasei* IMC 502, a uma concentração de 10 g/L (COMAN et al., 2018). Outro estudo avaliou os efeitos de extratos ricos em polifenóis de seis especiarias e ervas medicinais (canela da indonésia, cássia chinesa, orégano, knotweed japonês, casca de romã e cravo) sobre cinco bactérias láticas selecionadas, incluindo *L. acidophilus*, *L. delbrueckii* subsp. bulgaricus, *L. casei*, *L. plantarum* e *L. rhamnosus*. Os resultados demonstraram que nenhum dos extratos apresentou efeito inibitório sobre esses microrganismos, a uma concentração máxima de 2500 μg/mL (CHAN et al, 2018).

O efeito de extratos de maçã (polpa e cascas) ricos em quercetina sobre a adesão de duas cepas de *Lactobacillus* às células epiteliais intestinais Caco-2 e HT29-MTX também foi avaliado. Nesse estudo os extratos de polpa de maçã (10 e 20 mg/mL) diminuíram a adesão de *L. gasseri* R em 39,7% e 45,9%, e adesão de *L. casei* FMP em 6,8% e 19,4% às células

epiteliais, respectivamente. Enquanto, o extrato de casca de maçã (10 mg/mL) aumentou a adesão de *L. gasseri* R em 35,7% e a adesão de *L. casei* FMP em 28,2%. A casca de maçã a 20 mg/mL aumentou a adesão de ambas as cepas (VOLSTATOVA et al., 2017). Outro estudo avaliou a influência de (+) -catequina e ácido 3,4-diidroxifenilacético sobre a viabilidade de bactérias probióticas e o impacto desses polifenóis sobre a capacidade dos probióticos em inibirem a adesão de patógenos às células intestinais. Os resultados evidenciaram que a perda de viabilidade das cepas probióticas sob condições restritas a nutrientes foi atenuada pela presença dos polifenóis (50 e 250 µM); além disso, o uso combinado das cepas probióticas com os polifenóis aumentou a inibição da aderência de *E. coli* às células intestinais Caco-2 (LLANO, 2017).

Em contraste, outro estudo avaliou a influência de ácido gálico e catequina sobre o crescimento e algumas propriedades de *Streptococcus thermophilus* CHCC 3534, onde a catequina (0,3%) e o ácido gálico (0,8%) diminuíram a sobrevivência da cepa *S. thermophilus* CHCC 3534 em pH 2 e/ou 3. No mesmo estudo, catequina e ácido gálico aumentaram e diminuíram a sobrevivência de *S. thermophilus* CHCC 3534 na presença de 0,4% de sais biliares, respectivamente (KHALIL, 2010).

A partir disso, o uso combinado de polifenóis e probióticos parece ser uma estratégia vantajosa, em relação aos seus potenciais efeitos benéficos sobre a saúde humana. Como estudos que avaliam a influência desses compostos sobre crescimento e funcionalidades fisiológicas de cepas probióticas ainda são escassos, e com resultados muito variados. Torna-se necessário a realização de estudos que investiguem os efeitos de polifenóis isolados sobre diferentes propriedades relacionadas às funcionalidades de cepas probióticas específicas.

3 MATERIAL E MÉTODOS

3.1 LOCAL DE EXECUÇÃO

Os experimentos foram realizados no Laboratório de Microbiologia e Bioquímica de Alimentos/Departamento de Nutrição/Centro de Ciências da Saúde/Universidade Federal da Paraíba.

3.2 CEPAS TESTE

Seis cepas de *Lactobacillus*, *L. plantarum* 49, *L. plantarum* 53, *L. paracasei* 106, *L. paracasei* 108, *L. fermentum* 263 e *L. fermentum* 296, isoladas de subprodutos de processamento de frutas e previamente caracterizadas como potencialmente probióticas, por uma série de testes de segurança e funcionalidade fisiológica *in vitro* foram utilizados como cepas de teste (ALBUQUERQUE et al., 2017; GARCIA et al., 2016). Estas cepas foram identificadas usando a análise da sequência do gene 16S rRNA (GARCIA et al., 2016). Os estoques foram armazenados a –20 °C no caldo Mann, Rogosa e Sharpe (MRS) (HiMedia, Mumbai, Índia) contendo glicerol (Sigma-Aldrich, St. Louis, EUA; 20 mL/100 mL). Antes da utilização nos ensaios, cada cepa foi cultivada anaerobicamente (Anaerobic System Anaerogen, Oxoid, Hampshire, UK) em caldo MRS (HiMedia, Mumbai, Índia) a 37 °C por 20 - 24 h (fase de crescimento estacionária), centrigugada (4500 *g* × 15 min e 4 ºC), lavada em solução salina estéril (0,85 g/100 mL) e ressuspensa, para obter suspensões celulares com uma leitura de Densidade Óptica a 625 nm (DO_{625nm}) de 0,5. Esta suspensão proporcionou contagens viáveis de aproximadamente 7 - 8 log UFC/mL para cada cepa quando inoculada em ágar MRS (HiMedia, Mumbai, Índia).

Cepas de *Listeria monocytogenes* (INCQS 00266, originalmente ATCC 7644) e *Escherichia coli* (INCQS 00219, originalmente ATCC 8739) utilizadas nos ensaios de atividade antagônica e coagregação foram obtidas do Instituto Nacional de Controle de Qualidade em Saúde (Fundação Oswaldo Cruz, Rio de Janeiro, Brasil). Os estoques foram armazenados em caldo Brain Heart Infusion (BHI) (HiMedia, Mumbai, Índia) contendo glicerol (Sigma-Aldrich, St. Louis, EUA; 20 mL/100 mL) a -20 °C. Antes da utilização, cada cepa foi cultivada aerobicamente em caldo BHI a 37 °C durante 20 – 24 h (fase de crescimento estacionária), colhida por centrifugação (4500 *g* × 10 min e 4 ºC), lavada duas vezes em solução salina estéril e ressuspensa em solução salina estéril para obtenção de suspensões celulares com uma DO_{660nm} de 0,1 Esta suspensão proporcionou contagens de

células viáveis de aproximadamente 7 - 8 log UFC/mL para cada cepas, quando inoculadas em ágar BHI (HiMedia, Mumbai, India).

3.3 QUERCETINA E RESVERATROL

Quercetina (QUE) e resveratrol (RES) foram obtidos da Sigma-Aldrich (pureza ≥ 95%; St. Louis, USA). As soluções destes polifenóis foram preparadas em caldo MRS (HiMedia, Mumbai, Índia), com dimetilsulfóxido (DMSO, 10%, v/v), imediatamente antes do uso em ensaios, em uma quantidade suficiente para fornecer uma concentração inicial de 2048 e 1400 μg/mL. As concentrações de QUE e RES utilizadas no estudo, foram definidas considerando estudos anteriores que avaliaram os efeitos *in vitro* destes compostos fenólicos sobre microrganismos, bem como a concentração destes polifenóis encontrados em matrizes alimentares (DUDA-CHODAK, 2012; VOLSTATOVA et al., 2017).

3.4 DETERMINAÇÃO DA CONCENTRAÇÃO INIBITÓRIA MÍNIMA (CIM)

Os valores de CIM de QUE e RES sobre as cepas de *Lactobacillus* testadas foram determinados usando o método de microdiluição em caldo (CLSI, 2015). As culturas de cada cepa de *Lactobacillus* foram cultivadas anaerobicamente em caldo MRS (HiMedia, Mumbai, Índia) (20 – 24 h, 37 ºC, Anaerobic System Anaerogen, Oxoid, Hampshire, UK). Alíquotas de 100 μL das diferentes soluções de QUE ou RES foram dispensadas em poços de uma microplaca de 96 poços e cada concentração inicial foi então diluída em série para alcançar pelo menos oito concentrações testadas finais. Posteriormente, 100 μL de uma suspensão (7 - 8 log UFC/mL) da amostra de *Lactobacillus* foram adicionados a cada poço. As concentrações finais testadas foram de 87,5 - 1024μg/mL de QUE e RES. A microplaca foi incubada a 30 °C por 24 h. Cada microplaca incluiu um controle positivo, no qual a cepas foram inoculadas sem a adição dos polifenóis, e um conjunto de controles negativos, que se trata da incubação do MRS (HiMedia, Mumbai, Índia) e/ou QUE ou RES, sem a adição do inóculo bacteriano. O crescimento aceitável (botão de ≥2 mm ou turbidez definitiva) ocorreu controle positivo. A CIM foi considerada a menor concentração de cada polifenol testado capaz de causar inibição do crescimento visual da cepa testada.

3.5 EFEITOS SOBRE A TOLERÂNCIA A DIFERENTES VALORES DE pH E SAIS BILIARES

As culturas de cada cepa de *Lactobacillus* foram cultivadas anaerobicamente em caldo MRS (HiMedia, Mumbai, Índia) (20 – 24 h, 37 ºC, Anaerobic System Anaerogen, Oxoid, Hampshire, UK). A tolerância a diferentes valores de pH e concentrações de sais biliares foi avaliada pela inoculação de alíquotas de 1 mL da suspensão de inóculo (7 – 8 log UFC/ml) de cada cepa de *Lactobacillus*, em 10 mL de *Phosphate Buffered Saline* (PBS, 50 mM K_2HPO_4/KH_2PO_4) contendo diferentes concentrações (CIM, 1/2 CIM e 1/4 CIM) de QUE ou RES, com pH ajustado para 2, 3 ou 5 usando HCl a 1 M ou suplementado com 0,15, 0,3 ou 1% (p/v) de sais biliares (Sigma-Aldrich, St. Louis, EUA).

As suspensões foram incubadas anaerobicamente a 37 ºC sob agitação (150 rpm) em incubadora com agitação orbital (TECNAL TE-424, São Paulo, Brasil). Em diferentes períodos de incubação (1, 2 e 3 horas) uma alíquota de 1 mL foi tomada da suspensão, diluída de forma seriada em 0,15% (p/v) de água de peptona esterilizada e inoculada, por meio da técnica de microgota, em ágar MRS (HiMedia, Mumbai, Índia), para enumeração da viabilidade celular. Após o período de incubação de 48 horas a 37 ºC em anaerobiose, a contagem de células viáveis foi realizada e os resultados expressos em log de unidade formadora de colônia por mL (log UFC/mL). Como controle, as cepas de *Lactobacillus* foram cultivadas em PBS com pH 7,2 ajustado usando solução de NaOH a 1 M (JACOBSEN et al., 1999; MONTEAGUDO-MERA et al., 2012). Para avaliar as influências de QUE e RES sobre a capacidade de tolerar diferentes valores de pH e sais biliares, comparou-se as contagens viáveis das cepas de *Lactobacillus* cultivadas em sistemas com e sem QUE ou RES, submetidos a diferentes valores de pH e concentrações de sais biliares.

3.6 HIDROFOBICIDADE DA SUPERFÍCIE CELULAR

As culturas de cada cepa de *Lactobacillus* foram cultivadas anaerobicamente em caldo MRS (HiMedia, Mumbai, Índia) (20 – 24 h, 37 ºC, Anaerobic System Anaerogen, Oxoid, Hampshire, UK), centrifugadas (4500 *g* × 10 min, 4 ºC), lavadas, ressuspensas e diluídas em PBS, contendo diferentes concentrações (MIC,1/2 MIC ou 1/4 MIC) de QUE ou RES, para obter uma DO_{560nm} de 1.0 de absorbância (A_0).

Posteriormente, o solvente orgânico N-hexadecano (Sigma-Aldrich, Saint Louis, EUA) foi adicionado à suspensão de células, e a mistura foi homogeneizada utilizando vortex.

A DO_{560nm} foi mensurada novamente (A) após a incubação (37 ºC/60 min). A hidrofobicidade da superfície celular foi calculada de acordo com a equação: % Hidrofobicidade = $[(A_0 – A)/A_0] \times 100$, onde A_0 refere-se aos valores de absorbância inicial e A refere-se aos valores de absorbância após 60 min de incubação (SANTOS et al., 2015). Para avaliar os efeitos de QUE ou RES sobre a hidrofobicidade da superfície celular, comparou-se a hidrofobicidade da superfície celular de amostras de *Lactobacillus* tratadas e não tratadas com QUE ou RES.

3.7 AUTOAGREGAÇÃO E COAGREGAÇÃO

Para avaliar a capacidade de autoagregação, as células de cada cepa de *Lactobacillus* foram cultivadas anaerobicamente em caldo MRS (HiMedia, Mumbai, Índia) (20 – 24 h, 37 ºC, Anaerobic System Anaerogen, Oxoid, Hampshire, UK), centrifugadas (4500 *g* × 10 min, 4 ºC), lavadas, ressuspensas e diluídas em solução salina estéril (NaCl 0,85 g/100 mL), contendo diferentes concentrações (MIC,1/2 MIC ou 1/4 MIC) de QUE ou RES, para obter uma DO_{660nm} de 0.3 (absorbância). Em seguida, a suspensão foi incubada, e a DO_{660nm} registrada novamente. A autoagregação foi determinada usando a seguinte equação: % Autoagregação = $[(DO_0 – DO_{60})/ DO_0] \times 100$, onde DO_0 refere-se à DO inicial e DO_{60} refere-se à DO determinada após 60 minutos (TODOROV et al., 2008).

Para a avaliação da coagregação as linhagens de *Lactobacillus* foram cultivadas em caldo MRS (HiMedia, Mumbai, Índia) (20 – 24 h, 37 ºC, Anaerobic System Anaerogen, Oxoid, Hampshire, UK), centrifugadas (4500 *g* × 10 min e 4 ºC), lavadas, ressuspensas e diluídas em solução salina (NaCl 0,85 g/100 mL) contendo diferentes concentrações (MIC,1/2 MIC ou 1/4 MIC) de QUE ou RES, para atingir um valor de DO_{660nm} de 0.3. Posteriormente, uma alíquota de 750 µL da suspensão de *Lactobacillus* foi misturada com o mesmo volume da suspensão de *L. monocytogenes* (INCQS 00266, origem ATCC 7644) ou *E. coli* (INCQS 00219, origem ATCC 8739) por 30 segundos em vortex. O valor de DO_{660nm} foi determinado no tempo zero (logo após a mistura das suspensões) e após 60 min de incubação a 37 °C. A coagregação foi calculada usando a seguinte equação: % Coagregação = $[(DO_0 – DO_{60})/ DO_0] \times 100$. DO_0 refere-se à DO inicial, mensurada imediatamente após a mistura das cepas, e DO_{60} refere-se à DO determinada após os 60 minutos de incubação (TODOROV et al., 2008). Para avaliar a influência dos polifenóis sobre a capacidade de autoagregação e coagregação, comparou-se os valores de autoagregação ou de coagregação das cepas de *Lactobacillus* tratadas e não tratadas com QUE ou RES.

3.8 A ATIVIDADE ANTAGONISTA FRENTE A PATÓGENOS

A atividade antagonista de cada cepa de *Lactobacillus* contra as cepas bacterianas indicadoras foi avaliada usando o *agar spot test*. As cepas foram cultivadas anaerobicamente em caldo MRS (HiMedia, Mumbai, Índia) (20 – 24 h, 37 ºC, Anaerobic System Anaerogen, Oxoid, Hampshire, UK), e suplementadas com diferentes concentrações (MIC, 1/2 MIC e 1/4 MIC) de QUE ou RES. Uma alíquota de 10 µL das culturas com cada cepa de *Lactobacillus* (7 – 8 log UFC/ml), cultivadas como descrito anteriormente, foi transferida para superfícies de ágar MRS (HiMedia, Mumbai, Índia) contendo 0.2% (p/v) de glicose (Sigma-Aldrich, Saint Louis, EUA) e 1.2% (p/v) de ágar bacteriológico (HiMedia, Mumbai, Índia) por meio da técnica de microgota, seguindo-se por incubação anaeróbica (Anaerobic System Anaerogen, Oxoid, Hampshire, UK) por 24 horas a 37 °C. Posteriormente, uma alíquota de 1 mL de cada suspensão de bactéria indicadora (7 – 8 log UFC/ml) foi misturada com 18 mL de ágar BHI semissólido [contendo 0,7% (p/v) de ágar bacteriológico], e a mistura vertida sobre o ágar MRS (HiMedia, Mumbai, Índia) inoculado anteriormente com a cepa de *Lactobacillus* testada. As placas foram novamente incubadas a 37 ºC por 48 horas em aerobiose. A atividade antagonista foi registada como o diâmetro (mm) de halos de inibição de crescimento desenvolvido em torno de cada cultura de cepa *spot*, zonas livres de crescimento com diâmetro maior que 1 mm (ao redor do ponto ou poço) foi considerada como atividade inibitória positiva (JACOBSEN et al., 1999). Para avaliar os efeitos de QUE ou RES sobre atividade antagonista, o diâmetro das zonas de inibição de crescimento em relação às bactérias indicadoras causadas por cepas de *Lactobacillus* tratadas e não tratadas com QUE ou RES foram comparadas.

3.9 EFEITOS SOBRE A SOBREVIVÊNCIA A EXPOSIÇÃO ÀS CONDIÇÕES GASTROINTESTINAIS SIMULADAS

Cepas de *Lactobacillus* foram cultivadas anaerobicamente em caldo MRS (HiMedia, Mumbai, Índia) (20 – 24 h, 37 ºC, Anaerobic System Anaerogen, Oxoid, Hampshire, UK) foram expostas a condições gastrointestinais simuladas em caldo MRS com e sem QUE (1/2 CIM) ou RES (1/2 CIM). Inicialmente, alíquotas de 10 mL de caldo MRS foram colocadas em frascos de vidro (50 mL) e inoculadas com a cepa de *Lactobacillus* examinada (6 - 7 log UFC/mL).

A simulação nesses frascos foi realizada continuamente em fases simulando mastigação e condições no esôfago-estômago, duodeno e íleo. A agitação mecânica foi utilizada para simular os movimentos peristálticos e o teste foi realizado em incubadora a 37 °C com ajuste de rotação em cada fase. A mastigação foi simulada usando 100 U de α-amilase diluída em 1 mL de $CaCl_2$ mL a 1 mM, com pH ajustado para 6,9 com $NaHCO_3$ a 1 M e tempo de exposição de 2 min, a 200 rpm; condições de esôfago-estômago com 25 mg de pepsina diluída em 1 mL de HCl a 1 M, adicionado a uma concentração de 0,05 mL/mL, pH com diminuição gradual usando HCl a 1 M (pH 5,5/10 min; pH 4,6/10 min; pH 3,8/10 min; pH 2,8/20 min; pH 2,3/20 min e pH 2/20 min) sob agitação (130 rpm).

Por fim, as condições duodenais foram simuladas com 2 g de pancreatina/L de $NaHCO_3$ a 1 M e 12 g de sais biliares bovinos/L de $NaHCO_3$ a 1 M, pH ajustado para 5,0 com $NaHCO_3$ a 1 M e tempo de exposição de 30 min sob agitação (45 rpm); as condições do íleo foram simuladas com pH ajustado para 6,5 utilizando $NaHCO_3$ a 1 M, tempo de exposição de 60 min sob agitação (45 rpm). Todas as enzimas e sais biliares bovinos foram adquiridos da Sigma-Aldrich (St. Louis, EUA). Após cada fase da digestão gastrointestinal simulada, alíquotas de 100 μL de caldo MRS (HiMedia, Mumbai, Índia) com ou sem QUE ou RES foram diluídas em série em solução salina estérilizada (NaCl 0,85 g/100 mL) e plaqueadas, por meio da técnica de microgota, em ágar MRS (HiMedia, Mumbai, Índia) (MEIRA et al., 2015).

Após um período de incubação de 48 h a 37 ºC sob anaerobiose, as células viáveis foram enumeradas e os resultados expressos como log UFC/mL. O caldo MRS inoculado mantido a 37 ºC sem QUE ou RES foi utilizado como controle. Um limite de detecção de 1 log UFC/mL foi usado nestes ensaios.

3.10 ANÁLISES ESTATÍSTICAS

Todos os ensaios foram realizados em triplicata e em três experimentos independentes, sendo os resultados expressos como a média ± desvio padrão dos dados obtidos. As análises estatísticas foram realizadas para determinar diferenças significativas ($p \leq 0,05$) entre os resultados obtidos usando ANOVA, seguido do teste de Tukey. Estas análises foram realizadas utilizando o software GraphPad Prism 5.0 (San Diego, CA, USA).

