PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
MESTRADO EM CIÊNCIA ANIMAL

TÉCNICA DE FILTRAGEM EM MEMBRANA PARA AVALIAÇÃO QUANTITATIVA DA AGLUTINAÇÃO DE LINHAGENS DE *E. COLI* EM MANANOLIGOSSACARÍDEO

PAULA MARIOTO PEREZ

Presidente Prudente - SP
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Dissertação apresentada Pró-Reitoria de Pesquisa e Pós-Graduação, Universidade do Oeste Paulista, como parte dos requisitos para obtenção do título de Mestre em Ciência Animal – Área de concentração: Fisiopatologia Animal.

Orientador:
Prof. Dr. Hermann Bremer Neto

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Presidente Prudente, 26 de abril de 2017

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DEDICATÓRIA

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“Se enxerguei mais longe, foi porque me apoiei sobre ombros de gigantes”.

(Autor desconhecido)

RESUMO

Técnica de Filtragem em Membrana para Avaliação Quantitativa da Aglutinação de Linhagens de *E. Coli* in Mananoligossacarídeo

O objetivo deste estudo foi padronizar uma técnica quantitativa para avaliar a capacidade *in vitro* de aglutinação de diferentes mananoligossacarídeos (MOS) à cepa de *Escherichia coli* isoladas de casos de diarreia infantil, de crianças de 0 a 5 anos de vida. A pesquisa para a expressão da fímbria tipo 1 presente nos isolados foi realizada pelo teste de microhemoaglutinação. Para realização dos ensaios de aglutinação de oligossacarídeos utilizou-se três marcas comerciais de MOS, extraídos de *S. cerevisae,* grupos 1, 2 e 3, filtrados através de um microfiltro, posteriormente diluídos para semeadura superficial em Ágar cromogênico *E. coli*. Para avaliação microscópica da capacidade aglutinante do MOS foram submetidas a testes de aglutinação em lâmina.Para estimar a contagem bacteriana total (CBT), calculou-se a média das contagens do conjunto de três placas. Pressupostos de homogeneidade de variâncias e normalidade de dados foram validados, respectivamente, pelo teste de Levene e Shapiro-Wilk. Variáveis homocedásticas foram comparadas por análise de variância em uma via (ANOVA *one-way*), com contrastes pelo método de Tukey. Variáveis heterocedásticas foram comparadas com ANOVA *one-way* com aplicação da correção de Welche contrastes pelo método de Games-Howell. As correlações entre CBTs, Títulos hemaglutinantes, diâmetros e áreas dos glóbulos foram avaliados pelo teste de correlação de Pearson. Todas as análises foram conduzidas no software R, considerando-se probabilidade de erro tipo 1= 5%. Das 30 cepas testadas, 25 (83,3%) expressaram capacidade hemaglutinante com títulos variando entre 1:4 e 1:16. Os resultados sugerem que o MOS apresentou ação efetiva na aglutinação das bactérias, visto que as estimativas de CBTs das cepas tratadas com MOS foram inferiores as contagens da amostra pura. O grau de aglutinação pode variar segundo a composição do MOS, visto que o produto 1 apresentou CBTs superiores aos demais, denotando que os glóbulos aglutinados não foram capazes de reter bactérias nos poros dos filtros de forma similar aos produtos 2 e 3.Observou-se correlação significativa e negativa entre a hemaglutinação sensível à manose e aglutinação de MOS para os produtos 1 e 2, o que sugere que intensidade da aglutinação por bactérias pode estar relacionada a expressão de fímbrias do tipo 1. Conclui-se que a técnica de filtragem e cultura pode ser utilizada para avaliar diferentes graus de aglutinação de produtos a base de MOS e a quantidade de bactérias retidas nos filtros parece estar relacionada mais com a expressão de fímbrias tipo 1 mano-sensíveis do que com os tamanhos dos glóbulos produzidos.

Palavras-chave: Aglutinação; *Escherichia coli*; Mananoligossacarídeo.

ABSTRACT

Membrane Filtration Technique for Quantitative Evaluation of Agglutination of E. Coli Lines in Mananoligosacarídeo

The objective of this study was to standardize a quantitative technique to evaluate the in vitro capacity of agglutination of different mannanoligosaccharides (MOS) to the strain of Escherichia coli isolated from cases of infantile diarrhea in children from 0 to 5 years of age. The research for the expression of fimbria Type 1 present in the isolates was performed by the microhemoagglutination test. For the oligosaccharide agglutination assays three MOS markers were extracted from *S. cerevisae*, groups 1, 2 and 3, filtered through a microfilter, later diluted for surface sowing in chromogenic Agar *E. coli*. For microscopic evaluation of binder capacity of the MOS were subjected to slide agglutination tests. In order to estimate the total bacterial count (CBT), the counts of the three-plate set were calculated. Assumptions of homogeneity of variances and normality of data were validated, respectively, by the Levene and Shapiro-Wilk test. Homozygous variables were compared by analysis of variance in one way (ANOVA one-way), with contrasts by the Tukey method. Heterocedastic variables were compared with one-way ANOVA with application of the Welche contrast contrasts by the Games-Howell method. Correlations between CBTs, Titres hemagglutinants, diameters and areas of the globules were evaluated by the Pearson correlation test. All analyzes were conducted in software R, considering probability of error type 1 = 5% Of the 30 strains tested, 25 (83.3%) expressed hemagglutinating capacity with titers ranging from 1: 4 to 1: 16. The results suggest that the MOS showed effective action on the agglutination of the bacteria, since the estimates of CBTs of the strains treated with MOS were inferior the counts of the pure sample. The degree of agglutination may vary according to the composition of the MOS, since product 1 showed higher CBTs than the others, indicating that the agglutinated beads were not able to retain bacteria in the pores of the filters similarly to products 2 and 3. It was observed significant correlation between mannose-sensitive haemagglutination and MOS agglutination for products 1 and 2, suggesting that the intensity of bacterial agglutination may be related to the expression of type 1 fimbriae. It is concluded that the filtration and culture technique can be used to evaluate different degrees of agglutination of MOS products and the amount of bacteria retained in the filters appears to be more related to the expression of mano-sensitive type 1 fimbriae than to the sizes of the globules produced.

Keywords: Agglutination. *Escherichia coli*. Mannanoligosaccharide.

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1 ARTIGO

Padronização da técnica de análise quantitativa da aglutinação de diferentes mananoligossacarídeos a linhagens de *E. coli* patogênicas

Standardization of the technique of quantitative analysis of the agglutination of different mannanoligosaccharides to pathogenic *E. coli* strains

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Resumo

O objetivo deste estudo foi padronizar uma técnica quantitativa para avaliar a capacidade in vitro de aglutinação de diferentes mananoligossacarídeos (MOS) à cepa de Escherichia coli isoladas de casos de diarreia infantil, de crianças de 0 a 5 anos de vida.A pesquisa para a expressão da fímbria tipo 1 presente nos isolados foi realizada pelo teste de microhemoaglutinação. Para realização dos ensaios de aglutinação de oligossacarídeos utilizou-se três marcas comerciais de MOS, extraídos de *S. cerevisae*,grupos 1, 2 e 3, filtrados através de um microfiltro, posteriormente diluídos para semeadura superficial em Ágar cromogênico*E. coli*. Para avaliação microscópica da capacidade aglutinante do MOS foram submetidas a testes de aglutinação em lâmina.Para estimar a contagem bacteriana total (CBT), calculou-se a média das contagens do conjunto de três placas. Pressupostos de homogeneidade de variâncias e normalidade de dados foram validados, respectivamente, pelo teste de Levene e Shapiro-Wilk. Variáveis homocedásticas foram comparadas por análise de variância em uma via (ANOVA *one-way*), com contrastes pelo método de Tukey. Variáveis heterocedásticasforam comparadas com ANOVA *one-way* com aplicação da correção de Welche contrastes pelo método de Games-Howell. Ascorrelações entre CBTs, Títuloshemaglutinantes, diâmetros e áreas dos glóbulos foram avaliados pelo teste de correlação de Pearson. Todas as análises foram conduzidas no software R, considerando-se probabilidade de erro tipo 1= 5%Das 30 cepas testadas, 25 (83,3%)expressaram capacidade hemaglutinante com títulos variando entre 1:4 e 1:16.Os resultados sugerem que o MOS apresentou ação efetiva na aglutinação das bactérias, visto que as estimativas de CBTs das cepas