REFERÊNCIAS

ABUSHELAIBI, A. et al. Characterization of potential probiotic lactic acid bacteria isolated from camel milk. **LWT-Food Science and Technology**, v. 79, n. 1, p. 316-325, 2017.

ABRAHAMSSON, T. R. et al. Low diversity of the gut microbiota in infants with atopic eczema. **Journal of Allergy and Clinical Immunology**, v. 129, n. 2, p. 434-440, 2012.

ALARD, J. et al. Beneficial metabolic effects of selected probiotics on diet-induced obesity and insulin resistance in mice are associated with improvement of dysbiotic gut microbiota. **Environmental Microbiology**, v. 18, n. 5, p. 1484-1497, 2016.

ALBUQUERQUE, T. M. R. et al. *In Vitro* Characterization of *Lactobacillus* Strains Isolated from Fruit Processing By-Products as Potential Probiotics. **Probiotics and Antimicrobial Proteins**, v. 10, n 4, p. 704–716, 2017.

ALVES, J. L. B. et al. New insights on the use of dietary polyphenols or probiotics for the management of arterial hypertension. **Frontiers in physiology**, v. 7, n. 448, p. 1-8, 2016.

ANGMO, K. et al. Probiotic characterization of lactic acid bacteria isolated from fermented foods and beverage of Ladakh. **Food Science and Technology**, v. 66, n.1, p. 428–435, 2016.

ANHÊ, F. F. et al. A polyphenol-rich cranberry extract protects from diet-induced obesity, insulin resistance and intestinal inflammation in association with increased Akkermansia spp. population in the gut microbiota of mice. **Gut**, v. 64, n. 6, p. 872-883, 2015.

AOUDIA, N. et al. Biofilms of *Lactobacillus plantarum* and *Lactobacillus fermentum*: Effect on stress responses, antagonistic effects on pathogen growth and immunomodulatory properties. **Food Microbiology**, v. 53, n.1, p. 51-59, 2016.

BANERJEE, A.; DHAR, P. Amalgamation of polyphenols and probiotics induce health promotion. **Critical Reviews in Food Science and Nutrition**, p. 1-24, 2018.

BODE, L. M. et al. *In vivo* and *in vitro* metabolism of *trans*-resveratrol by human gut microbiota. **The American Journal of Clinical Nutrition**, v. 97, n. 2, p. 295-309, 2013.

BRON, P. A. et al. Can probiotics modulate human disease by impacting intestinal barrier function? **British Journal of Nutrition**, v. 117, n. 1, p. 93-107, 2017.

CAMPANELLA, D. et al. Exploitation of grape marc as functional substrate for lactic acid bacteria and bifidobacteria growth and enhanced antioxidant activity. **Food Microbiology**, v. 65, n.1, p. 25-35, 2017.

CASAROTTI, S. N. et al. *In vitro* assessment of safety and probiotic potential characteristics of *Lactobacillus* strains isolated from water buffalo mozzarella cheese. **Annals of Microbiology**, v. 67, n. 4, p. 289-301, 2017.

CHAN, C. L. et al. Polyphenols from selected dietary spices and medicinal herbs differentially affect common food-borne pathogenic bacteria and lactic acid bacteria. **Food Control**, v. 92, n.1, p. 437-443, 2018.

CHEN, J. et al. Multiple sclerosis patients have a distinct gut microbiota compared to healthy controls. **Scientific Reports**, v. 6, n. 1, p. 28484, 2016.

CHOI, E. A.; CHANG, H. C. Cholesterol-lowering effects of a putative probiotic strain *Lactobacillus plantarum* EM isolated from kimchi. **LWT - Food Science and Technology,** v. 62, n. 1, p. 210-217, 2015.

CLEMENTE, J. C.; MANASSON, J.; SCHER, J. U. The role of the gut microbiome in systemic inflammatory disease. **British Medical Journal**, v. 360, n.1, p. 5145, 2018.

CLINICAL AND LABORATORY STANDARDS INSTITUTE**. Performance Standards for Antimicrobial Susceptibility Testing; Twenty- Second Informational Supplement. CLSI document M100-S22**. Clinical and Laboratory Standards Institute, Pennsylvania, United States of America, 2015.

COMAN, M. M. et al. Polyphenol content and *in vitro* evaluation of antioxidant, antimicrobial and prebiotic properties of red fruit extracts. **European Food Research and Technology**, v. 244, n. 4, p. 735-745, 2018.

CORBO, M. R. et al. Selection of wild lactic acid bacteria for sausages: Design of a selection protocol combining statistic tools, technological and functional properties. **Food Science and Technology**, v. 81, n.1, p. 144-152, 2017.

CRASCÌ, L. et al. Natural antioxidant polyphenols on inflammation management: anti-glycation activity vs metalloproteinases inhibition. **Critical Reviews in Food science and Nutrition**, v. 58, n. 6, p. 893-904, 2018.

CUEVA, C. et al. An integrated view of the effects of wine polyphenols and their relevant metabolites on gut and host health. **Molecules**, v. 22, n. 1, p. 99, 2017.

DAS, P.; KHOWALA, S.; BISWAS, S. *In vitro* probiotic characterization of *Lactobacillus casei* isolated from marine samples. **Food Science and Technology**, v. 73, n. 1, p. 383-390, 2016.

DAVIS, K. L. Low Gut Microbiota Diversity in Early Infancy Precedes Asthma at School Age. **Pediatrics**, v. 136, n. Supplement 3, p. S232-S232, 2015.

DOBSON, A. et al. Bacteriocin production: a probiotic trait? **Applied and Environmental Microbiology**. v. 78, n. 1, p. 106, 2012.

DOLINSKY, V. W. et al. Resveratrol prevents hypertension and cardiac hypertrophy in hypertensive rats and mice. **Biochimica et Biophysica Acta-Molecular Basis of Disease**, v. 1832, n. 10, p. 1723-1733, 2013.

DOMINGOS-LOPES, M. F. P. et al. Genetic diversity, safety and technological characterization of lactic acid bacteria isolated from artisanal Pico cheese. **Food Microbiology,** v. 63, n. 1, p. 178-190, 2017.

DUDA-CHODAK, A. The inhibitory effect of polyphenols on human gut microbiota. **Journal of Physiology and Pharmacology**, v. 63, n. 5, p. 497-503, 2012.

ESPINOSA, R. R. et al. Antioxidant activity of phenolic compounds added to a functional emulsion containing omega-3 fatty acids and plant sterol esters. **Food Chemistry**, v. 182, n. 1, p. 95-104, 2015.

FARHADI, K. et al. Determination of phenolic compounds content and antioxidant activity in skin, pulp, seed, cane and leaf of five native grape cultivars in West Azerbaijan province, Iran. **Food Chemistry**, v. 199, n. 1, p. 847-855, 2016.

FOOD AND AGRICULTURE ORGANIZATION (FAO) / WORLD HEALTH ORGANIZATION (WHO). Probiotics in food. Health and nutritional properties and guidelines for evaluation. Rome: **FAO Food and Nutrition Paper**. N° 85, 2006.

GHARBI, Y. et al. *In-vitro* characterization of potentially probiotic *Lactobacillus* strains isolated from human microbiota: interaction with pathogenic bacteria and the enteric cell line HT29. **Annals of Microbiology**, v. 69, n. 1, p. 61-72, 2019.

GARCIA, E. F. et al. Identification of lactic acid bacteria in fruit pulp processing byproducts and potential probiotic properties of selected *Lactobacillus* strains. **Frontiers in microbiology**, v. 7, n. 1, p. 1371, 2016.

GELEN, V. et al. Therapeutic effect of quercetin on renal function and tissue damage in the obesity induced rats. **Biomedicine & Pharmacotherapy**, v. 89, n. 1, p. 524-528, 2017.

GÉRARD, P. Gut microbiota and obesity. **Cellular and Molecular Life Sciences**, v. 73, n. 1, p. 147-162, 2016

GRANATO, D. et al. Effects of geographical origin, varietal and farming system on the chemical composition and functional properties of purple grape juices: A review. **Trends in Food Science & Technology**, v. 52, n. 1, p. 31-48, 2016.

HARNLY, J. M.; BHAGWAT, S.; LIN, L. Profiling methods for the determination of phenolic compounds in foods and dietary supplements. **Analytical and Bioanalytical Chemistry**, v. 389, n. 1, p. 47-61, 2007.

HERNÁNDEZ-ALCÁNTARA, A. M. et al. Probiotic properties and stress response of thermotolerant lactic acid bacteria isolated from cooked meat products. **Food Science and Technology**, v. 91, n. 1, p. 249-257, 2018.

HOUGHTON, D. et al. Systematic review assessing the effectiveness of dietary intervention on gut microbiota in adults with type 2 diabetes. **Diabetologia**, v. 61, n. 8, p. 1700–1711, 2018.

JACOBSEN, C. Nexmann et al. Screening of probiotic activities of forty-seven strains of *Lactobacillus* spp. by *in vitro* techniques and evaluation of the colonization ability of five selected strains in humans. **Applied and Environmental Microbiology**, v. 65, n. 11, p. 4949-4956, 1999.

JAY, J. M. LOESSNER, M. J.; GOLDEN, D. A. **Modern Food Microbiology.** 7ª ed. New York: Springer, p. 175-195, 2005.

KAUSHANSKY, K. et al. **Williams Hematology.** 8ª Ed. The McGraw-Hill Companies, p. 346-358, 2010.

KHALIL, R. K. S. Influence of gallic acid and catechin polyphenols on probiotic properties of *Streptococcus thermophilus* CHCC 3534 strain. **World Journal of Microbiology and Biotechnology**, v. 26, n. 11, p. 2069-2079, 2010.

KUMAR, R.; VIJAYALAKSHMI, S.; NADANASABAPATHI, S. Health Benefits of Quercetin. **Defense Life Science Journal**, v. 2, n. 2, p. 142-151, 2017.

LACEY, A. M. L. et al. Survival and metabolic activity of probiotic bacteria in green tea. **LWT-Food Science and Technology**, v. 55, n. 1, p. 314-322, 2014a.

LACEY, A. M. L. et al. Biotransformation and resulting biological properties of green tea polyphenols produced by probiotic bacteria. **LWT-Food Science and Technology**, v. 58, n. 2, p. 633-638, 2014b.

LLANO, D. G. et al. Reciprocal beneficial effects between wine polyphenols and probiotics: an exploratory study. **European Food Research and Technology**, v. 243, n. 3, p. 531-538, 2017.

LLANO, D. G. et al. Some Contributions to the Study of Oenological Lactic Acid Bacteria through Their Interaction with Polyphenols. **Beverages**, v. 2, n. 4, p. 27, 2016.

LUKENS, J. R. et al. Critical roles for microbiota-mediated regulation of Th17 responses in a maternal immune activation model of autism. **The Journal of Immunology**, v. 200, n. 1, p. 166.36; 2018

LUO, X. M. et al. Gut microbiota in human systemic lupus erythematosus and a mouse model of lupus. **Applied and Environmental Microbiology.**, v. 84, n. 4, p. e02288-17, 2018

MA et al. Stimulatory effects of tea supplements on the propagation of *Lactobacillus casei* in milk. **International Dairy Journal**, v. 43, n. 1, p. 1-6, 2015.

MAGALHÃES, A. J. et al. How to select a probiotic? A review and update of methods and criteria. **Biotechnology advances**, v. 36, n. 1, p. 2060–2076, 2018.

MAJEED, M. et al. Evaluation of the *in vitro* cholesterol-lowering activity of the probiotic strain *Bacillus coagulans* MTCC 5856. **International Journal of Food Science & Technology**, v. 54, n. 1, p. 212-220, 2019.

MAZLOOM, K.; SIDDIQI, I.; COVASA, M. Probiotics: How Effective Are They in the Fight against Obesity? **Nutrients**, v. 11, n. 2, p. 258, 2019.

MEIRA, Q. G. S. et al. Effects of added *Lactobacillus acidophilus* and *Bifidobacterium lactis* probiotics on the quality characteristics of goat ricotta and their survival under simulated gastrointestinal conditions. **Food Research International**, v. 76, n. 1, p. 828-838, 2015.

MOHANTY, D. et al. *In vitro* evaluation of adherence and anti-infective property of probiotic *Lactobacillus plantarum* DM 69 against *Salmonella enterica*. **Microbial Pathogenesis**, v. 126, n. 1, p. 212-217, 2019.

MOJGANI, N.; HUSSAINI, F.; VASEJI, N. Characterization of indigenous *Lactobacillus* strains for probiotic properties. **Jundishapur Journal of Microbiology**, v. 8, n. 2, 2015.

MONTEAGUDO-MERA, A. et al. *In vitro* evaluation of physiological probiotic properties of different lactic acid bacteria strains of dairy and human origin. **Journal of Functional Foods**, v. 4, n. 2, p. 531–541, 2012.

MORENO-INDIAS, I. et al. Red wine polyphenols modulate fecal microbiota and reduce markers of the metabolic syndrome in obese patients. **Food & Function**, v. 7, n. 4, p. 1775-1787, 2016.

MUELLER, M. et al. Rhamnosidase activity of selected probiotics and their ability to hydrolyse flavonoid rhamnoglucosides. **Bioprocess and biosystems engineering**, v. 41, n. 2, p. 221-228, 2018.

NEVEU, et al. Phenol-Explorer: an online comprehensive database on polyphenol contents in foods. **Database**, v. 2010, 2010.

NEYRINCK, A. M. et al. Polyphenol-rich extract of pomegranate peel alleviates tissue inflammation and hypercholesterolaemia in high-fat diet-induced obese mice: potential implication of the gut microbiota. **British Journal of Nutrition**, v. 109, n. 5, p. 802-809, 2013.

OAK, J. S.; JHA, R. The effects of probiotics in lactose intolerance: A systematic review. **Critical Reviews in Food Science and Nutrition**, p. 1-9, n.1, 2018.

ONGOL, M. P. Lactic acid bacteria in health and disease. **Rwanda Journal of Health Sciences**, v. 1, n. 1, p. 39-50, 2012.

OZDAL, T. et al. The reciprocal interactions between polyphenols and gut microbiota and effects on bioaccessibility. **Nutrients**, v. 8, n. 2, p. 78, 2016.

PACHECO-ORDAZ, R. et al. Effect of phenolic compounds on the growth of selected probiotic and pathogenic bacteria. **Letters in Applied Microbiology**, v. 66, n. 1, p. 25-31, 2018.

PASINETTI, G. M. et al. Roles of resveratrol and other grape-derived polyphenols in Alzheimer's disease prevention and treatment. **Biochimica et Biophysica Acta -Molecular Basis of Disease**, v. 1852, n. 6, p. 1202-1208, 2015.

PEREIRA-CARO, G. et al. Chronic administration of a microencapsulated probiotic enhances the bioavailability of orange juice flavanones in humans. **Free Radical Biology and Medicine**, v. 84, n. 1, p. 206-214, 2015.

PESAVENTO, G. et al. Prevalence and antibiotic resistance of *Enterococcus* spp. isolated from retail cheese, ready to-eat salads, ham, and raw meat. **Food Microbiology**, v. 41, n. 1, p. 1-7, 2014.

PLAZA-DÍAZ, J. et al. Evidence of the anti-inflammatory effects of probiotics and synbiotics in intestinal chronic diseases. **Nutrients**, v. 9, n. 6, p. 555, 2017.

PLAZA-DIAZ, J. et al. Mechanisms of action of probiotics. **Advances in Nutrition**, v. 10, n. suppl_1, p. S49-S66, 2019.

POSSEMIERS, S. et al. The intestinal microbiome: a separate organ inside the body with the metabolic potential to influence the bioactivity of botanicals. **Fitoterapia**, v. 82, n. 1, p. 53-66, 2011.

RAMOS, C. L. et al. Strain-specific probiotics properties of *Lactobacillus fermentum*, *Lactobacillus plantarum* and *Lactobacillus brevis* isolates from Brazilian food products. **Food microbiology**, v. 36, n. 1, p. 22-29, 2013.

RAZMPOOSH, E. et al. Probiotics as beneficial agents in the management of diabetes mellitus: a systematic review. **Diabetes/metabolism research and reviews**, v. 32, n. 2, p. 143-168, 2016.

RIZZOLI, René. Nutritional influence on bone: role of gut microbiota. **Aging Clinical and Experimental Research**, p. 1-9, n. 1, 2019.

ROBLES-VERA, I. et al. Antihypertensive Effects of Probiotics. **Current Hypertension reports**, v. 19, n. 4, p. 26, 2017.

ROBLES-VERA, Iñaki et al. The probiotic *Lactobacillus fermentum* prevents dysbiosis and vascular oxidative stress in rats with hypertension induced by chronic nitric oxide blockade. **Molecular Nutrition & Food Research**, v. 62, n. 19, p. 1800298, 2018.

RODRÍGUEZ-PÉREZ, C. et al. Antibacterial activity of isolated phenolic compounds from cranberry (*Vaccinium macrocarpon*) against *Escherichia coli*. **Food & Function**, v. 7, n. 3, p. 1564-1573, 2016.

ROOPCHAND, D. E. et al. Dietary polyphenols promote growth of the gut bacterium *Akkermansia muciniphila* and attenuate high fat diet-induced metabolic syndrome. **Diabetes**, p. db141916, 2015.

ROSSI, M. et al. Potential impact of probiotic consumption on the bioactivity of dietary phytochemicals. **Journal of Agricultural and Food Chemistry**, v. 61, n. 40, p. 9551-9558, 2013.

SANTOS, K. M. O. et al. Artisanal Coalho cheeses as source of beneficial *Lactobacillus plantarum* and *Lactobacillus rhamnosus* strains. **Dairy Science & Technology**, v. 95, n. 2, p. 209–230, 2015.

SCHER, J. U. et al. Decreased bacterial diversity characterizes the altered gut microbiota in patients with psoriatic arthritis, resembling dysbiosis in inflammatory bowel disease. **Arthritis & Rheumatology**, v. 67, n. 1, p. 128-139, 2015.

SHINDE, T. S., J. D. B., SUN-WATERHOUSE D. Preparation and use of apple skin polyphenol extracts in milk: enhancement of the viability and adhesion of probiotic *Lactobacillus acidophilus* (ATCC 1643) bacteria. **International Journal Food Science and Technology,** v. 50, n. 1, p. 1303**–**1310, 2015

SCHIRRU, S. et al. Sardinian goat's milk as source of bacteriocinogenic potential protective cultures. **Food Control,** v. 25, n. 1, p. 309-320, 2012

SHARMA, A. Importance of Probiotics in Cancer Prevention and Treatment. In: **Recent Developments in Applied Microbiology and Biochemistry**. Academic Press, 2019. p. 33-45.

SHARMA, P. et al. Antibiotic resistance of *Lactobacillus* sp isolated from commercial probiotic preparations. **Journal of Food Safety**, v. 36, n. 1, p. 38-51, 2016.

SHEHATA, M. G. et al. Screening of isolated potential probiotic lactic acid bacteria for cholesterol lowering property and bile salt hydrolase activity. **Annals of Agricultural Science**, v. 61, n. 1, p. 65–75, 2016.

SILVA, B. V.; BARREIRA, J. C. M.; OLIVEIRA, M. B. P. P. Natural phytochemicals and probiotics as bioactive ingredients for functional foods: Extraction, biochemistry and protected-delivery technologies. **Trends in Food Science & Technology**, v. 50, n. 1, p. 144-158, 2016.

SINGH, D. et al. Antibacterial activity of resveratrol structural analogues: A mechanistic evaluation of the structure-activity relationship. **Toxicology and Applied Pharmacology**, v. 367, n. 1, p. 23-32 2019.

SINHA, D. et al. Resveratrol for breast cancer prevention and therapy: Preclinical evidence and molecular mechanisms. **Seminars in Cancer Biology**, v. 40–41, n. 1, p. 209-232, 2016

SOUZA, E. L. et al. Potential interactions among phenolic compounds and probiotics for mutual boosting of their health-promoting properties and food functionalities–A review. **Critical Reviews in FoodSscience and Nutrition**, p. 1-15, 2018.

STEVENS, J. F.; MAIER, C. S. The chemistry of gut microbial metabolism of polyphenols. **Phytochemistry Reviews**, v. 15, n. 3, p. 425-444, 2016.

TABASCO, R. et al. Effect of grape polyphenols on lactic acid bacteria and bifidobacteria growth: resistance and metabolism. **Food Mcrobiology**, v. 28, n. 7, p. 1345-1352, 2011.

TESTER, R. F.; AL-GHAZZEWI, F. H. Role of prebiotics and probiotics in oral health. **Nutrition & Food Science**, v. 48, n. 1, p. 16-29, 2018.

TODOROV, S. D. et al. Boza, a natural source of probiotic lactic acid bacteria. **Journal of Applied Microbiology**, v. 104, n. 2, p. 465-477, 2008.

TUO, Y. et al. Aggregation and adhesion properties of 22 *Lactobacillus* strains. **Journal Dairy Science**, v. 96, n. 7, p. 4252–4257, 2013.

VALDES, A. M. et al. Role of the gut microbiota in nutrition and health. **British Medical Journal**, v. 361, n. 1, p. k2179, 2018.

VIJAYAKUMAR, M. et al. *In-vitro* assessment of the probiotic potential of *Lactobacillus plantarum* KCC-24 isolated from Italian rye-grass (*Lolium multiflorum*) forage. **Anaerobe**, v. 32, n. 1, p. 90-97, 2015.

VITETTA, L.; VITETTA, G.; HALL, S. Immunological tolerance and function: associations between intestinal bacteria, probiotics, prebiotics, and phages. **Frontiers in Immunology**, v. 9, 2018.

VOLSTATOVA, T. et al. Effect of apple extracts and selective polyphenols on the adhesion of potential probiotic strains of *Lactobacillus gasseri* R and *Lactobacillus casei* FMP. **Journal of Functional Foods**, v. 35, n. 1, p. 391-397, 2017.

WESTFALL, S. et al. Microbiome, probiotics and neurodegenerative diseases: deciphering the gut brain axis. **Cellular and Molecular Life Sciences**, v. 74, n. 20, p. 3769-3787, 2017.

WANG, J. et al. Modulation of gut microbiota during probiotic-mediated attenuation of metabolic syndrome in high fat diet-fed mice. **The ISME Journal**, v. 9, n. 1, p. 1, 2015.

WU, Q. et al. Different antitumor effects of quercetin, quercetin-3′-sulfate and quercetin-3-glucuronide in human breast cancer MCF-7 cells. **Food & Function**, v. 9, n. 3, p. 1736-1746, 2018.

YANG, Chen et al. Sinapic acid and resveratrol alleviate oxidative stress with modulation of gut microbiota in high-fat diet-fed rats. **Food Research International**, 2018.

YERLIKAYA, O. Probiotic potential and biochemical and technological properties of *Lactococcus lactis* ssp. lactis strains isolated from raw milk and kefir grains. **Journal of Dairy Science**, v. 102, n. 1, p. 124-134, 2019.

YI, J. et al. Study on antitumor, antioxidant and immunoregulatory activities of the purified polyphenols from pinecone of *Pinus koraiensis* on tumor-bearing S180 mice in vivo. **International Journal of Biological Macromolecules**, v. 94, n. 1, p. 735-744, 2017.

YUKSEKDAG, Z. et al. β-Glucosidase activity and bioconversion of isoflavone glycosides to aglycones by potential probiotic bacteria. **International Journal of Food Properties**, v. 20, n. sup3, p. S2878-S2886, 2017.

ZHANG, H. et al. The association of biofilm formation with antibiotic resistance in lactic acid bacteria from fermented foods. **Journal of Food Safety**, v. 33, n. 2, p. 114-120, 2013.

ZHOU, J. S.; GOPAL, P. K.; HILL, H. S. Potential probiotic lactic acid bacteria *Lactobacillus rhamnosus* (HN001), *Lactobacillus acidophilus* (HN017) and *Bifidobacterium lactis* (HN019) do not degrade gastric mucin *in vitro*. **International Journal of Food Microbiology**, v. 63, n. 1-2, p. 81–90, 2001.

ZOUMPOPOULOU, G. et al. Dairy probiotics: Beyond the role of promoting gut and immune health. **International Dairy Journal**, v. 67, n. 1, p. 46-60, 2017.

ZUO, T; NG, S. C. The gut microbiota in the pathogenesis and therapeutics of inflammatory bowel disease. **Frontiers in Microbiology**, v. 9, n. 1, p. 2247, 2018.

APÊNDICES

Effects of quercetin and resveratrol on *in vitro* properties related to the functionality of potentially probiotic *Lactobacillus* strains

Abstract

This study assessed the effects of the polyphenols quercetin (QUE) and resveratrol (QUE) on some *in vitro* functionality-related properties of six potentially probiotic *Lactobacillus* strains. The tested *Lactobacillus* strains presented low susceptibility to QUE and RES since the minimum inhibitory concentration (MIC) of these polyphenols reached >1024 μg/mL. In most cases, QUE and RES, at all tested concentrations (i.e., MIC, 1/2 MIC and 1/4 MIC), did not affect the tolerance of the *Lactobacillus* strains to acidic pH and bile salts. QUE increased the cell surface hydrophobicity of most of the tested *Lactobacillus* strains, while increases or decreases in cell surface hydrophobicity varied in the presence of different RES concentrations among some of the strains. QUE and RES increased the ability of the tested *Lactobacillus* strains to aggregate and coaggregate with pathogens. These compounds did not negatively affect the antagonistic activity of the tested *Lactobacillus* strains against pathogens and did not decrease their survival when exposed to *in vitro* digestion. In a few cases, the ability of some of the tested *Lactobacillus* strains to antagonize pathogens as well as to survive specific steps of the *in vitro* digestion were increased by QUE or RES. The combined use of QUE or RES with probiotic lactobacilli could improve the functional properties exerted by these bacteria on the host; however, the concentration of these compounds should be carefully considered to achieve these desirable effects and vary according to the selected probiotic strain.

Keywords: Polyphenols, probiotics, physiological properties, modulatory effects.

1. Introduction

Polyphenols are widely distributed in fruits, vegetables, herbs, seeds, cereals, honey and beverages (Hossen et al., 2017). Among the polyphenols present in foods, quercetin (QUE) and resveratrol (RES) have received increased interest because of the strong evidence associating their intake with a variety of health benefits (Dolinsk et al., 2013; Gelen et al., 2017). QUE is one of the major representatives of the class of flavonoids, being naturally found in apples and red wine, while RES is the main representative of the class of the stilbenes, being naturally present in the bark and seeds of grapes and wine (de Brito-Alves et al., 2016; Dolinsky et al., 2013).

The beneficial bioactivities (e.g., antioxidant and anti-inflammatory properties) of polyphenols are primarily related to the presence of an aromatic structure and multiple hydroxyl groups that are able to donate electrons or hydrogen atoms to free radicals and reactive oxygen species (ROS) (Espinosa et al., 2015; Farhadi et al., 2016). The presence of hydroxyl groups in polyphenol molecules has also been associated with the reported potential antimicrobial properties of these compounds (Singh et al., 2019).

Polyphenols may exert health benefits at a local level when they act directly during passage through the gastrointestinal tract as well as at a systemic level after their absorption (Gelen et al., 2017; Sinha et al., 2016). Polyphenol digestion and absorption is limited, and it is estimated that only 5–10% of total ingested polyphenols can be absorbed in the small intestine (Cueva et al., 2017; Llano et al., 2016). The metabolism of polyphenols by specific microbial groups forming the gut microbiota has been cited to increase the bioavailability of dietary polyphenols, in addition to stimulating the growth of beneficial bacteria in the intestine (Moreno-Indias et al., 2016; Pereira-Caro et al., 2015).

Interestingly, probiotics are among the microorganisms potentially capable of metabolizing polyphenols in the intestine (Llano et al., 2017), and it has been proposed that

the combined ingestion of polyphenols and probiotics could be an effective strategy to boost their biological functionalities when used in food or nutraceutical formulations (de Souza et al., 2018; Westfall et al., 2018). Among the lactic acid bacteria, *Lactobacillus* species have been intensively studied for the selection of probiotic strains (Albuquerque et al., 2017; Gharbi et al., 2019) and recently for their ability to metabolize dietary polyphenols (Mueller et al., 2018).

The potential ability of probiotics to exert health benefits on the host has been commonly associated with their physiological functionalities (e.g., acid and bile salt tolerance, cell surface hydrophobicity, autoaggregation, coaggregation with pathogens and antagonistic activity against pathogens) and ability to survive during exposure to gastrointestinal conditions (Abushelaibi et al., 2017; Albuquerque et al., 2017). This consideration reflects the fact that the impacts on the growth and/or biological *in vitro* functionalities of probiotics resulting from the interactions of these microorganisms with one or more compounds coexisting in the same environment could indicate a potential influential factor to reach the desired beneficial effects on the host.

Although some researchers have indicated the combined use of dietary polyphenols and probiotics as an advantageous strategy (Georgakouli et al., 2016; Lacey et al., 2014), studies evaluating the influence of these compounds on the growth and physiological functionalities of probiotic strains remain scarce. Only a few studies have evaluated the influence of polyphenol-rich foods/beverages or individual polyphenols on the growth and/or some probiotic-related functionalities of lactic acid bacteria, including *Lactobacillus* species, with variable results (Chan et al., 2018; Llano et al., 2017; Pacheco-Ordaz et al., 2018). However, none of these studies have investigated the effects of QUE and RES on different properties related to the functionalities of potentially probiotic *Lactobacillus* strains.

This study first evaluated the susceptibility of six previously proven potentially probiotic *Lactobacillus* strains to QUE and RES. Second, the effects of different amounts of QUE and RES on the ability of these *Lactobacillus* strains to tolerate different pH values and bile salt concentrations, as well as on their ability to autoaggregate, coaggregate with and antagonize pathogens, were evaluated. Finally, the effects of QUE and RES on the survival of the tested *Lactobacillus* strains when exposed to simulated gastrointestinal digestion was assessed.

2. Materials and methods

2.1 Test strains and inoculum preparation

Six strains of *Lactobacillus* species, namely, *L. plantarum* 49, *L. plantarum* 53, *L. paracasei* 106, *L. paracasei* 108, *L. fermentum* 263 and *L. fermentum* 296, isolated from fruit processing byproducts and previously characterized as potentially probiotic in a series of safety and physiological functionality *in vitro* tests were used in this study (Albuquerque et al., 2017; Garcia et al., 2016). These strains were identified using 16S rRNA gene sequence analysis (Garcia et al., 2016). Stocks were stored at −20 °C in de Mann, Rogosa and Sharpe (MRS) broth (HiMedia, Mumbai, India) containing glycerol (Sigma-Aldrich, St. Louis, USA; 20 mL/100 mL). Working cultures were maintained aerobically on MRS agar (HiMedia, Mumbai, India) at 4 °C and transferred to new media monthly. Prior to use, each strain was cultivated anaerobically (using the AnaeroGen Anaerobic System, Oxoid, Hampshire, UK) in MRS broth at 37 °C for 20 – 24 h (stationary growth phase). These cultures were harvested through centrifugation (4500 *g* × 15 min, and 4 °C), washed twice and resuspended in sterile saline solution (0.85 g/100 mL) to obtain cell suspensions with an OD reading at 625 nm (OD625) of 0.5. This suspension provided viable counts of in the

range of 7 - 8 log CFU/mL for each strain when spread plated in MRS agar (HiMedia, Mumbai, India).

Strains of *Listeria monocytogenes* (INCQS 00266, originally ATCC 7644) and *Escherichia coli* (INCQS 00219, originally ATCC 8739) used in assays of antagonistic activity and coaggregation were obtained from the National Institute for Quality Control in Health (Oswaldo Cruz Foundation, Rio de Janeiro, Brazil). Stocks were stored in brain heart infusion (BHI) broth (HiMedia, Mumbai, India) containing glycerol (Sigma-Aldrich, St. Louis, USA; 20 mL/100 mL) at -20 °C. Prior to use, each strain was aerobically grown in BHI broth at 37 °C for 20 – 24 h (stationary growth phase), harvested through centrifugation (4500 *g* × 15 min, and 4 ºC), washed twice and resuspended in sterile saline solution to obtain cell suspensions with an OD625 of 0.1. This suspension provided viable cell counts in the range of 7 – 8 log CFU/mL when pour plated on BHI agar (HiMedia, Mumbai, India).

2.2 QUE and RES

QUE and RES were obtained from Sigma-Aldrich (purity ≥ 95%; St. Louis, USA). The solutions of QUE and RES were prepared in MRS broth with dimethyl sulfoxide (DMSO, 10%, v/v) immediately before use in assays in an amount sufficient to provide initial QUE or RES concentrations of 2048 and 1400 μg/mL.

2.3 Determination of the minimum inhibitory concentration (MIC)

The MIC values of QUE and RES on the tested *Lactobacillus* strains were determined using a microdilution in broth procedure (CLSI, 2015). Initially, 100 μL-aliquots of the different solutions of QUE or RES were dispensed into wells of a 96-well microplate, and each initial concentration was then serially diluted to provide at least eight

different final concentrations. Subsequently, 100 μL of a suspension (7 – 8 log CFU/mL) of the test *Lactobacillus* strain was added to each well. The final tested concentrations were in the range of 1024 – 87.5 μg/mL for QUE and RES. The microplate with lid was statically incubated at 30 °C for 24 h. Each microplate included a set of positive and negative controls. MIC was considered the lowest concentration of QUE or RES capable of causing visual growth inhibition of the target strain.

2.4 Assessment of the effects of QUE and RES on probiotic-related physiological properties in vitro

2.4.1 Tolerance to different pH values and bile salt concentrations

The tolerance to different pH values and bile salt concentrations was assessed by inoculating 1 mL-aliquots of the inoculum suspension of each tested *Lactobacillus* strain (grown anaerobically in MRS broth, 20 – 24 h, 37 °C, using the AnaeroGen Anaerobic System, Oxoid, Hampshire, UK) in 10 mL PBS (50 mM K_2HPO_4/KH_2PO_4; final viable cell counts in the range of 6 - 7 log CFU/mL) containing different concentrations (MIC, 1/2 MIC or 1/4 MIC) of QUE or RES with pH adjusted to 2, 3 or 5 using 1 M HCl or supplemented with 0.15, 0.3 or 1% (w/v) bile salts (Sigma-Aldrich, St. Louis, USA). The mixtures were incubated aerobically at 37 °C under stirring (150 rpm). At different incubation time intervals (1, 2 and 3 h), 1 mL-aliquots were removed from each mixture, serially diluted in sterile peptone (0.15 g/100 mL) water and spread plated onto MRS agar for enumeration of viable cells. After an incubation period of 48 h at 37 °C under anaerobiosis (using the Anaerobic System AnaeroGen, Oxoid Hampshire, UK), the viable cells were counted, and the results were expressed as the log CFU/mL. For controls, *Lactobacillus* strains were cultivated in PBS with pH 7.2 adjusted using 1 M NaOH in MRS without bile salts and QUE or RES (Jacobsen et al., 1999; Monteagudo-Mera et al.,

2012). To determine whether QUE or RES affect acid and bile salt tolerance, the viable counts of tested *Lactobacillus* strains at a specific pH or bile salt concentration in the presence or absence of QUE or RES were compared.

2.4.2 Cell surface hydrophobicity

Lactobacillus cells grown anaerobically in MRS broth (20 – 24 h, 37 °C, using the Anaerogen Anaerobic System, Oxoid, Hampshire, UK) were centrifuged (4500 *g* × 15 min, and 4 ºC), washed twice and resuspended in PBS containing different concentrations (MIC, 1/2 MIC or 1/4 MIC) of QUE or RES to achieve an OD at 560 nm of 1.0, named the A560 value (A0).

n-Hexadecane (Sigma-Aldrich, St. Louis, USA) was mixed (1:5) with the cell suspension and vortexed for 2 min. After a 1 h incubation at 37 °C, the A560 value (A) of the formed aqueous layer was measured again. The cell surface hydrophobicity was calculated using the equation: %H = [(A0–A)/A0] × 100, where A0 and A refer to the absorbance values determined before and after the extraction with *n*-hexadecane, respectively (Santos et al., 2015). To determine whether QUE or RES affect cell surface hydrophobicity, the cell surface hydrophobicity of *Lactobacillus* strains treated and not treated with QUE or RES was compared.

2.4.3 Autoaggregation and coaggregation

For the evaluation of autoaggregation capacity, *Lactobacillus* strains grown anaerobically in MRS broth (20 – 24 h, 37 °C, using the AnaeroGen Anaerobic System, Oxoid, Hampshire, UK) were harvested by centrifugation (4,500 *g* × 10 min, 20 °C), washed, resuspended and diluted in saline solution (NaCl 0.85 g/100 mL) containing different concentrations (MIC, 1/2 MIC or 1/4 MIC) of QUE or RES to achieve an OD at

660 nm (OD660nm) of 0.3. After a 60 min incubation at 37 °C, the OD660nm value was measured again. The autoaggregation was determined using the equation: % autoaggregation = [(OD0–OD60)/OD0] × 100, where OD0 refers to the initial OD value, and OD60 refers to the OD value determined after the 60 min incubation (Todorov et al., 2008).

For the evaluation of coaggregation capacity, the *Lactobacillus* strains were similarly grown in MRS broth, harvested by centrifugation (4,500 *g* × 10 min, 20 °C), washed, resuspended and diluted in saline solution (NaCl 0.85 g/100 mL) containing different concentrations of QUE or RES to achieve an OD660nm value of 0.3. A 750 µL-aliquot of a suspension of the tested *Lactobacillus* was mixed with the same volume of a suspension of the coaggregation partner (*L. monocytogenes* INCQS 00266 or *E. coli* INCQS 00219) and vortexed for 30 s. The OD660nm value was determined at time zero (baseline - just after mixing the suspensions) and after 60 min incubation at 37 °C. Coaggregation was measured using the equation: % coaggregation = [(OD0–OD60)/OD0] × 100, where OD0 refers to the initial OD value determined at time zero and OD60 refers to the OD value of the supernatant after 60 min of incubation (Todorov et al., 2008). To determine whether QUE or RES affect the autoaggregation or coaggregation capacity, the autoaggregation or coaggregation capacity of test *Lactobacillus* strains treated and not treated with QUE or RES were compared.

2.4.4 Antagonistic activity against pathogens

The antagonistic activity of each *Lactobacillus* strain against the indicator bacterial strains (*L. monocytogenes* INCQS 00266 and *E. coli* INCQS 00219) was evaluated using an agar spot test. The strains were cultivated anaerobically in MRS both (20 – 24 °C, 37 °C, using the AnaeroGen Anaerobic System, Oxoid, Hampshire, UK), followed by

supplementation of the medium with different concentrations (MIC, 1/2 MIC or 1/4 MIC) of QUE or RES. Subsequently, a 10-µL aliquot of this mixture (viable cell counts in the range of 7 – 8 log CFU/mL) was spotted on the surface of MRS agar containing 0.2% (w/v) glucose and 1.2% (w/v) bacteriological agar (HiMedia, Mumbai, India) and incubated anaerobically (using the AnaeroGen Anaerobic System, Oxoid, Hampshire, UK) for 24 h at 37 °C. A 1-mL aliquot of each indicator bacterium suspension was mixed with 18-mL soft BHI agar (with 0.7% agar) and poured over the spot-inoculated MRS agar. The plates were incubated aerobically at 37 °C for 48 h. The antagonistic activity was recorded as the diameter (mm) of growth inhibition zones around each spot. A free growth inhibition zone with a diameter greater than 1 mm (around the spot) was considered as positive inhibitory activity (Jacobsen et al., 1999). Non-inoculated MRS agar and MRS agar without QUE or RES were used as negative controls (Jacobsen et al., 1999). To determine whether QUE or RES affect the antagonistic activity, the diameter of the growth inhibition zones of indicator bacteria caused by the *Lactobacillus* strains treated and not treated with QUE or RES were compared.

2.4.5 Survival under simulated gastrointestinal conditions

Lactobacillus strains grown anaerobically in MRS broth (20 – 24 h, 37 °C, using the AnaeroGen Anaerobic System, Oxoid, Hampshire, UK) were exposed to simulated gastrointestinal conditions in MRS broth with and without QUE (1/2 MIC) or RES (1/2 MIC). Initially, 10 mL-aliquots of MRS broth were placed in glass flasks (50 mL) and inoculated with the examined *Lactobacillus* strain (final viable count in the range of 6 – 7 log CFU/mL). The simulation in these flasks was performed continuously in phases mimicking mastication and conditions in the esophagus-stomach, duodenum and ileum. Mechanical agitation was used to simulate the peristaltic movements, and the test was

performed in an incubator at 37 °C with rotation adjustment in each phase. Mastication was simulated using 100 U α-amylase diluted in 1 mL of 1 mM $CaCl_2$, pH adjusted to 6.9 with 1 M $NaHCO_3$ and exposure time of 2 min at 200 rpm; esophagus-stomach conditions were simulated with 25 mg of pepsin diluted in 1 mL of 0.1 M HCl, added at a rate of 0.05 mL/mL, with gradually decreasing pH achieved using 1 M HCl (pH 5.5/10 min; pH 4.6/10 min; pH 3.8/10 min; pH 2.8/20 min; pH 2.3/20 min; and pH 2/20 min) under stirring (130 rpm). Duodenal conditions were simulated with 2 g pancreatin/L of 0.1 M $NaHCO_3$ and 12 g bovine bile salts/L of 0.1 M $NaHCO_3$, pH adjusted to 5 with 0.1 M $NaHCO_3$ and an exposure time of 30 min under stirring (45 rpm); the ileal conditions were simulated with pH adjusted to 6.5 using 0.1 M $NaHCO_3$, exposure time of 60 min under stirring (45 rpm). All the enzymes and bovine bile salts were purchased from Sigma-Aldrich (St. Louis, USA). After each simulated gastrointestinal condition, 100 μL-aliquots of inoculated MRS broth with or without QUE or RES were serially diluted in sterile saline solution (NaCl 0.85 g/100 mL) and plated on MRS agar (Meira et al., 2015).

After an incubation period of 48 h at 37 ºC under anaerobiosis (using the AnaeroGen Anaerobic System, Oxoid, Hampshire, UK), the viable cells were counted, and the results were expressed as log CFU/mL. Inoculated MRS broth maintained at 37 °C without QUE or RES was used as a control. A detection limit of 1 log cfu/mL was used in these assays. To determine whether QUE or RES affect the survival of the tested *Lactobacillus* strains when exposed to simulated gastrointestinal digestion, the viable counts of these strains when exposed to each step of the simulated digestion in media with or without QUE or RES were compared.

2.5 Statistical analysis

All assays were performed in triplicate in three independent experiments, and the results are expressed as the average of the obtained data. Statistical analyses were performed to determine significant differences ($p \leq 0.05$) among the obtained results using ANOVA, followed by Tukey's test. The analyses were performed using the software GraphPad Prism 5.0 (San Diego, CA, USA).

3. Results

3.1 MIC determination

QUE showed an MIC of 1024 µg/mL against *L. plantarum* 49, *L. plantarum* 53 and *L. paracasei* 106 and 512 µg/mL against *L. fermentum* 263 and *L. fermentum* 296. RES presented an MIC of 1024 µg/mL against the tested *Lactobacillus* strains, with the exception of *L. paracasei* 106, for which the MIC was >1024 µg/mL. These results show that QUE and RE present high MIC values against all tested *Lactobacillus* strains.

Concentrations referring to MIC, 1/2 MIC and 1/4 MIC of QUE and RES against each of the examined potentially probiotic *Lactobacillus* strains were included in assays to measure the effects of these compounds on probiotic-related *in vitro* physiological properties.

3.2 Acid and bile salt tolerance

The examined *Lactobacillus* strains presented an overall good ability to survive at pH 7.2, 5, 3 and 2 during the 3 h of exposure, with the exception of *L. paracasei* 108, which presented viable counts of <1 log CFU/mL when exposed to pH 3 and 2 for 1, 2 or 3 h (data in supplementary material, S1 and Table 1). In most cases, the presence of QUE or

RES at either of the tested concentrations did not affect ($p>0.05$) the survival of the examined *Lactobacillus* strains when exposed to pH 7.2 and 5.

Specifically, *L. paracasei* 108 presented lower viable counts ($p\leq0.05$) at pH 5 after 1 h in the presence of QUE at the MIC when compared to control, as well as after 2 h in the presence of QUE at the MIC and 1/2 MIC; the same behavior was observed for *L. paracasei* 108 at pH 5 after 2 h in the presence of RES at the 1/2 MIC or 1/4 MIC. For *L. paracasei* 106, the viable counts were lower ($p\geq0.05$) at pH 5 after 3 h in the presence of RES at either of the tested concentrations when compared to the control; similar results were observed for *L. fermentum* 263 after 2 and 3 h in the presence of RES and QUE at the MIC (data in supplementary material, S1).

Specifically, *L. plantarum* 53 presented higher counts ($p\leq0.05$) at pH 2 after 2 h of exposure to QUE and RES at the MIC and 1/4 MIC when compared to the control (Table 1). Similarly, *L. paracasei* 106 and *L. fermentum* 263 demonstrated higher counts at pH 2 after 3 h in the presence of QUE or RES at either of the tested concentrations. *L. fermentum* 296 also presented higher counts at pH 3 after 2 h in the presence of QUE at the 1/2 MIC and 1/4, and at pH 2 after 1 h in the presence of QUE at MIC and 1/2 MIC, when compared to the control. Overall, these data indicated that the presence of QUE or RES was more effective in exerting protective effects on some of the tested *Lactobacillus* strains when exposed to more acidic pH values (i.e., pH 2 or 3).

The six examined *Lactobacillus* strains presented a good ability to survive in the presence of 0.15% bile salts during the measured 3 h exposure period, as well as in the presence of 0.3% bile salts up to 1 h of exposure. Only *L. paracasei* 108 had a sharp decrease in viable counts (<1 log cfu/mL) in the presence of 0.3% bile salts after 2 or 3 h of exposure, as well as in the presence of 1% bile salts after 1, 2 or 3 h of exposure (data in supplementary material, S2). Specifically, *L. plantarum* 49 presented higher counts

($p \leq 0.05$) in the presence of 1% bile salts after 3 h of exposure to QUE at the 1/2 MIC and 1/4 MIC, and RES at the MIC and 1/4 MIC, when compared to the control. In contrast, *L. paracasei* 108 presented lower counts ($p \leq 0.05$) in the presence of 0.3% bile salts after 1 h of exposure to QUE at all concentrations, when compared to the control (data in supplementary material, S2). Overall, the presence of QUE or RES at either of the tested concentrations did not affect the viable counts of the examined *Lactobacillus* strains when exposed to 0.15, 0.3 or 1% bile salts during the measured 3 h exposure period.

3.3 Cell surface hydrophobicity

The cultivation of *L. paracasei* 106, *L. paracasei* 108, *L. fermentum* 263 and *L. fermentum* 296 in the presence of QUE at either of the tested concentrations induced increased cell surface hydrophobicity ($p \leq 0.05$) when compared to control cells. An increase in the cell surface hydrophobicity of *L. plantarum* 49 and *L. plantarum* 53 was induced by the MIC of QUE, while the MIC and 1/2 MIC of QUE increased the cell surface hydrophobicity of *L. plantarum* 53. RES, at all tested concentrations, increased ($p \leq 0.05$) the cell surface hydrophobicity of *L. fermentum* 296, and the 1/2 MIC of RES increased the cell surface hydrophobicity of *L. paracasei* 106 and *L. paracasei* 108. However, the 1/4 MIC of QUE and all tested concentrations of RES reduced ($p \leq 0.05$) the cell surface hydrophobicity of *L. plantarum* 49. Nevertheless, the 1/2 MIC and/or 1/4 MIC of RES decreased the cell surface hydrophobicity of *L. plantarum* 53 and *L. paracasei* 108. RES at all tested concentrations exerted no influence on the cell surface hydrophobicity of *L. fermentum* 263 (Table 2).

3.4 Autoaggregation and coaggregation

All examined *Lactobacillus* strains presented the ability to autoaggregate and coaggregate with *E. coli* and *L. monocytogenes*. In most cases, no decrease ($p \leq 0.05$) in autoaggregation capacity was caused by QUE or RES at either of the tested concentrations, with the exception of the *L. paracasei* 108, which an decrease in autoaggregation capacity was caused by 1/4 MIC of QUE and 1/2 MIC and 1/4 MIC of RES, when compared to control. QUE at all tested concentrations increased ($p \leq 0.05$) the autoaggregation capacity of *L. plantarum* 49 and *L. fermentum* 296, and the MIC and 1/2 MIC of QUE increased the autoaggregation capacity of *L. plantarum* 53 and *L. fermentum* 263. RES at the 1/2 MIC increased the autoaggregation capacity of *L. plantarum* 49 and *L. plantarum* 53. QUE and RES at all tested concentrations did not alter ($p > 0.05$) the autoaggregation capacity of *L. paracasei* 106. RES at all tested concentrations did not alter ($p > 0.05$) the autoaggregation capacity of *L. fermentum* 263 and *L. fermentum* 296, as well as the MIC and/or 1/4 MIC of RES did not alter this property in *L. plantarum* 49 and *L. plantarum* 53 (Table 2).

No decrease in coaggregation capacity was caused by QUE or RES at either of the tested concentrations. QUE at all tested concentrations increased ($p \leq 0.05$) the ability of *L. plantarum* 49, *L. plantarum* 53 and *L. paracasei* 108 to coaggregate with *L. monocytogenes* and/or *E. coli*. RES at all tested concentrations increased the ability of *L. plantarum* 49, *L. plantarum* 53 and *L. paracasei* 108 to coaggregate with *E. coli*, as well as the MIC and 1/2 MIC of RES increased the ability of *L. plantarum* 49, *L. plantarum* 53 e *L. fermentum* 263 to coaggregate with *L. monocytogenes* (Table 2).

3.5 Antagonistic activity against pathogens

The six examined *Lactobacillus* strains presented inhibitory effects against *L. monocytogenes* and *E. coli*. In most cases, the presence of QUE or RES did not affect

($p \leq 0.05$) the antagonistic activity of the *Lactobacillus* strains against the target bacterial strains. In particular, the presence of the MIC and/or 1/2 MIC of QUE increased ($p \leq 0.05$) the antagonistic activity of *L. plantarum* 53 against *L. monocytogenes* and *E. coli*; of *L. paracasei* 106 against *L. monocytogenes*; and of *L. paracasei* 108 against *E. coli*. QUE at all concentrations and RES at 1/2 MIC and 1/4 MIC increased ($p \leq 0.05$) the antagonistic activity of *L. fermentum* 296 against *L. monocytogenes* (Table 3).

3.6 Survival during exposure to simulated gastrointestinal conditions

The average viable counts of the examined *Lactobacillus* strains in growth medium with and without QUE (1/2 MIC) or RES (1/2 MIC) were in the range of 3.2 – 7.7 log CFU/mL up to the 7th step of the *in vitro* digestion (duodenum condition). Average viable counts in the range of 4.6 – 7.4 and 4.4 – 7.4 CFU/mL were observed for the examined *Lactobacillus* strains in medium with QUE or RES, respectively. The decreases in viable counts were always <2 log CFU/mL up to the 7th step of the *in vitro* digestion despite the presence of QUE or RES.

The exposure of *L. plantarum* 49 to the 8th (duodenal conditions) and 9th steps (ileal conditions) of the *in vitro* digestion in media with or without QUE or RES resulted in viable counts of <1 log CFU/mL. Counts of <1 log CFU/mL were also observed for *L. paracasei* 108 when exposed to the 8th and 9th steps of the *in vitro* digestion in these media with QUE, as well as when exposed to the 9th step in medium with RES. The *L. plantarum* 53, *L. paracasei* 106, *L. plantarum* 263 and *L. plantarum* 296 counts were in the range of 4.4 – 6.4 log CFU/mL when exposed to the 8th and 9th steps of the *in vitro* digestion in growth medium with QUE or RES. The average counts of these strains in medium without QUE or RES when exposed to the 8th and 9th steps were in the range of 3.2 – 5.9 log CFU/mL.

The presence of QUE or RES overall did not affect ($p>0.05$) the ability of the examined *Lactobacillus* strains to survive the exposure to the different *in vitro* digestion stages. However, the counts of *L. paracasei* 108 when exposed to the 8th step of the *in vitro* digestion were higher ($p \leq 0.05$) in medium with RES than in medium without RES. Higher counts ($p \leq 0.05$) were also observed for *L. plantarum* 296 when exposed to the 8th and 9th steps of the *in vitro* digestion in medium with QUE or RES.

4. Discussion

QUE and RES presented high MIC values against all six examined potentially probiotic *Lactobacillus* strains, indicating low susceptibility of these strains to both tested polyphenols. The available literature has shown variable results considering the effects of polyphenols and phenolic-rich extracts on the growth of lactic acid bacteria. An early study reported no inhibitory effects of phenolic-rich extracts of spices and medicinal plants (313 - 2500 μg/mL) against different lactic acid bacteria, including *Lactobacillus* species (Chan et al., 2018), as well as of caffeic acid, gallic acid, tannic acid, catechin, epicatechin and QUE (5000 μg/disk) against *Lactobacillus acidophilus* CECT 903 (Hervert-Hernandez et al., 2009). Furthermore, polyphenol/anthocyanin-rich ethanol red fruit extract (0.24 – 250 mg/mL) has shown stimulatory effects on the growth of *Lactobacillus rhamnosus* IMC 501 and *L. paracasei* IMC 502 (Coman et al., 2018), while wine polyphenols have shown to protect the viability of different lactic acid bacteria in a probiotic formulation (Llano et al., 2017).

However, an early study reported that flavan-3-ol-enriched grape seed extract (1 mg/mL) inhibited the growth of *L. plantarum*, *Lactobacillus casei* and *Lactococcus bulgaricus*. These inhibitory effects were associated with the high amounts of gallate-derived compounds (e.g., (-)-epicatechin-3-O-gallate) in the grape seed extract (Tabasco et

al., 2011). The inhibitory effects exerted by some phenolic compounds (mostly in high concentrations) on specific bacterial species have been tentatively related to their ability to cause alterations in the structure of the plasma membrane with changes in polarization and fluidity (Mora-Pale et al., 2015; Wang et al., 2017).

The tolerance to acidic pH and bile salts are important characteristics of probiotics. However, the capability of different *Lactobacillus* strains to survive in acidic conditions as well as in the presence of bile salts is variable (Albuquerque et al., 2017; Monteagudo-Mera et al., 2012). The results of this study showed that QUE and RES exerted no influence on the survival of the tested *Lactobacillus* strains at pH 7.2, but at some tested concentrations, these compounds caused decreases in the survival of three (*L. paracasei* 106, *L. paracasei* 108 and *L. fermentum* 263) of the examined strains when exposed to pH 5.

In contrast, QUE and RES, at some tested concentrations, increased the survival of *L. paracasei* 106, *L. fermentum* 263 and/or *L. fermentum* 296 when exposed to pH 2 or 3 after one of the measured exposure time intervals, indicating that these polyphenols could exert protective effects on probiotic survival in more acidic environments. It has been suggested that food components, including polyphenols, could act as buffers in acidic environments, shielding probiotics against pH values as low as 2 and 3 (Monteagudo-Mera et al., 2012). In disagreement with the findings of this study, an early investigation reported that catechin (0.3%) and gallic acid (0.8%) decreased the survival of the starter lactic acid culture *Streptococcus thermophilus* CHCC 3534 at pH 2 and/or 3. In the same study, catechin and gallic acid increased and decreased the survival of *S. thermophilus* CHCC 3534 in the presence of 0.4% bile salts, respectively (Khalil, 2010).

QUE, at all tested concentrations, increased the cell surface hydrophobicity of *L. paracasei* 106, *L. paracasei* 108, *L. fermentum* 263 and *L. fermentum* 296 when measured

by adhesion to *n*-hexadecane, which is a valid tool to estimate the capability of bacteria to adhere to epithelial cells (Mohanty et al., 2019; Souza et al., 2018). In most cases, QUE and RES caused improvements in the autoaggregation and coaggregation capacity of the examined *Lactobacillus* strains. Aggregation is an important feature for biofilm formation by probiotic bacteria, assisting them in adhering to intestinal mucosa (Souza et al., 2018). The maintenance or improvement of the capacity of probiotics to coaggregate with pathogens should be considered a positive feature of QUE and RES since it is one of the steps to eliminate pathogenic microorganisms from the host gastrointestinal tract (Todorov and Dicks, 2008; Souza et al., 2018).

Few studies have assessed the effects of polyphenols on the adhesion capacity of probiotics. Apple pulp extract (10 and 20 mg/mL) decreased the adhesion of *Lactobacillus grasseri* R to Caco-2 cells, while apple peel extract (10 and 20 mg/mL) and QUE (20 µg/mL) increased the adhesion of *L. grasseri* R and *Lactobacillus casei* FMP to these cells. These data indicate that apple peel extract and QUE could positively affect probiotic-epithelium interactions (Volstatova et al., 2017). In addition, the adaptation of *S. thermophiles* CHCC 3534 in media containing catechin (0.3%) resulted in decreased bacterial adherence to *n*-hexane (Khalil, 2010). Both cell surface hydrophobicity and aggregation are strongly related to the ability of probiotic strains to colonize intestinal mucosa, which reinforces the importance of the overall positive modulatory effects exerted by QUE and RES on these physiological features in the *Lactobacillus* strains used in this study. Phenolic compounds seem to influence bacterial adhesion because of the presence of hydroxyl groups in their structure, enabling protein-protein interactions, which are key factors in the adhesion of bacteria to intestinal mucin and/or epithelial cells (Izquierdo et al., 2009; Bustos et al., 2012).

QUE and RES presented little influence on the antagonistic activity of the examined *Lactobacillus* species against *L. monocytogenes* and *E. coli*. The antagonistic activities of *L. plantarum* 53 against *L. monocytogenes* and *E. coli*, as well as of *L. paracasei* 108 against *E. coli*, were increased by MIC and 1/2 MIC, while the antagonistic activity of *L. fermentum* 296 against *L. monocytogenes*, were increased by QUE at all concentrations and RES at 1/2 MIC and 1/4 MIC. Only one previous study reported that the combined use of a commercial phenolic-rich green tea extract with probiotics exerted increased inhibitory effects toward *Staphylococcus aureus* and *Streptococcus pyogenes*. The authors stated that the occurrence of an enhanced inhibitory effect from the action of probiotics toward pathogens induced by the presence of phenolic compounds could be due to additional mechanisms offered by the latter to act on target cells (Su et al., 2008).

QUE and RES exerted no negative impact on the survival of the examined *Lactobacillus* strains when exposed to simulated *in vitro* digestion. Specifically, QUE and RES increased the survival of *L. paracasei* 108 when exposed to the duodenal conditions. Early studies have reported that phenolic-rich matrices could protect probiotic *Lactobacillus* strains during the passage through the gastrointestinal tract. Grape marc protected *L. plantarum* 12A, *L. plantarum* PU1, *L. paracasei* 14A and *Bifidobacterium breve* 15A when exposed to a stomach-mimicking condition (Campanella et al., 2017), and mashed tomato protected *Lactobacillus reuteri* ATCC 55730 when exposed to simulated gastrointestinal conditions (García-Hernández et al., 2018). These possible protective effects of phenolic compounds have been primarily attributed to their antioxidant properties, which could protect probiotic cells from the damage caused by exposure to the harsh conditions found in the gastrointestinal tract (Maukonen and Saarela, 2015). The fact that QUE and RES did not exert any negative impact on the survival of the potentially probiotic *Lactobacillus* strains is an interesting finding because only the bioactive

components that resist the stomach and small intestine conditions can reach the large intestine and exert their beneficial effects on the host (García Hernández et al., 2018).

It is noteworthy to cite that the different effects exerted by QUE and RES on the measured *in vitro* properties of the tested potentially probiotic *Lactobacillus* strains could be associated with the specific hydroxylation pattern of the molecules of these polyphenols. Indeed, the number and position of these hydroxyl groups in the phenolic ring seem to be associated with the importance of different biological effects exerted by polyphenols (Oh et al., 2015; de Souza et al., 2018). QUE presents more hydroxyl groups in molecule than RES, which could be responsible for the strongest protective effects exerted by the former on some of the measured *in vitro* properties in tested *Lactobacillus* strains.

In conclusion, the results obtained in this study showed that the six tested potentially probiotic *Lactobacillus* strains present low susceptibility to QUE and RES, being necessary high concentrations of these polyphenols to cause growth inhibition. The presence of QUE and RES at the various tested concentrations (MIC, 1/2 MIC and 1/4 MIC) in most cases exerted no influence or improved the different measured *in vitro* functionality-related properties of the examined *Lactobacillus* strains, as well as their ability to survive experimental conditions mimicking the gastrointestinal tract. However, in some conditions, the presence of QUE or RES decreased the cell surface hydrophobicity and the ability to survive moderate pH in some of the tested *Lactobacillus* species. When the presence of QUE or RES resulted in increased or decreased performance in the measured functionality-related properties, the effects varied relative to the type of polyphenol, concentration assayed and *Lactobacillus* strain tested. Finally, these results indicate that the combined use of QUE or RES with probiotic *Lactobacillus* strains could result in some advantage with respect to the potential beneficial effects exerted on the host.

However, the concentration of these compounds should be cautiously considered to achieve these desirable effects and will vary according to the probiotic strain selected.

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References

Abushelaibi, A., Al-Mahadin, S., El-Tarabily, K., Shah, N.P., Ayyash, M., 2017. Characterization of potential probiotic lactic acid bacteria isolated from camel milk. LWT - Food Sci. Technol. 79, 316-325.

Albuquerque, T.M.R., Garcia, E.F., de Oliveira Araújo, A., Magnani, M., Saarela, M., de Souza, E.L., 2017. In vitro characterization of *Lactobacillus* strains isolated from fruit processing by-products as potential probiotics. Probiotics Antimicrob. Proteins 10, 704 - 716.

Bustos, I., Garcia-Cayuela, T., Hernández-Ledesma, B., Peláez, C., Requena, T., Martínez-Cuesta, M. C., 2012. Effect of flavan-3-ols on the adhesion of potential probiotic lactobacilli to intestinal cells. J. Agric. Food Chem. 60, 9082-9088.

Campanella, D., Rizzello, C.G., Fasciano, C., Gambacorta, G., Pinto, D., Marzani, B., Gobbetti, M., 2017. Exploitation of grape marc as functional substrate for lactic acid bacteria and bifidobacteria growth and enhanced antioxidant activity. Food Microbiol. 65, 25-35.

Chan, C. L., Gan, R.Y., Shah, N.P., Corke, H., 2018. Polyphenols from selected dietary spices and medicinal herbs differentially affect common food-borne pathogenic bacteria and lactic acid bacteria. Food Cont. 92, 437-443.

CLSI, 2012. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-second Informational Supplement (CLSI M100–S22). Clinical and Laboratory Standards Institute, Wayne, PA.

Coman, M.M., Oancea, A.M., Verdenelli, M.C., Cecchini, C., Bahrim, G.E., Orpianesi, C., Silvi, S., 2018. Polyphenol content and *in vitro* evaluation of antioxidant, antimicrobial and prebiotic properties of red fruit extracts. Eur. Food Res. Technol. 244, 735-745.

Cueva, C., Gil-Sánchez,I., Ayuda-Durán, B., González-Manzano, S., González-Paramás, A.M., Santos-Buelga, C., Moreno-Arribas, M., 2017. An integrated view of the effects of wine polyphenols and their relevant metabolites on gut and host health. Molecules 22, 99.

de Brito-Alves, J.L., de Sousa, V. P., Cavalcanti Neto, M.P., Magnani, M., Braga, V.A., Costa-Silva, J.H.D., Pirola, L., 2016. New insights on the use of dietary polyphenols or probiotics for the management of arterial hypertension. Front. Physiol. 7, 448.

de Souza, E.L., Albuquerque, T.M.R., dos Santos, A.S., Massa, N.M.L., de Brito-Alves, J.L., 2018. Potential interactions among phenolic compounds and probiotics for mutual boosting of their health-promoting properties and food functionalities–A review. Crit. Rev. Food. Sci. Nutr. 1-15.

Dolinsky, V.W., Chakrabarti, S., Pereira, T.J., Oka, T., Zordoky, B.N., Morton, J.S., Dyck, J.R., 2013. Resveratrol prevents hypertension and cardiac hypertrophy in hypertensive rodents. Can. J. Diabetes 37, S23.

Espinosa, R.R., Inchingolo, R., Alencar, S.M., Rodriguez-Estrada, M.T., Castro, I.A., 2015. Antioxidant activity of phenolic compounds added to a functional emulsion containing omega-3 fatty acids and plant sterol esters. Food Chem. 182, 95-104.

Farhadi, K., Esmaeilzadeh, F., Hatami, M., Forough, M., Molaie, R., 2016. Determination of phenolic compounds content and antioxidant activity in skin, pulp, seed, cane and leaf of five native grape cultivars in West Azerbaijan province, Iran. Food Chem. 199, 847-855.

Garcia, EF., Luciano, W.A., Xavier, D.E., Costa, W.K.A., Oliveira, K., Franco, O., Morais, M., Lucena, B., Picão, R., Magnani, M., Saarela, M., de Souza, E., 2016. Identification of lactic acid bacteria in fruit pulp processing byproducts and potential probiotic properties of selected *Lactobacillus* strains. Front. Microbiol. 7, 1371. doi: 10.3389/fmicb.2016.01371

García-Hernández, J., Hernández-Pérez, M., Peinado, I., Andrés, A., Heredia, A., 2018. Tomato-antioxidants enhance viability of *L. reuteri* under gastrointestinal conditions while the probiotic negatively affects bioaccessibility of lycopene and phenols. J. Funct. Foods 43, 1-7.

Gelen, V., Şengül, E., Gedikli, S., Gür, C., Özkanlar, S., 2017. Therapeutic effect of quercetin on renal function and tissue damage in the obesity induced rats. Biomed. Pharmacother. 89, 524-528.

Georgakouli, K., Mpesios, A., Kouretas, D., Petrotos, K., Mitsagga, C., Giavasis, I., & Jamurtas, A., 2016. The effects of an olive fruit polyphenol-enriched yogurt on body composition, blood redox status, physiological and metabolic parameters and yogurt microflora. Nutrients 8, 344.

Gharbi, Y., Fhoula, I., Ruas-Madiedo, P., Afef, N., Boudabous, A., Gueimonde, M., Ouzari, H. I., 2019. *In-vitro* characterization of potentially probiotic *Lactobacillus* strains isolated from human microbiota: interaction with pathogenic bacteria and the enteric cell line HT29. Ann. Microbiol. 69, 61–72.

Hervert-Hernández, D., Pintado, C., Rotger, R., Goñi, I., 2009. Stimulatory role of grape pomace polyphenols on *Lactobacillus acidophilus* growth. Int. J. Food. Microbiol. 136, 119-122.

Hossen, MS., Ali, M.Y., Jahurul, M.H.A., Abdel-Daim, M.M., Gan, S.H., Khalil, M.I., 2017. Beneficial roles of honey polyphenols against some human degenerative diseases: A review. Pharmacol. Rep. 69, 1194-1205.

Izquierdo, E., Horvatovich, P., Marchioni, E., Aoude-Werner, D., Sanz, Y., Ennahar, S., 2009. 2-DE and MS analysis of key proteins in the adhesion of *Lactobacillus plantarum*, a first step toward early selection of probiotics based on bacterial biomarkers. Electrophoresis 30, 949-956.

Jacobsen, C.N., Nielsen, V.R., Hayford, A.E., Møller, P.L., Michaelsen, K.F., Paerregaard, A., Jakobsen, M., 1999. Screening of probiotic activities of forty-seven strains of *Lactobacillus* spp. by in vitro techniques and evaluation of the colonization ability of five selected strains in humans. Appl. Environ. Microbiol. 65, 4949-4956.

Khalil, R.K., 2010. Influence of gallic acid and catechin polyphenols on probiotic properties of *Streptococcus thermophilus* CHCC 3534 strain. World J. Microbiol. Biotechnol. 26, 2069-2079.

Lacey, A.M.L., Pérez-Santín, E., López-Caballero, M.E., Montero, P., 2014. Biotransformation and resulting biological properties of green tea polyphenols produced by probiotic bacteria. LWT –Food Sci. Technol. 58, 633-638.

Llano, D.G., Gil-Sánchez, I., Esteban-Fernández, A., Ramos, A.M., Fernández-Díaz, M., Cueva, C., Bartolomé, B., 2017. Reciprocal beneficial effects between wine polyphenols and probiotics: an exploratory study. Eur. Food Res. Technol. 243, 531-538.

Llano, D.G., Gil-Sánchez, I., Esteban-Fernández, A., Ramos, A.M., Cueva, C., Moreno-Arribas, M., Bartolomé, B., 2016. Some contributions to the study of oenological lactic acid bacteria through their interaction with polyphenols. Beverages 2, 27.

Maukonen, J., Saarela, M., 2015. Human gut microbiota: does diet matter? Proc. Nutr. Soc. 74, 23-36.

Meira, Q.G.S., Magnani, M., de Medeiros Júnior, F.C., Egito, R.C.R.E., Madruga, M.S., Gullón, B., de Souza, E.L., 2015. Effects of added *Lactobacillus acidophilus* and

Bifidobacterium lactis probiotics on the quality characteristics of goat ricotta and their survival under simulated gastrointestinal conditions. Food Res. Int. 76, 828-838.

Mohanty, D., Panda, S., Kumar, S., Ray, P., 2019. *In vitro* evaluation of adherence and anti-infective property of probiotic *Lactobacillus plantarum* DM 69 against *Salmonella enterica*. Microb. Pathog. 126, 212-217.

Monteagudo-Mera A., Rodrıguez-Aparicio L., Rua J., Martinez B. H., Navasa N, Garcia-Armesto R.M., Ferrero M.A., 2012. *In vitr*o evaluation of physiological probiotic properties of different lactic acid bacteria strains of dairy and human origin. J. Funct. Foods 4, 531–541

Mora-Pale, M., Bhan, N., Masuko, S., James, P., Wood, J., McCallum, S., Koffas, M.A., 2015. Antimicrobial mechanism of resveratrol-trans-dihydrodimer produced from peroxidase-catalyzed oxidation of resveratrol. Biotechnol. Bioeng. 112, 2417-2428.

Moreno-Indias, I., Sánchez-Alcoholado, L., Pérez-Martínez, P., Andrés-Lacueva, C., Cardona, F., Tinahones, F., Queipo-Ortuño, M.I., 2016. Red wine polyphenols modulate fecal microbiota and reduce markers of the metabolic syndrome in obese patients. Food Funct. 7, 1775-1787.

Mueller, M., Zartl, B., Schleritzko, A., Stenzl, M., Viernstein, H., Unger, F.M., 2018. Rhamnosidase activity of selected probiotics and their ability to hydrolyse flavonoid rhamnoglucosides. Bioprocess Biosyst. Eng. 41, 221-228.

Oh, E., Jeon, B., 2015. Synergistic anti-*Campylobacter jejuni* activity of fluoroquinolone and macrolide antibiotics with phenolic compounds. Front. Microbiol. 6, 1129. doi:10.3389/fmicb.2015.01129.

Pacheco-Ordaz, R., Wall-Medrano, A., Goñi, M.G., Ramos-Clamont-Montfort, G., Ayala-Zavala, J.F., González-Aguilar, G.A., 2018. Effect of phenolic compounds on the growth of selected probiotic and pathogenic bacteria. Lett. Appl. Microbiol. 66, 25-31.

Pereira-Caro, G., Oliver, C.M., Weerakkody, R., Singh, T., Conlon, M., Borges, G., Augustin, M.A., 2015. Chronic administration of a microencapsulated probiotic enhances the bioavailability of orange juice flavanones in humans. Free Radic. Biol. Med. 84, 206-214.

Santos, K.M.O., Vieira, A.D.S., Buriti, F.C.A., Nascimento, J.C.F., de Melo, M.E.S., Bruno, L.M., Todorov, S.D., 2015. Artisanal Coalho cheeses as source of beneficial *Lactobacillus plantarum* and *Lactobacillus rhamnosus* strains. Dairy Sci. Technol. 95, 209-230.

Sinha, D., Sarkar, N., Biswas, J., Bishayee, A., 2016. Resveratrol for breast cancer prevention and therapy: Preclinical evidence and molecular mechanisms. Semin. Cancer Biol. 40, 209-232.

Singh, D., Mendonsa, R., Koli, M., Subramanian, M., Nayak, S.K., 2019. Antibacterial activity of resveratrol structural analogues: A mechanistic evaluation of the structure-activity relationship. Toxicol. Appl. Pharmacol. 367, 23-32

Souza, B.M.S., Borgonovi, T.F., Casarotti, S.N., Todorov, S.D., Penna, A.L.B., 2018. *Lactobacillus casei* and *Lactobacillus fermentum* strains isolated from mozzarella cheese: Probiotic potential, safety, acidifying kinetic parameters and viability under gastrointestinal tract conditions. Probiotics Antimicrob. Proteins, 1, 1-15.

Su, P., Henriksson, A., Nilsson, C., Mitchell, H., 2008. Synergistic effect of green tea extract and probiotics on the pathogenic bacteria, *Staphylococcus aureus* and *Streptococcus pyogenes*. World J. Microbiol. Biotechnol. 24, 1837-1842.

Tabasco, R., Sánchez-Patán, F., Monagas, M., Bartolomé, B., Moreno-Arribas, M.V., Peláez, C., Requena, T., 2011. Effect of grape polyphenols on lactic acid bacteria and bifidobacteria growth: resistance and metabolism. Food Microbiol. 28, 1345-1352.

Todorov, S. D., Botes, M., Guigas, C., Schillinger, U., Wiid, I., Wachsman, M.B., Dicks, L.M.T., 2008. Boza, a natural source of probiotic lactic acid bacteria. J. Appl. Microbiol. 104, 465-477.

Todorov, SD., Dicks, L. M., 2008. Evaluation of lactic acid bacteria from kefir, molasses and olive brine as possible probiotics based on physiological properties. Ann. Microbiol. 58, 661.

Volstatova, T., Marsik, P., Rada, V., Geigerova, M., Havlik, J., 2017. Effect of apple extracts and selective polyphenols on the adhesion of potential probiotic strains of *Lactobacillus gasseri* R and *Lactobacillus casei* FMP. J. Funct. Foods 35, 391-397.

Wang, S., Yao, J., Zhou, B., Yang, J., Chaudry, M.T., Wang, M., Yin, W., 2017. Bacteriostatic effect of Quercetin as an antibiotic alternative in vivo and its antibacterial mechanism in vitro. J. Food. Prot. 81, 68-78.

Westfall, S., Lomis, N., Prakash, S., 2018. A novel polyphenolic prebiotic and probiotic formulation have synergistic effects on the gut microbiota influencing Drosophila melanogaster physiology. Artif. Cells Nanomed. Biotechnol. 1-15.

Table 1. Counts (n = 9; average ± standard deviation; log CFU/mL) of potentially probiotic *Lactobacillus* strains when exposed to pH 3 and 2 in media with and without different concentrations of quercetin (QUE) or resveratrol (RES) for different time intervals.

Strains/treatments	pH values/exposure times					
	pH 3			pH 2		
	1 h	2 h	3 h	1 h	2 h	3 h
	L. plantarum 49					
MIC QUE	5.6 ± 0.4^{ABb}	6.4 ± 0.2^{Aa}	2.5 ± 0.6^{Ac}	2.9 ± 0.3^{Bb}	4.0 ± 0.5^{Aa}	3.3 ± 0.7^{Aa}
1/2 MIC QUE	5.4 ± 0.2^{Ba}	4.4 ± 0.4^{Bb}	2.6 ± 0.5^{Ac}	3.5 ± 0.3^{Ba}	2.4 ± 0.2^{Bb}	2.4 ± 0.4^{Ab}
1/4 MIC QUE	6.2 ± 0.2^{Aa}	4.5 ± 0.5^{Bb}	3.2 ± 0.2^{Ac}	3.0 ± 0.3^{Ba}	3.6 ± 0.2^{Aa}	1.4 ± 0.3^{Bb}
MIC RES	5.2 ± 0.2^{Ba}	4.4 ± 0.4^{Bb}	3.4 ± 0.3^{Ac}	4.8 ± 0.4^{Aa}	3.8 ± 0.2^{Ab}	1.5 ± 0.4^{Bc}
1/2 MIC RES	3.3 ± 0.2^{Ca}	3.0 ± 0.4^{Ca}	3.4 ± 0.3^{Aa}	3.6 ± 0.3^{Ba}	2.5 ± 0.7^{ABb}	$< 1 \pm 0.0^{Bc}$
1/4 MIC RES	5.2 ± 0.2^{Ba}	3.2 ± 0.2^{Cb}	3.3 ± 0.4^{Ab}	4.1 ± 0.4^{Aba}	3.4 ± 0.2^{Ab}	2.2 ± 0.4^{Ac}
Control	4.5 ± 0.8^{Ba}	3.4 ± 0.6^{BCab}	3.0 ± 0.3^{Ab}	3.4 ± 0.6^{Ba}	3.2 ± 0.3^{Aa}	2.4 ± 0.2^{Ab}
	L. plantarum 53					
MIC QUE	5.5 ± 0.7^{Aa}	3.2 ± 0.2^{Bc}	4.1 ± 0.5^{Ab}	3.2 ± 0.3^{Ab}	4.4 ± 0.5^{Aa}	2.5 ± 0.7^{ABb}
1/2 MIC QUE	4.4 ± 0.5^{Aa}	3.4 ± 0.6^{ABab}	3.0 ± 0.4^{Bb}	2.0 ± 0.7^{Bb}	3.3 ± 0.5^{BCa}	1.8 ± 1.1^{Bab}
1/4 MIC QUE	3.3 ± 0.4^{Ba}	3.6 ± 0.5^{ABa}	3.3 ± 0.4^{ABa}	3.1 ± 0.2^{Aa}	3.6 ± 0.3^{ABa}	3.8 ± 0.6^{Aa}
MIC RES	4.4 ± 0.3^{Aa}	3.4 ± 0.6^{ABb}	2.0 ± 0.5^{Cc}	2.0 ± 0.6^{Bb}	3.4 ± 0.6^{ABa}	2.2 ± 0.3^{Bb}
1/2 MIC RES	3.3 ± 0.5^{Ba}	3.2 ± 0.3^{Ba}	3.2 ± 0.3^{Ba}	2.3 ± 0.4^{Ba}	2.0 ± 0.6^{Ca}	$< 1 \pm 0.0^{Cb}$
1/4 MIC RES	3.3 ± 0.5^{Ba}	3.6 ± 0.6^{Aa}	$< 1 \pm 0.0^{Db}$	3.4 ± 0.5^{Aab}	3.6 ± 0.3^{ABa}	2.6 ± 0.4^{Bb}
Control	4.7 ± 0.9^{Aa}	4.5 ± 0.4^{Aa}	2.5 ± 0.7^{BCb}	2.2 ± 0.2^{Ba}	2.0 ± 0.8^{Ca}	2.5 ± 0.6^{Ba}
	L. paracasei 106					
MIC QUE	3.4 ± 0.9^{Ab}	5.0 ± 0.4^{Aa}	3.0 ± 0.5^{Bb}	3.2 ± 0.6^{BCa}	3.1 ± 0.5^{ABa}	2.4 ± 0.5^{Aa}
1/2 MIC QUE	4.1 ± 1.1^{Aa}	4.2 ± 0.5^{Aa}	4.2 ± 0.2^{Aa}	3.4 ± 0.3^{Ba}	3.1 ± 0.3^{Aab}	2.3 ± 0.5^{Ab}
1/4 MIC QUE	3.3 ± 0.4^{Aa}	4.1 ± 0.4^{Ba}	3.4 ± 0.5^{ABa}	2.2 ± 0.5^{Ca}	2.2 ± 0.2^{Ca}	1.2 ± 0.3^{Bb}
MIC RES	3.2 ± 0.2^{Aa}	3.5 ± 0.4^{BCa}	2.9 ± 0.5^{Ba}	3.1 ± 0.4^{BCa}	3.4 ± 0.3^{Aa}	2.2 ± 0.3^{Ab}
1/2 MIC RES	3.2 ± 0.4^{Ab}	4.5 ± 0.3^{Aa}	4.1 ± 0.2^{Aa}	5.5 ± 1.3^{Aa}	2.1 ± 1.1^{ABCb}	2.3 ± 0.5^{Ab}
1/4 MIC RES	3.3 ± 0.5^{Aa}	3.3 ± 0.5^{BCa}	3.2 ± 0.3^{Ba}	5.2 ± 1.0^{Aa}	2.3 ± 0.4^{BCb}	2.2 ± 0.3^{Ab}
Control	2.6 ± 0.4^{Aa}	3.1 ± 0.2^{Ca}	3.3 ± 0.3^{Ba}	3.1 ± 0.4^{BCa}	2.3 ± 0.4^{BCa}	$< 1 \pm 0.0^{Cb}$
	L. paracasei 108					

MIC QUE	< 1 ± 0.0	< 1 ± 0.0	< 1 ± 0.0	< 1 ± 0.0	< 1 ± 0.0	< 1 ± 0.0
MIC QUE	< 1 ± 0.0	< 1 ± 0.0	< 1 ± 0.0	< 1 ± 0.0	< 1 ± 0.0	< 1 ± 0.0
1/2 MIC QUE	< 1 ± 0.0	< 1 ± 0.0	< 1 ± 0.0	< 1 ± 0.0	< 1 ± 0.0	< 1 ± 0.0
1/4 MIC QUE	< 1 ± 0.0	< 1 ± 0.0	< 1 ± 0.0	< 1 ± 0.0	< 1 ± 0.0	< 1 ± 0.0
MIC RES	< 1 ± 0.0	< 1 ± 0.0	< 1 ± 0.0	< 1 ± 0.0	< 1 ± 0.0	< 1 ± 0.0
1/2 MIC RES	< 1 ± 0.0	< 1 ± 0.0	< 1 ± 0.0	< 1 ± 0.0	< 1 ± 0.0	< 1 ± 0.0
1/4 MIC RES	< 1 ± 0.0	< 1 ± 0.0	< 1 ± 0.0	< 1 ± 0.0	< 1 ± 0.0	< 1 ± 0.0
	L. fermentum 263					
MIC QUE	6.1 ± 0.4^{Aa}	5.4 ± 0.2^{Bb}	5.4 ± 0.2^{Ab}	5.4 ± 0.3^{Aa}	3.5 ± 0.6^{ABb}	2.2 ± 0.4^{Ac}
1/2 MIC QUE	6.3 ± 0.6^{Aa}	5.6 ± 0.4^{Bab}	5.3 ± 0.3^{Ab}	3.7 ± 0.4^{Ba}	2.7 ± 0.5^{Bb}	2.2 ± 0.2^{Ab}
1/4 MIC QUE	6.5 ± 0.2^{Aa}	5.4 ± 0.3^{Bb}	3.2 ± 0.8^{Bc}	4.8 ± 0.3^{Aa}	1.2 ± 0.3^{Cb}	1.2 ± 0.3^{Bb}
MIC RES	5.2 ± 0.2^{Bb}	6.5 ± 0.3^{Aa}	$< 1 \pm 0.0^{Cc}$	3.1 ± 0.2^{Ba}	3.7 ± 0.4^{Aa}	2.3 ± 0.4^{Ab}
1/2 MIC RES	6.9 ± 0.5^{Aa}	5.3 ± 0.5^{BCb}	4.3 ± 1.1^{ABb}	5.2 ± 0.2^{Aa}	4.5 ± 0.5^{Aa}	2.9 ± 0.9^{Ab}
1/4 MIC RES	4.5 ± 0.2^{Cb}	5.2 ± 0.3^{Ba}	5.5 ± 0.2^{Aa}	3.6 ± 0.5^{Ba}	4.2 ± 0.3^{Aa}	2.5 ± 0.3^{Ab}
Control	6.6 ± 0.2^{Aa}	4.4 ± 0.4^{Cb}	4.2 ± 0.2^{Bb}	5.3 ± 0.4^{Aa}	3.7 ± 0.3^{Ab}	$< 1 \pm 0.0^{Bc}$
	L. fermentum 296					
MIC QUE	6.6 ± 0.4^{Aa}	5.6 ± 0.8^{ABa}	2.3 ± 0.5^{Bb}	3.7 ± 0.3^{Ba}	3.2 ± 0.3^{Aa}	1.3 ± 0.4^{Bb}
1/2 MIC QUE	3.8 ± 1.2^{Bb}	6.2 ± 0.2^{Aa}	3.9 ± 0.3^{Ab}	5.0 ± 0.7^{Aa}	4.1 ± 1.1^{Aa}	2.1 ± 0.5^{ABb}
1/4 MIC QUE	7.0 ± 0.7^{Aa}	6.7 ± 0.3^{Aa}	3.0 ± 0.5^{ABb}	3.0 ± 0.5^{BCa}	3.5 ± 0.7^{Aa}	1.2 ± 0.3^{Bb}
MIC RES	3.7 ± 1.0^{Ba}	4.2 ± 0.6^{Ba}	3.1 ± 0.5^{ABa}	4.6 ± 0.3^{Aa}	3.2 ± 0.2^{Ab}	2.4 ± 0.6^{Ab}
1/2 MIC RES	6.1 ± 0.2^{Aa}	3.9 ± 0.5^{Bb}	2.4 ± 0.6^{Bc}	2.3 ± 0.5^{Ca}	2.0 ± 0.7^{Ba}	1.4 ± 0.6^{ABa}
1/4 MIC RES	7.0 ± 0.8^{Aa}	4.3 ± 0.3^{Bb}	3.1 ± 0.6^{ABc}	2.7 ± 0.7^{BCa}	2.4 ± 0.6^{ABa}	1.1 ± 0.4^{Bb}
Control	6.0 ± 1.4^{ABa}	5.0 ± 0.6^{Ba}	3.5 ± 0.7^{ABb}	2.4 ± 0.6^{Ca}	2.1 ± 0.9^{ABa}	2.5 ± 0.5^{Aa}

Control: 0 µg/mL of QUE or RES.

For *L. plantarum* 49, *L. plantarum* 53, *L. paracasei* 106 and *L. fermentum* 263 - MIC of QUE: 1024 µg/mL, 1/2 MIC of QUE: 512 µg/mL, 1/4 MIC of QUE: 256 µg/mL, MIC of RES: 1024 µg/mL, 1/2 MIC of RES: 512 µg/mL, 1/4 MIC of RES: 256 µg/mL; For *L. paracasei* 108 and *L. fermentum* 296 - MIC of QUE: 512 µg/mL, 1/2 MIC of QUE: 256 µg/mL, 1/4 MIC of QUE: 128 µg/mL, MIC of RES: 1024 µg/mL, 1/2 MIC: of RES 512 µg/mL, 1/4 MIC of RES: 256 µg/mL.

$^{A-C}$: Different superscript capital letters in the same column denote differences ($p \leq 0.05$) between counts for the tested *Lactobacillus* strain when exposed to a pH in media with or without QUE or RES, based on Tukey's test.

$^{a-c}$: Different superscript lower case letters in the same row denote differences ($p \leq 0.05$) in counts for the tested *Lactobacillus* strains when exposed for a pH and a concentration of QUE or RES for different time intervals, based on Tukey's test.

Table 2. Effects of different concentrations of quercetin (QUE) and resveratrol (RES) on cell surface hydrophobicity, autoaggreation and coaggregation properties (n = 9; average ± standard deviation) of potentially probiotic *Lactobacillus* strains.

Treatments	Hydrophobicity (%)	Autoaggregation (%)	Coaggregation (%)	
			L. monocytogenes	*E. coli*
	L. plantarum 49			
MIC QUE	52.5 ± 3.5*	51,2 ± 3.1*	70.0 ± 0.7*	74.8 ± 3.9*
1/2 MIC QUE	22.0 ± 2.8	22.3 ± 3.3*	54.7 ± 6.6*	50.0 ± 2.9*
1/4 MIC QUE	14.0 ± 1.4*	13.8 ± 1.1*	55.6 ± 7.9*	50.4 ± 2.0*
MIC RES	8.9 ± 1.6*	10.6 ± 2.0	31.7 ± 2.3*	38.6 ± 1.9*
1/2 MIC RES	7.3 ± 0.1*	17.0 ± 1.4*	31.2 ± 1.1*	32.9 ± 0.5*
1/4 MIC RES	14.1 ± 1.6*	13.7 ± 1.8*	21.4 ± 2.3	29.0 ± 1.4*
Control	19.1 ± 1.3	9.2 ± 1.2	15.0 ± 4.2	19.0 ± 1.4
	L. plantarum 53			
MIC QUE	44.0 ± 5.7*	41.0 ± 2.8*	51.5 ± 2.1*	28.5 ± 2.1*
1/2 MIC QUE	46.1 ± 1.5*	29.5 ± 0.7*	61.1 ± 7.2*	18.5 ± 2.1*
1/4 MIC QUE	29.0 ± 1.4	22.5 ± 3.5	58.5 ± 7.8*	5.4 ± 2.3*
MIC RES	25.8 ± 1.1	22.6 ± 1.9	48.8 ± 1.7*	7.0 ± 2.1*
1/2 MIC RES	17.0 ± 4.2*	35.8 ± 1.1*	28.5 ± 3.4*	20.3 ± 3.2*
1/4 MIC RES	9.8 ± 1.1*	25.8 ± 0.3	19.9 ± 0.2	17.1 ± 3.0*
Control	26.7 ± 2.4	25.0 ± 1.3	17.3 ± 0.9	2.5 ± 0.1
	L. paracasei 106			
MIC QUE	53.6 ± 5.0*	42.5 ± 3.5	71.1 ± 1.6*	68.0 ± 2.8*
1/2 MIC QUE	43.6 ± 5.1*	30.9 ± 1.3	65.0 ± 4.2*	56.6 ± 2.3*
1/4 MIC QUE	39.0 ± 1.4*	33.5 ± 2.1	71.3 ± 4.7*	31.1 ± 5.5
MIC RES	30.7 ± 0.9	36.6 ± 2.3	62.3 ± 6.1*	42.3 ± 3.3
1/2 MIC RES	46.5 ± 3.5*	33.3 ± 1.1	40.7 ± 6.1	37.3 ± 0.9
1/4 MIC RES	28.5 ± 2.1	31.5 ± 4.9	28.4 ± 0.6	39.2 ± 1.8
Control	29.8 ± 2.9	38.5 ± 9.2	26.1 ± 8.4	38.7 ± 3.6
	L. paracasei 108			
MIC QUE	48.5 ± 2.1*	49.8 ± 2.5*	41.5 ± 2.1*	57.0 ± 2.9*
1/2 MIC QUE	46.9 ± 1.3*	31.8 ± 4.5	35.4 ± 0.9*	62.2 ± 3.1*
1/4 MIC QUE	27.1 ± 4.1*	23.0 ± 4.2*	30.6 ± 2.1*	39.3 ± 1.8*

MIC RES	9.9 ± 1.6	47.7 ± 0.5*	38.7 ± 2.4*	33.0 ± 4.2*
1/2 MIC RES	23.2 ± 2.5*	17.2 ± 3.9*	11.6 ± 2.2	33.1 ± 1.3*
1/4 MIC RES	9.2 ± 1.1*	22.0 ± 2.8*	12.3 ± 2.5	37.9 ± 4.1*
Control	13.8 ± 1.7	38.0 ± 2.9	11.6 ± 1.9	22.7 ± 1.9
	L. fermentum 263			
MIC QUE	37.5 ± 3.5*	39.0 ± 1.4*	40.0 ± 2.8*	43.0 ± 1.4*
1/2 MIC QUE	26.8 ± 1.7*	36.5 ± 2.2*	47.0 ± 4.2*	39.3 ± 1,0*
1/4 MIC QUE	18.6 ± 1.9*	21.1 ± 4.1	52.8 ± 3.9*	39.7 ± 2.5*
MIC RES	10.9 ± 1.6	21.1 ± 1.6	45.2 ± 3.1*	46.2 ± 3.1*
1/2 MIC RES	8.2 ± 1.2	20.0 ± 2.8	37.2 ± 3.1*	36.9 ± 2.7*
1/4 MIC RES	8.2 ± 1.1	17.0 ± 2.8	33.4 ± 3.5	19.9 ± 1.3
Control	8.9 ± 2.9	17.5 ± 6.4	23.5 ± 2.1	21.0 ± 2.9
	L. fermentum 296			
MIC QUE	32.1 ± 1.2*	31.7 ± 2.5*	79.9± 2.7*	68.5± 2.2*
1/2 MIC QUE	26.1 ±1.2*	32.1 ± 3.0*	33.0± 4.3*	34.1± 1.3
1/4 MIC QUE	29.4 ± 2.0*	26.2 ± 1.1*	22.2± 9.7	30.6± 0.8
MIC RES	11.1 ± 1.3*	12.6 ± 3.4	29.1± 1.3	40.7± 3.8*
1/2 MIC RES	9.9 ± 1.1*	15.9 ± 0.6	25.2± 1.1	32.1± 2.9
1/4 MIC RES	13.8 ± 2.5*	15.0 ± 1.4	23.5± 2.1	18.9± 5.8
Control	5.6 ± 1.5	17.8 ± 3.1	18.9± 4.8	29.8± 5.2

Control: 0 µg/mL of QUE or RES.
For *L. plantarum* 49, *L. plantarum* 53, *L. paracasei* 106 and *L. fermentum* 263 - MIC of QUE: 1024 µg/mL, 1/2 MIC of QUE: 512 µg/mL, 1/4 MIC of QUE: 256 µg/mL, MIC of RES: 1024 µg/mL, 1/2 MIC of RES: 512 µg/mL, 1/4 MIC of RES: 256 µg/mL; For *L. paracasei* 108 and *L. fermentum* 296 - MIC of QUE: 512 µg/mL, 1/2 MIC of QUE: 256 µg/mL, 1/4 MIC of QUE: 128 µg/mL, MIC of RES: 1024 µg/mL, 1/2 MIC of RES: 512 µg/mL, 1/4 MIC of RES: 256 µg/mL.
(*): means difference ($p \leq 0.05$) of the measured property in the tested strain in comparison to the control, based on Tukey's test.

Table 3. Antagonistic activities, as measured by agar spot test, of different potentially probiotic *Lactobacillus* strains against *Listeria monocytogenes* INCQS 00266 and *Escherichia coli* INCQS 00219 indicator bacteria in the presence or absence of quercetin (QUE) or resveratrol (RES). The results are expressed as the diameter (mm) of growth inhibition zones (n = 9; average ± standard deviation).

Strains	*L. monocytogenes* INCQS 00266							*E. coli* INCQS 00219						
	Control	MIC QUE	1/2 MIC QUE	1/4 MIC QUE	MIC RES	1/2 MIC RES	MIC/4 RES	Control	MIC QUE	1/2 MIC QUE	MIC/4 QUE	MIC RES	MIC/2 RES	MIC/4 RES
L. plantarum 49	18.5±2.1	16.0±2.8	16.0±2.8	18.0±2.8	20.0±2.8	17.5±2.1	18.0±2.8	16.0±2.8	16.0±1.4	16.5±2.1	20.0±2.8	14.5±3.5	17.5±2.1	17.5±3.5
L. plantarum 53	14.5±2.1	23.0±2.8*	18.5±1.5*	17.5±2.1	18.0±2.8	18.5±3.5	16.5±2.1	14.5±0.7	18.0±1.4*	17.5±2.1	21.0±1.4*	14.5±0.7	15.5±0.7	17.0±2.8
L. paracasei 106	15.5±2.1	17.5±2.1	19.5±0.7*	18.5±0.7	14.0±1.4	17.0±1.4	16.5±2.1	17.5±3.5	14.5±2.1	16.5±0.7	17.0±2.8	16.5±2.1	15.5±2.1	18.0±2.8
L. paracasei 108	16.0±1.4	19.0±1.4	18.0±2.8	16.5±2.1	19.0±1.4	17.5±2.1	18.0±2.8	14.5±0.7	18.5±0.7*	17.5±1.5*	18.0±2.8	16.5±1.7	15.5±0.7	12.0±1.4
L. fermentum 263	19.0±1.4	15.5±1.1	15.5±2.1	17.5±0.8	18.0±2.8	18.5±0.7	18.5±0.7	16.5±3.5	16.5±0.7	16.5±2.1	15.5±3.5	17.0±1.4	18.0±0.7	17.5±3.5
L. fermentum 296	11.0±1.4	16.5±0.7*	21.5±2.1*	21.5±0.7*	14.0±1.4	16.5±2.1*	16.0±1.8*	16.5±2.1	17.5±0.8	18.0±1.4	14.0±2.8	15.0±1.4	13.5±0.9	16.5±2.1

Control: 0 µg/mL of QUE or RES.

For *L. plantarum* 49, *L. plantarum* 53, *L. paracasei* 106 and *L. fermentum* 263 - MIC of QUE: 1024 µg/mL, 1/2 MIC of QUE: 512 µg/mL, 1/4 MIC of QUE: 256 µg/mL, MIC of RES: 1024 µg/mL, 1/2 MIC of RES: 512 µg/mL, 1/4 MIC of RES: 256 µg/mL; For *L. paracasei* 108 and *L. fermentum* 296 - MIC of QUE: 512 µg/mL, 1/2 MIC of QUE: 256 µg/mL, 1/4 MIC of QUE: 128 µg/mL, MIC of RES: 1024 µg/mL, 1/2 MIC of RES: 512 µg/mL, 1/4 MIC of RES: 256 µg/mL.

(*): means difference ($p \leq 0.05$) of the measured inhibition zone caused by the tested *Lactobacillus* strain in the presence of QUE or RES in comparison to the control, based on Tukey's test.

Table 4. Counts (n = 9; average ± standard deviation; log CFU/mL) of different potentially probiotic *Lactobacillus* strains when exposed to the different phases of simulated gastrointestinal digestion in de Mann, Rogosa and Sharpe (MRS) broth with (1/2 MIC) and without quercetin (QUE) or resveratrol (RES).

L. plantarum 49			
Phase	MRS	MRS + QUE (512 µg/mL)	MRS + RES (512 µg/mL)
1	7.2 ± 0.8^{A}	7.3 ± 0.5^{A}	7.3 ± 0.4^{A}
2	7.0 ± 0.6^{A}	7.1 ± 0.2^{A}	7.2 ± 0.2^{A}
3	7.0 ± 0.2^{A}	7.0 ± 0.8^{A}	7.2 ± 0.3^{A}
4	6.9 ± 0.4^{A}	7.1 ± 0.8^{A}	7.2 ± 0.8^{A}
5	6.9 ± 0.6^{A}	7.1 ± 0.2^{A}	6.9 ± 0.8^{A}
6	6.9 ± 0.3^{A}	6.9 ± 0.4^{A}	6.5 ± 1.0^{A}
7	6.2 ± 0.9^{A}	6.1 ± 0.2^{A}	6.7 ± 2.2^{A}
8	< 1 ± 0.0^{A}	< 1 ± 0.0^{A}	< 1 ± 0.0^{A}
9	< 1 ± 0.0^{A}	< 1 ± 0.0^{A}	< 1 ± 0.0^{A}
L. plantarum 53			
Phase	MRS	MRS + QUE (512 µg/mL)	MRS + RES (512 µg/mL)
1	7.3 ± 0.4^{A}	6.9 ± 0.2^{A}	6.5 ± 0.6^{A}
2	7.1 ± 0.6^{A}	6.8 ± 0.4^{A}	7.1 ± 0.2^{A}
3	7.5 ± 0.3^{A}	6.9 ± 0.5^{A}	6.8 ± 0.8^{A}
4	6.9 ± 0.2^{A}	6.8 ± 0.8^{A}	6.7 ± 0.4^{A}
5	6.9 ± 0.9^{A}	6.7 ± 0.2^{A}	6.6 ± 0.5^{A}
6	7.2 ± 0.5^{A}	6.6 ± 0.3^{A}	6.7 ± 0.2^{A}
7	6.8 ± 0.1^{A}	6.7 ± 0.2^{A}	6.3 ± 0.1^{A}
8	5.9 ± 0.8^{A}	5.8 ± 1.1^{A}	5.5 ± 0.7^{A}
9	5.9 ± 0.7^{A}	5.7 ± 0.3^{A}	5.8 ± 0.2^{A}
L. paracasei 106			
Phase	MRS	MRS + QUE (512µg/mL)	MRS + RES (512 µg/mL)
1	7.2 ± 0.2^{A}	7.2 ± 0.5^{A}	7.0 ± 0.7^{A}
2	7.1 ± 0.2^{A}	6.9 ± 0.4^{A}	6.8 ± 0.2^{A}
3	7.2 ± 0.4^{A}	7.0 ± 0.6^{A}	6.7 ± 0.3^{A}
4	7.0 ± 0.5^{A}	7.4 ± 0.6^{A}	6.9 ± 0.8^{A}
5	6.9 ± 0.3^{A}	6.9 ± 0.7^{A}	6.7 ± 0.9^{A}
6	6.9 ± 0.2^{A}	6.5 ± 0.5^{A}	6.8 ± 0.2^{A}
7	6.9 ± 0.8^{A}	6.8 ± 0.2^{A}	6.8 ± 0.5^{A}
8	5.8 ± 0.3^{A}	5.4 ± 0.5^{A}	5.9 ± 0.6^{A}
9	4.9 ± 0.8^{A}	5.2 ± 0.8^{A}	5.2 ± 0.2^{A}
L. paracasei 108			
Phase	MRS	MRS + QUE (256 µg/mL)	MRS + RES (512 µg/mL)
1	6.9 ± 0.8^{A}	7.1 ± 0.2^{A}	7.1 ± 0.4^{A}
2	6.8 ± 0.3^{A}	6.8 ± 0.4^{A}	7.1 ± 0.2^{A}
3	6.7 ± 0.6^{A}	6.9 ± 0.3^{A}	7.1 ± 0.1^{A}
4	6.6 ± 0.4^{A}	6.9 ± 0.6^{A}	7.1 ± 0.2^{A}
5	5.9 ± 0.7^{A}	6.8 ± 0.3^{A}	6.9 ± 0.5^{A}
6	5.9 ± 1.2^{A}	6.9 ± 0.2^{A}	6.7 ± 0.3^{A}
7	6.2 ± 0.3^{A}	6.7 ± 0.4^{A}	6.5 ± 0.2^{A}
8	< 1 ± 0.0^{B}	< 1 ± 0.0^{B}	6.4 ± 1.3^{A}
9	< 1 ± 0.0^{A}	< 1 ± 0.0^{A}	< 1 ± 0.0^{A}
L. fermentum 263			
Phase	MRS	MRS + QUE (512 µg/mL)	MRS + RES (512 µg/mL)
1	6.7 ± 0.2^{A}	6.7 ± 0.2^{A}	7.4 ± 0.2^{A}
2	6.8 ± 0.5^{A}	7.4 ± 0.4^{A}	7.1 ± 0.3^{A}
3	6.6 ± 0.3^{A}	6.8 ± 0.3^{A}	7.4 ± 0.4^{A}
4	6.8 ± 0.2^{A}	6.5 ± 0.6^{A}	6.7 ± 0.5^{A}
5	6.9 ± 0.1^{A}	6.7 ± 0.2^{A}	7.0 ± 0.2^{A}
6	6.8 ± 0.3^{A}	6.5 ± 0.5^{A}	6.7 ± 0.4^{A}
7	6.9 ± 0.6^{A}	6.1 ± 0.7^{A}	6.5 ± 0.2^{A}

8	5.9 ± 0.8^{A}	5.9 ± 0.3^{A}	6.2 ± 0.4^{A}
9	5.5 ± 0.7^{A}	5.6 ± 0.2^{A}	5.6 ± 0.1^{A}
L. fermentum 296			
Phase	MRS	MRS + QUE (256 µg/mL)	MRS + RES (512 µg/mL)
1	7.4 ± 0.7^{A}	7.3 ± 0.3^{A}	7.3 ± 0.2^{A}
2	7.6 ± 0.5^{A}	7.1 ± 0.2^{A}	7.1 ± 0.4^{A}
3	7.7 ± 0.4^{A}	7.0 ± 0.9^{A}	7.0 ± 0.5^{A}
4	7.5 ± 0.6^{A}	7.2 ± 0.5^{A}	7.0 ± 0.6^{A}
5	7.0 ± 0.4^{A}	6.9 ± 0.6^{A}	6.9 ± 0.3^{A}
6	6.9 ± 0.2^{A}	6.3 ± 0.5^{A}	5.8 ± 0.2^{B}
7	7.1 ± 0.4^{A}	6.3 ± 0.7^{A}	6.5 ± 0.8^{A}
8	3.9 ± 0.2^{B}	5.8 ± 0.2^{A}	5.4 ± 0.2^{A}
9	3.2 ± 0.3^{B}	4.6 ± 0.2^{A}	4.4 ± 0.2^{A}

Phase 1: mouth conditions, pH 6.9, exposure time 2 min; phase 2: esophagus-stomach conditions, pH 5.5, exposure time 12 min; phase 3: esophagus-stomach conditions, pH 4.6, exposure time 22 min; phase 4: stomach conditions, pepsin, pH 3.8, exposure time 32 min; phase 5: stomach conditions, pepsin, pH 2.8, exposure time 52 min; phase 6: stomach conditions, pepsin, pH 2.3, exposure time 72 min; phase 7: stomach conditions, pepsin, pH 2, exposure time 92 min; phase 8: duodenum conditions, pancreatin + bile salts, pH 5, exposure time 122 min; phase 9: ileum conditions, pH 6.5, exposure time 182 min.

$^{A\ -\ B}$: different superscript capital letters in the same row denote differences ($p \leq 0.05$) in counts of the tested *Lactobacillus* strain when exposed to the same simulated digestion phase in MRS with and without QUE or RES.

S1. Counts (n = 9; average ± standard deviation; log CFU/mL) of potentially probiotic *Lactobacillus* strains when exposed to pH 7.2 and 5 in media with and without different concentrations of quercetin (QUE) or resveratrol (RES) for different time intervals.

Strains/treatments	pH values/exposure times					
	pH 7.2			pH 5		
	1 h	2 h	3 h	1 h	2 h	3 h
L. plantarum 49						
MIC QUE	7.4 ± 0.6^{Aa}	7.5 ± 0.6^{Aa}	7.4 ± 0.5^{Aa}	7.3 ± 0.6^{Aa}	7.4 ± 0.4^{Aa}	7.5 ± 0.5^{Aa}
1/2 MIC QUE	7.4 ± 0.5^{Aa}	7.7 ± 0.9^{Aa}	6.8 ± 0.4^{Aa}	7.2 ± 0.3^{Aa}	6.7 ± 0.4^{Aa}	6.4 ± 0.5^{Aa}
1/4 MIC QUE	7.2 ± 0.3^{Aa}	7.7 ± 0.8^{Aa}	6.5 ± 0.6^{Aa}	7.6 ± 0.2^{Aa}	7.3 ± 0.5^{Aa}	7.3 ± 0.2^{Aa}
MIC RES	7.4 ± 0.2^{Aa}	7.4 ± 0.5^{Aa}	7.4 ± 0.3^{Aa}	7.5 ± 0.2^{Aa}	7.4 ± 0.5^{Aa}	7.2 ± 0.2^{Aa}
1/2 MIC RES	7.2 ± 0.2^{Aa}	6.6 ± 0.6^{Aa}	7.4 ± 0.4^{Aa}	7.1 ± 0.2^{Aa}	7.2 ± 0.5^{Aa}	7.2 ± 0.3^{Aa}
1/4 MIC RES	6.8 ± 0.2^{Aa}	6.7 ± 0.5^{Aa}	6.6 ± 0.5^{Aa}	7.4 ± 0.2^{Aa}	7.3 ± 0.2^{Aa}	7.3 ± 0.4^{Aa}
Control	7.3 ± 0.7^{Aa}	7.6 ± 0.5^{Aa}	7.4 ± 0.3^{Aa}	7.5 ± 0.2^{Aa}	7.1 ± 0.3^{Aa}	7.4 ± 0.3^{Aa}
L. plantarum 53						
MIC QUE	7.2 ± 0.6^{Aa}	6.6 ± 0.2^{Aab}	6.3 ± 0.2^{Ab}	6.2 ± 0.8^{Ab}	7.6 ± 0.4^{Aa}	5.9 ± 0.5^{Ab}
1/2 MIC QUE	7.3 ± 0.2^{Aa}	6.7 ± 0.3^{Ab}	6.4 ± 0.2^{Ab}	6.4 ± 0.6^{Aa}	6.6 ± 0.3^{Ba}	6.2 ± 0.2^{Aa}
1/4 MIC QUE	7.0 ± 0.3^{Aa}	6.5 ± 0.7^{Aa}	6.5 ± 0.4^{Aa}	6.6 ± 0.3^{Aa}	6.4 ± 0.5^{Ba}	6.1 ± 0.2^{Aa}
MIC RES	7.3 ± 0.4^{Aa}	6.7 ± 0.2^{Aa}	6.6 ± 0.2^{Ab}	7.3 ± 0.6^{Aa}	6.6 ± 0.3^{Ba}	5.9 ± 0.2^{Ab}
1/2 MIC RES	6.8 ± 0.5^{Aa}	6.4 ± 0.6^{Aa}	6.3 ± 0.2^{Aa}	6.6 ± 0.5^{Aab}	6.7 ± 0.2^{Ba}	6.2 ± 0.2^{Ab}
1/4 MIC RES	6.6 ± 0.4^{Aa}	6.7 ± 0.5^{Aa}	6.5 ± 0.3 ^{Aa}	6.8 ± 0.3^{Aa}	6.7 ± 0.3^{Ba}	5.7 ± 0.3^{Ab}
Control	7.4 ± 0.5^{Aa}	6.8 ± 0.3^{Aa}	6.5 ± 0.5^{Aa}	6.8 ± 0.2^{Ab}	7.5 ± 0.2^{Aa}	6.4 ± 0.4^{Ab}
L. paracasei 106						
MIC QUE	6.6 ± 0.3^{Aa}	6.5 ± 0.7^{ABa}	6.7 ± 0.5^{Aa}	6.4 ± 0.8^{Aa}	6.2 ± 0.2^{Aa}	6.1 ± 0.7^{Aa}
1/2 MIC QUE	6.7 ± 0.4^{Aa}	6.2 ± 0.3^{Ba}	6.4 ± 0.6^{Aa}	6.5 ± 0.4^{Aa}	6.3 ± 0.3^{Aa}	4.4 ± 0.5^{Bb}
1/4 MIC QUE	6.3 ± 0.2^{Aa}	6.8 ± 0.3^{ABa}	6.6 ± 0.6^{Aa}	6.3 ± 0.7^{Aa}	5.9 ± 0.4^{Aa}	5.4 ± 0.7^{ABa}
MIC RES	6.1 ± 0.6^{Aa}	6.7 ± 0.3^{ABa}	6.8 ± 0.4^{Aa}	6.3 ± 0.4^{Aa}	6.1 ± 0.3^{Aa}	5.2 ± 0.4^{Bb}
1/2 MIC RES	6.7 ± 0.6^{Aa}	6.5 ± 0.7^{ABa}	6.1 ± 0.7^{Aa}	6.2 ± 0.2^{Aa}	6.1 ± 0.6^{Aa}	4.6 ± 0.6^{Bb}
1/4 MIC RES	6.3 ± 0.3^{Aa}	6.5 ± 0.6^{ABa}	6.7 ± 0.5^{Aa}	6.4 ± 0.4^{Aa}	6.4 ± 0.8^{Aa}	4.2 ± 0.7^{Bb}
Control	6.6 ± 0.5^{Aa}	7.1 ± 0.5 ^{Aa}	6.7 ± 0.3^{Aa}	6.4 ± 0.3^{Aa}	6.3 ± 0.3^{Aa}	6.3 ± 0.4^{Aa}
L. paracasei 108						

MIC QUE	7.2 ± 0.5^{Aa}	5.2 ± 0.3^{Bb}	5.2 ± 0.4^{Bb}	4.1 ± 0.8^{Bb}	5.5 ± 0.5^{Ba}	4.8 ± 1.1^{ABab}
1/2 MIC QUE	7.2 ± 0.8^{Aa}	6.6 ± 0.3^{Aa}	5.9 ± 1.3^{ABa}	5.7 ± 0.7^{ABa}	4.8 ± 0.4^{BCa}	4.9 ± 1.3^{ABa}
1/4 MIC QUE	7.5 ± 0.7^{Aa}	6.5 ± 0.6^{ABa}	6.6 ± 0.4^{Aa}	5.6 ± 0.7^{ABab}	6.6 ± 0.9^{ABa}	4.4 ± 0.6^{ABb}
MIC RES	6.4 ± 0.6^{Aa}	5.0 ± 0.6^{ABb}	6.5 ± 0.7^{Aa}	5.9 ± 0.4^{Ab}	7.3 ± 0.5^{Aa}	5.6 ± 0.6^{Ab}
1/2 MIC RES	6.7 ± 0.4^{Aa}	5.4 ± 0.3^{ABb}	5.8 ± 1.0^{ABab}	6.2 ± 0.9^{Aa}	4.3 ± 0.9^{BCb}	4.4 ±0.4^{Bb}
1/4 MIC RES	6.8 ± 0.3^{Aa}	5.5 ± 0.2^{ABb}	5.5 ± 0.7^{ABb}	6.3 ± 1.1^{Aa}	4.2 ± 0.4^{Cb}	4.6 ±0.5^{ABb}
Control	6.3 ± 0.5^{Aa}	5.8 ± 1.3^{Aba}	5.9 ± 0.9^{ABa}	6.3 ± 0.4^{Aab}	6.6 ± 0.2^{Aa}	5.4 ± 0.7^{ABb}
L. fermentum 263						
MIC QUE	6.8 ± 0.9^{Aa}	7.5 ± 0.7^{Aa}	5.6 ± 0.2^{Bb}	7.5 ± 0.9^{ABa}	5.2 ± 0.2^{Bb}	5.5 ± 0.4^{Bb}
1/2 MIC QUE	6.2 ± 0.8^{Aab}	6.9 ± 0.4^{Aba}	5.4 ± 0.6^{Bb}	7.3 ± 0.6^{ABa}	6.4 ± 0.5^{Aab}	5.8 ± 0.6^{ABb}
1/4 MIC QUE	7.1 ± 1.0^{Aab}	7.4 ± 0.3^{Aa}	6.2 ± 0.5^{Bb}	7.6 ± 0.7^{ABa}	6.5 ± 0.4^{Aa}	5.0 ± 0.6^{Bb}
MIC RES	6.9 ± 0.2^{Aa}	6.4 ± 0.7^{Bab}	5.6 ± 0.4^{Bb}	7.0 ± 0.4^{ABa}	5.3 ± 0.4^{Bb}	4.7 ± 0.5^{Bb}
1/2 MIC RES	7.4 ± 0.5^{Aa}	6.6 ± 0.4^{Bab}	5.9 ± 0.7^{Bb}	6.1 ± 0.8^{Bab}	6.9 ± 0.2^{Aa}	5.9 ± 0.7^{ABb}
1/4 MIC RES	7.5 ± 0.7^{Aa}	7.6 ± 0.6^{Aba}	6.5 ± 0.5^{ABa}	7.3 ± 0.3^{ABa}	7.4 ± 0.6^{Aa}	6.3 ± 0.2^{Ab}
Control	7.4 ± 0.3^{Aa}	7.5 ± 0.5^{Aba}	7.5 ± 0.5^{Aa}	7.6 ± 0.3^{Aa}	7.3 ± 0.5^{Aa}	6.6 ± 0.3^{Aa}
L. fermentum 296						
MIC QUE	6.5 ± 0.4^{Ba}	6.4 ± 0.5^{Aa}	4.3 ± 1.1^{Bb}	7.4 ± 0.3^{Aa}	5.8 ± 2.3^{Aa}	7.7 ± 0.5^{Aa}
1/2 MIC QUE	7.7 ± 0.3^{Aa}	6.2 ± 0.4^{Ab}	5.5 ± 0.5^{ABb}	6.3 ± 0.4^{Ba}	6.6 ± 0.3^{Aa}	6.9 ± 1.6^{Aa}
1/4 MIC QUE	6.1 ± 0.2^{Ba}	6.5 ± 0.7^{Aab}	5.6 ± 0.2^{Bb}	7.2 ± 0.3^{Aa}	5.9 ± 2.0^{Aa}	5.7 ± 1.8^{Aa}
MIC RES	6.5 ± 0.9^{ABa}	6.5 ± 0.6^{Aa}	6.2 ± 0.5^{Aa}	6.5 ± 0.2^{Ba}	6.3 ± 1.2^{Aa}	7.7 ± 0.4^{Aa}
1/2 MIC RES	6.6 ± 0.4^{Ba}	5.9 ± 0.9^{Aa}	6.6 ± 0.6^{Aa}	6.5 ± 0.6^{ABa}	6.3 ± 0.8^{Aa}	6.9 ±1.5^{Aa}
1/4 MIC RES	6.5 ± 0.8^{Ba}	6.0 ± 0.5^{Aa}	6.5 ± 0.5^{Aa}	5.3 ± 0.5^{Cb}	6.5 ± 0.4^{Aa}	7.7 ± 0.5^{Aa}
Control	6.4 ± 0.4^{Ba}	5.4 ± 0.5^{Ab}	4.4 ± 0.5^{Bb}	7.4 ± 0.6^{Aa}	6.6 ± 0.3^{Aa}	6.9 ± 1.6^{Aa}

Control: 0 µg/mL of QUE or RES.

For *L. plantarum* 49, *L. plantarum* 53, *L. paracasei* 106 and *L. fermentum* 263: MIC of QUE 1024 µg/mL, 1/2 MIC of QUE 512 µg/mL, 1/4 MIC of QUE 256 µg/mL, MIC of RES 1024 µg/mL, 1/2 MIC of RES 512 µg/mL, 1/4 MIC of RES 256 µg/mL; For *L. paracasei* 108 and *L. fermentum* 296: MIC of QUE 512 µg/mL, 1/2 MIC of QUE 256 µg/mL, 1/4 MIC of QUE 128 µg/mL, MIC of RES 1024 µg/mL, 1/2 MIC of RES 512 µg/mL, 1/4 MIC of RES 256 µg/mL.

$^{A-C}$: Different superscript capital letters in the same column denote differences ($p \leq 0.05$) between counts for the tested *Lactobacillus* strain when exposed to a pH for a specific time interval in media with or without different concentrations of QUE or RES, based on Tukey's test.

$^{a-c}$: Different superscript small letters in the same row denote differences ($p \leq 0.05$) in counts for the tested *Lactobacillus* strains when exposed for a specific pH and a concentration of QUE or RES for different time intervals, based on Tukey's test.

S2. Counts (n = 9; average ± standard deviation; log CFU/mL) of potentially probiotic *Lactobacillus* strains when exposed to different bile salt concentrations (w/v) in media with and without different amounts of quercetin (QUE) or resveratrol (RES) for different time intervals.

Treatments	Bile salts concentrations/exposure time											
	0%			0.15%			0.3%			1%		
	1 h	2 h	3 h	1 h	2 h	3 h	1 h	2 h	3 h	1 h	2 h	3 h
L. plantarum 49												
MIC QUE	5.9 ± 0.8^{ABa}	5.1 ± 0.5^{Ba}	5.1 ± 0.8^{Aa}	4.9 ± 0.8^{ABa}	4.4 ± 0.8^{Aa}	4.2 ± 0.2^{Ba}	6.0 ± 0.7^{Aa}	5.5 ± 0.4^{Aa}	5.4 ± 0.3^{Aa}	6.2 ± 0.2^{Aa}	6.0 ± 0.9^{Aab}	5.0 ± 0.3^{Bb}
1/2 MIC QUE	5.4 ± 0.9^{ABa}	5.1 ± 0.5^{Ba}	5.7 ± 0.6^{Aa}	5.7 ± 0.3^{Aa}	4.4 ± 0.3^{Ab}	4.4 ± 0.2^{Bb}	6.1 ± 0.7^{Aa}	5.4 ± 0.7^{Aa}	5.0 ± 0.8^{Aa}	6.1 ± 0.2^{Aa}	6.5 ± 0.7^{Aa}	5.9 ± 0.3^{Aa}
1/4 MIC QUE	6.3 ± 0.2^{ABa}	5.8 ± 0.7^{ABa}	5.8 ± 0.6^{Aa}	4.8 ± 0.4^{Ba}	4.9 ± 0.4^{Aa}	5.6 ± 0.4^{Aa}	5.9 ± 0.7^{Aa}	5.5 ± 0.5^{Aa}	5.4 ± 0.3^{Aa}	6.0 ± 0.5^{Aa}	6.0 ± 0.9^{Aa}	5.9 ± 0.2^{Aa}
MIC RES	6.0 ± 0.3^{ABa}	5.6 ± 1.9^{ABa}	5.6 ± 0.3^{Aa}	5.1 ± 0.2^{Ba}	4.9 ± 0.3^{Aa}	4.7 ± 0.2^{Ba}	5.9 ± 0.8^{Aa}	5.5 ± 0.9^{Aa}	5.5 ± 0.5^{Aa}	6.2 ± 0.8^{Aa}	6.1 ± 0.2^{Aa}	5.9 ± 0.3^{Aa}
1/2 MIC RES	5.7 ± 0.4^{Ba}	5.5 ± 0.7^{Ba}	5.4 ± 0.5^{Aa}	5.4 ± 0.4^{ABa}	4.9 ± 0.3^{Aa}	4.7 ± 0.6^{ABa}	6.0 ± 0.8^{Aa}	5.5 ± 0.6^{Aa}	5.4 ± 0.4^{Aa}	6.0 ± 0.3^{Aa}	6.2 ± 0.4^{Aa}	5.7 ± 0.5^{ABa}
1/4 MIC RES	6.7 ± 0.5^{Aa}	7.1 ± 0.7^{Aa}	5.8 ± 0.2^{Ab}	5.3 ± 0.3^{ABa}	5.1 ± 0.4^{Aa}	4.8 ± 0.5^{ABa}	4.9 ± 0.8^{Aa}	5.5 ± 0.8^{Aa}	5.4 ± 0.2^{Aa}	5.5 ± 0.7^{Aa}	5.3 ± 0.7^{Aa}	5.9 ± 0.2^{Aa}
Control	6.6 ± 0.4^{Aa}	6.3 ± 0.7^{ABab}	5.5 ± 0.2^{Ab}	5.9 ± 0.4^{Aa}	4.8 ± 0.2^{Ab}	4.6 ± 0.4^{Bb}	5.8 ± 0.8^{Aa}	5.5 ± 0.3^{Aa}	5.5 ± 0.5^{Aa}	6.1 ± 0.4^{Aa}	6.1 ± 0.5^{Aa}	5.0 ± 0.2^{Bb}
L. plantarum 53												
MIC QUE	7.1 ± 0.4^{Aa}	7.1 ± 0.5^{Aa}	6.5 ± 0.9^{Aa}	5.9 ± 0.6^{Aa}	4.8 ± 0.9^{Aa}	4.8 ± 0.5^{Aa}	6.9 ± 0.8^{Aa}	5.9 ± 0.5^{ABa}	5.4 ± 0.9^{Aa}	5.6 ± 0.9^{ABa}	5.1 ± 0.5^{Aa}	4.9 ± 0.6^{Aa}
1/2 MIC QUE	6.7 ± 0.5^{Aa}	5.9 ± 0.4^{Ba}	5.5 ± 0.8^{Ba}	5.6 ± 0.9^{Aa}	5.1 ± 0.4^{Aa}	4.9 ± 0.8^{Aa}	6.3 ± 0.2^{Aa}	5.4 ± 0.3^{Bb}	4.9 ± 0.3^{Ab}	5.6 ± 0.4^{Aa}	5.7 ± 0.5^{Aa}	4.8 ± 0.7^{Aa}
1/4 MIC QUE	7.1 ± 0.5^{Aa}	6.7 ± 0.5^{ABab}	5.9 ± 0.3^{Bb}	6.1 ± 0.5^{Aa}	5.1 ± 0.7^{Aab}	4.8 ± 0.5^{Ab}	6.6 ± 0.5^{Aa}	5.7 ± 0.5^{ABa}	5.3 ± 0.9^{Aa}	5.6 ± 0.6^{Aa}	5.9 ± 0.9^{Aa}	5.7 ± 0.4^{Aa}
MIC RES	6.4 ± 0.6^{Aa}	5.6 ± 0.6^{Bab}	4.8 ± 0.8^{Bb}	5.7 ± 0.3^{Aa}	5.2 ± 0.4^{Aab}	4.7 ± 0.2^{Ab}	6.9 ± 0.8^{Aa}	6.8 ± 0.6^{Aa}	5.7 ± 0.7^{Aa}	5.2 ± 0.3^{ABa}	5.8 ± 0.5^{Aa}	5.6 ± 0.2^{Aa}
1/2 MIC RES	6.5 ± 0.4^{Aa}	5.5 ± 0.7^{Bab}	5.5 ± 0.2^{Bb}	5.5 ± 0.4^{Aa}	4.9 ± 0.8^{Aa}	4.9 ± 1.2^{Aa}	6.8 ± 0.6^{Aa}	6.3 ± 0.2^{Aa}	5.7 ± 0.8^{Aa}	5.2 ± 0.5^{ABa}	5.6 ± 0.2^{Aa}	5.2 ± 0.3^{Aa}
1/4 MIC RES	7.5 ± 0.6^{Aa}	6.5 ± 0.7^{ABab}	6.0 ± 0.3^{Bb}	6.2 ± 0.4^{Aa}	4.8 ± 1.2^{Aa}	4.8 ± 1.0^{Aa}	6.8 ± 0.4^{Aa}	6.1 ± 0.6^{ABa}	5.8 ± 0.6^{Aa}	4.2 ± 0.7^{Bb}	5.6 ± 0.5^{Aa}	5.2 ± 0.2^{Aa}
Control	7.2 ± 0.3^{Aab}	7.7 ± 0.6^{Aa}	6.9 ± 0.3^{Aa}	5.9 ± 0.3^{Aa}	5.4 ± 0.5^{Aa}	5.4 ± 1.1^{Aa}	6.6 ± 0.9^{Aa}	6.1 ± 0.5^{ABa}	5.3 ± 0.6^{Aa}	5.0 ± 1.0^{ABa}	5.7 ± 0.4^{Aa}	5.3 ± 0.4^{Aa}

L. paracasei 106												
MIC QUE	6.0 ± 0.9^{ABa}	5.1 ± 1.0^{Ba}	5.3 ± 0.5^{Aa}	6.6 ± 0.3^{Aa}	6.1 ± 0.5^{Aab}	5.7 ± 0.4^{Ab}	6.1 ± 0.5^{Aa}	5.2 ± 0.8^{Aa}	5.4 ± 0.7^{Aa}	4.3 ± 0.6^{Aa}	5.3 ± 0.8^{Aa}	4.4 ± 0.8^{Aa}
1/2 MIC QUE	6.3 ± 0.5^{ABa}	5.3 ± 0.8^{Ba}	5.6 ± 0.5^{Aa}	5.9 ± 0.6^{Aa}	6.1 ± 0.3^{Aa}	6.3 ± 0.5^{Aa}	6.3 ± 0.5^{Aa}	5.3 ± 1.0^{Aa}	5.9 ± 0.2^{Aa}	4.0 ± 0.4^{Aa}	5.2 ± 1.2^{Aa}	4.3 ± 0.9^{Aa}
1/4 MIC QUE	7.3 ± 0.5^{Aa}	6.1 ± 0.6^{ABb}	7.1 ± 1.4^{Aab}	6.5 ± 0.3^{Aa}	6.2 ± 0.4^{Aa}	5.9 ± 0.3^{Aa}	6.4 ± 0.2^{Aa}	5.2 ± 1.3^{Aab}	5.7 ± 0.2^{Ab}	3.9 ± 0.9^{Aa}	4.8 ± 1.1^{Aa}	4.9 ± 1.3^{Aa}
MIC RES	7.2 ± 0.5^{Aa}	5.7 ± 1.0^{ABa}	6.2 ± 0.4^{Ab}	6.6 ± 0.5^{Aa}	6.8 ± 0.4^{Aa}	5.4 ± 0.5^{Ab}	6.6 ± 0.5^{Aa}	5.5 ± 1.7^{Aa}	5.2 ± 1.0^{Aa}	4.0 ± 0.4^{Ab}	4.4 ± 0.2^{Ab}	5.3 ± 0.6^{Aa}
1/2 MIC RES	5.5 ± 0.5^{Ba}	5.6 ± 0.5^{Ba}	5.0 ± 0.4^{Ba}	6.6 ± 0.5^{Aa}	6.3 ± 0.5^{Aa}	5.9 ± 0.9^{Aa}	6.6 ± 0.8^{Aa}	5.5 ± 1.4^{Aa}	5.8 ± 0.2^{Aa}	4.1 ± 0.3^{Ab}	4.9 ± 0.9^{Aab}	5.1 ± 0.5^{Aa}
1/4 MIC RES	7.3 ± 0.5^{Aa}	7.1 ± 0.6^{Aa}	6.4 ± 0.6^{Aa}	6.6 ± 0.2^{Aa}	6.3 ± 0.4^{Aab}	5.7 ± 0.5^{Ab}	6.5 ± 0.4^{Aa}	5.5 ± 1.6^{Aab}	5.4 ± 0.6^{Ab}	4.0 ± 0.4^{Ab}	4.7 ± 0.5^{Aab}	5.0 ± 0.5^{Aa}
Control	6.4 ± 1.5^{ABa}	6.5 ± 0.9^{ABa}	6.5 ± 0.7^{Aa}	6.3 ± 1.2^{Aa}	6.2 ± 0.2^{Aa}	5.7 ± 0.9^{Aa}	6.3 ± 0.8^{Aa}	6.0 ± 0.4^{Aa}	5.7 ± 0.5^{Aa}	4.3 ± 0.7^{Aa}	5.3 ± 0.8^{Aa}	4.9 ± 1.2^{Aa}
L. paracasei 108												
MIC QUE	7.1 ± 0.4^{Aa}	6.4 ± 0.6^{Aa}	6.6 ± 0.2^{Aa}	4.8 ± 0.7^{Aa}	4.5 ± 0.5^{Aa}	4.0 ± 0.6^{Aa}	3.5 ± 0.6^{Ba}	< 1 ± 0.0^{Ab}	< 1 ± 0.0^{Ab}	< 1 ± 0.0	< 1 ± 0.0	< 1 ± 0.0
1/2 MIC QUE	7.1 ± 0.6^{Aa}	7.1 ± 0.7^{Aa}	6.8 ± 0.7^{Aa}	4.8 ± 0.7^{Aa}	4.3 ± 0.6^{Aa}	4.3 ± 0.6^{Aa}	4.6 ± 0.5^{Aa}	< 1 ± 0.0^{Ab}	< 1 ± 0.0^{Ab}	< 1 ± 0.0	< 1 ± 0.0	< 1 ± 0.0
1/4 MIC QUE	7.1 ± 0.5^{Aa}	6.9 ± 0.8^{Aa}	6.9 ± 0.9^{Aa}	4.6 ± 0.5^{Aa}	4.7 ± 1.4^{Aa}	4.3 ± 0.8^{Aa}	4.6 ± 0.2^{Aa}	< 1 ± 0.0^{Ab}	< 1 ± 0.0^{Ab}	< 1 ± 0.0	< 1 ± 0.0	< 1 ± 0.0
MIC RES	7.2 ± 0.8^{Aa}	7.1 ± 0.7^{Aa}	7.1 ± 0.4^{Aa}	4.9 ± 0.6^{Aa}	4.2 ± 1.1^{Aa}	3.4 ± 1.5^{Aa}	3.8 ± 0.2^{Ba}	< 1 ± 0.0^{Ab}	< 1 ± 0.0^{Ab}	< 1 ± 0.0	< 1 ± 0.0	< 1 ± 0.0
1/2 MIC RES	6.3 ± 0.4^{Aa}	6.1 ± 0.2^{Aa}	6.1 ± 0.6^{Aa}	4.4 ± 0.7^{Aa}	3.9 ± 0.6^{Aa}	3.4 ± 0.9^{Aa}	3.7 ± 0.3^{Ba}	< 1 ± 0.0^{Ab}	< 1 ± 0.0^{Ab}	< 1 ± 0.0	< 1 ± 0.0	< 1 ± 0.0
1/4 MIC RES	6.8 ± 0.7^{Aa}	6.8 ± 0.9^{Aa}	6.8 ± 0.8^{Aa}	5.1 ± 0.7^{Aa}	4.8 ± 1.2^{Aa}	4.5 ± 0.4^{Aa}	2.9 ± 0.3^{Ba}	< 1 ± 0.0^{Ab}	< 1 ± 0.0^{Ab}	< 1 ± 0.0	< 1 ± 0.0	< 1 ± 0.0
Control	6.9 ± 0.6^{Aa}	6.8 ± 1.0^{Aa}	7.0 ± 0.7^{Aa}	5.3 ± 0.6^{Aa}	5.7 ± 1.4^{Aa}	4.9 ± 0.7^{Aa}	4.9 ± 0.2^{Aa}	< 1 ± 0.0^{Ab}	< 1 ± 0.0^{Ab}	< 1 ± 0.0	< 1 ± 0.0	< 1 ± 0.0
L. fermentum 263												
MIC QUE	5.9 ± 1.2^{ABa}	6.5 ± 0.7^{ABa}	5.6 ± 0.7^{Ba}	5.5 ± 0.5^{Aa}	5.6 ± 0.8^{Aa}	5.5 ± 0.7^{Aa}	7.3 ± 0.6^{Aa}	7.3 ± 0.4^{Aa}	7.2 ± 0.5^{Aa}	7.0 ± 0.5^{Aa}	6.3 ± 0.5^{Aab}	5.4 ± 0.6^{Ab}
1/2 MIC QUE	6.6 ± 0.6^{ABa}	6.1 ± 0.6^{Ba}	5.7 ± 0.9^{ABa}	5.6 ± 0.5^{Aa}	6.3 ± 0.6^{Aa}	5.6 ± 1.0^{Aa}	7.2 ± 0.5^{Aa}	7.2 ± 0.2^{Aa}	6.9 ± 0.5^{Aa}	6.9 ± 0.5^{Aa}	6.5 ± 0.4^{Aa}	5.9 ± 0.7^{Aa}
1/4 MIC QUE	6.9 ± 0.4^{Aa}	6.8 ± 0.5^{ABa}	6.0 ± 0.6^{ABa}	5.5 ± 0.7^{Aa}	5.6 ± 0.6^{Aa}	5.6 ± 0.8^{Aa}	7.2 ± 0.9^{Aa}	7.3 ± 0.3^{Aa}	7.3 ± 0.6^{Aa}	7.0 ± 0.6^{Aa}	6.4 ± 0.5^{Aa}	6.0 ± 0.6^{Aa}
MIC RES	6.9 ± 0.5^{ABa}	6.7 ± 0.4^{ABa}	6.1 ± 0.5^{ABa}	5.6 ± 0.6^{Aa}	5.6 ± 0.5^{Aa}	6.4 ± 0.6^{Aa}	7.4 ± 0.7^{Aa}	7.1 ± 0.2^{Aa}	6.9 ± 0.5^{Aa}	7.1 ± 0.4^{Aa}	6.2 ± 0.6^{Aa}	4.9 ± 0.5^{Ab}

1/2 MIC RES	5.7 ± 0.7^{Ba}	5.9 ± 0.5^{Ba}	6.6 ± 0.6^{ABa}	5.6 ± 0.5^{Aa}	5.5 ± 0.7^{Aa}	5.6 ± 0.9^{Aa}	7.4 ± 0.6^{Aa}	7.2 ± 0.3^{Aa}	7.1 ± 0.6^{Aa}	7.1 ± 0.6^{Aa}	6.3 ± 0.6^{Aab}	5.1 ± 0.6^{Ab}
1/4 MIC RES	7.1 ± 0.9^{ABa}	7.1 ± 0.3^{Aa}	7.1 ± 0.5^{Aa}	5.6 ± 0.5^{Aa}	5.6 ± 0.6^{Aa}	5.6 ± 0.6^{Aa}	7.4 ± 0.5^{Aa}	7.0 ± 0.4^{Aa}	7.0 ± 0.5^{Aa}	7.1 ± 0.5^{Aa}	6.1 ± 0.6^{Aab}	4.9 ± 0.5^{Ab}
Control	7.4 ± 0.4^{Aa}	7.3 ± 0.2^{Aa}	7.0 ± 0.5^{Aa}	5.9 ± 1.0^{Aa}	5.8 ± 0.6^{Aa}	5.7 ± 0.5^{Aa}	7.0 ± 0.7^{Aa}	6.9 ± 0.2^{Aa}	6.7 ± 0.5^{Aa}	6.7 ± 0.6^{Aa}	6.3 ± 0.7^{Aab}	4.9 ± 0.6^{Ab}
L. fermentum 296												
MIC QUE	6.4 ± 0.6^{ABa}	6.1 ± 0.7^{ABa}	5.7 ± 0.4^{Ba}	5.8 ± 0.6^{Aa}	5.7 ± 0.4^{Aa}	5.4 ± 0.5^{Aa}	7.3 ± 0.6^{Aa}	7.0 ± 0.6^{Aa}	6.7 ± 0.3^{Aa}	7.1 ± 0.7^{Aa}	6.1 ± 0.7^{Aab}	5.3 ± 0.6^{Ab}
1/2 MIC QUE	5.7 ± 0.4^{Ba}	5.9 ± 0.5^{Ba}	5.7 ± 0.5^{Ba}	6.6 ± 0.6^{Aa}	5.8 ± 0.6^{Aa}	5.5 ± 0.6^{Aa}	7.4 ± 0.6^{Aa}	7.3 ± 0.6^{Aa}	6.7 ± 0.4^{Aa}	7.2 ± 0.6^{Aa}	6.3 ± 0.6^{Aab}	5.4 ± 0.5^{Ab}
1/4 MIC QUE	5.7 ± 0.5^{Ba}	6.1 ± 0.6^{Ba}	6.5 ± 0.7^{ABa}	6.5 ± 0.7^{Aa}	5.5 ± 0.9^{Aa}	5.3 ± 0.6^{Aa}	7.3 ± 0.6^{Aa}	7.4 ± 0.6^{Aa}	7.1 ± 0.6^{Aa}	7.1 ± 0.5^{Aa}	5.9 ± 0.5^{Ab}	5.3 ± 1.6^{Aab}
MIC RES	5.6 ± 0.6^{Ba}	5.1 ± 0.5^{Ba}	5.3 ± 0.6^{Ba}	6.5 ± 0.7^{Aa}	5.7 ± 0.5^{Aab}	4.9 ± 0.8^{Ab}	7.5 ± 0.8^{Aa}	7.2 ± 0.6^{Aa}	6.8 ± 0.4^{Aa}	7.2 ± 0.6^{Aa}	6.1 ± 1.0^{Aab}	5.4 ± 0.6^{Ab}
1/2 MIC RES	6.3 ± 0.6^{ABa}	5.7 ± 0.3^{Ba}	4.6 ± 0.7^{Bb}	6.6 ± 0.5^{Aa}	5.5 ± 0.7^{Aab}	5.3 ± 0.6^{Ab}	7.5 ± 0.7^{Aa}	7.3 ± 0.7^{Aa}	7.1 ± 0.6^{Aa}	7.3 ± 0.6^{Aa}	6.5 ± 0.2^{Aa}	5.2 ± 0.6^{Ab}
1/4 MIC RES	7.3 ± 0.6^{Aa}	7.5 ± 0.7^{Aa}	4.7 ± 0.8^{Bb}	6.5 ± 0.4^{Aa}	6.3 ± 0.6^{Aa}	5.6 ± 0.6^{Aa}	7.5 ± 0.7^{Aa}	7.2 ± 0.6^{Aa}	6.9 ± 0.6^{Aa}	7.3 ± 0.6^{Aa}	6.2 ± 0.6^{Aa}	6.4 ± 0.6^{Aa}
Control	6.5 ± 0.4^{ABa}	6.4 ± 0.8^{ABa}	6.6 ± 0.2^{Aa}	6.7 ± 0.6^{Aa}	6.7 ± 0.5^{Aa}	6.1 ± 0.3^{Aa}	6.9 ± 0.2^{Aa}	7.2 ± 0.4^{Aa}	6.7 ± 0.3^{Aa}	7.0 ± 0.5^{Aa}	6.6 ± 1.0^{Aab}	5.5 ± 0.6^{Ab}

Control: 0 µg/mL of QUE or RES.

For *L. plantarum* 49, *L. plantarum* 53, *L. paracasei* 106 and *L. fermentum* 263 - MIC of QUE: 1024 µg/mL, 1/2 MIC of QUE: 512 µg/mL, 1/4 MIC of QUE: 256 µg/mL, MIC of RES: 1024 µg/mL, 1/2 MIC of RES: 512 µg/mL, 1/4 MIC of RES: 256 µg/mL; For *L. paracasei* 108 and *L. fermentum* 296 - MIC of QUE: 512 µg/mL, 1/2 MIC of QUE: 256 µg/mL, 1/4 MIC of QUE: 128 µg/mL, MIC of RES: 1024 µg/mL, 1/2 MIC of RES: 512 µg/mL, 1/4 MIC of RES: 256 µg/mL.

$^{A-B}$: Same superscript capital letters in the same column denote no difference ($p \leq 0.05$) between counts for the tested *Lactobacillus* strain when exposed to a bile salts concentration for a specific time interval in media with or without different concentrations of QUE or RES, based on Tukey's test.

$^{a-b}$: Same superscript small letters in the same row denote differences ($p \leq 0.05$) in counts for the tested *Lactobacillus* strains when exposed for a bile salts concentration in media with different