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tratadas com MOS foram inferiores as contagens da amostra pura. O grau de aglutinação pode variar segundo a composição do MOS, visto que o produto 1 apresentou CBTs superiores aos demais, denotando que os glóbulos aglutinados não foram capazes de reter bactérias nos poros dos filtros de forma similar aos produtos 2 e 3.Observou-se correlação significativa enegativa entre a hemaglutinação sensível à manose e aglutinação de MOS para os produtos 1 e 2, o que sugere que intensidade da aglutinação por bactérias pode estar relacionada a expressão de fímbrias do tipo 1. Conclui-se que a técnica de filtragem e cultura pode ser utilizada para avaliar diferentes graus de aglutinação de produtos a base de MOS e a quantidade de bactérias retidas nos filtros parece estar relacionada mais com a expressão de fímbrias tipo 1 mano-sensíveis do que com os tamanhos dos glóbulos produzidos.

Palavras-chave: Aglutinação. Escherichia coli. MOS.

1. Introdução

A espécie *Escherichia coli* é uma bactéria gram-negativa, pertencente à família *Enterobacteriaceae*, amplamente disponível na natureza que compreende linhagens comensais que habitam o intestino de muitos animais e humanos, e linhagens, como *E. coli* diarreiogênicas, que podem causar uma grande variedade de doenças intestinais e extra-intestinais, como diarréia, variando de diarreias autolimitantes até síndromes crônicas graves, infecções do trato urinário, septicemia e meningite neonatal, com alta prevalência em crianças com idade de zero a cinco anos (Clermont et al., 2000; Pasquali et al., 2015).

Essas infecções entéricas associadas ao agente são mais comuns em países com baixos índices de desenvolvimento socioeconômico, sobretudo à pobreza, desmame precoce, deficiências nutricionais e menor nível de escolaridade maternal (Unicef, 2009).

As linhagens de *E. coli* diarreiogênicas são diferenciadas pela presença de fatores de virulência como adesinas fimbriais, toxinas e invasinas, e classificadas em: *E. coli* enteropatogênica (EPEC), *E. coli* enterotoxigênica (ETEC), *E. coli* enteroinvasora (EIEC), *E. coli* enterohemorrágica (EHEC) ou *E. coli* produtora da toxina de Shiga (STEC), *E. coli* enteroagregativa (AEAC) e *E. coli* aderente difusa (DAEC) (Nguyen et al., 2005; Teng et al., 2004).

A EPEC é a principal e mais versátil entre as categorias diarreiogênicas e uma das principais causas de diarréia em crianças menores de cinco anos de idade (Fagundes-Neto and Scaletsky, 2000; Rodríguez-Angeles, 2002).

O mecanismo da patogênese envolve três estágios de interação entre EPEC e a célula: 1) aderência localizada (AL) mediada pela fímbria BFP (bundle-formingpilus) e codificada pelo gene plasmidial EAF; 2) sinais de transdução; e 3) aderência íntima promovida pela intimina (gene aea) (Nataro and Kaper, 1998).

Dentre os principais fatores dessa aderência, destacam-se as fímbrias tipo 1: filamentos proteicos requeridos para a aderência de *E. coli* a receptores glicopeptídicos que expressam manose, presentes

na superfície da mucosa intestinal (Westerlund-Wikström and Korhonen, 2005). Considerando-se que a adesão ao epitélio intestinal é um passo essencial para a expressão de outros fatores de virulência que incluem principalmente toxinas, o bloqueio dos receptores de aderência com moléculas de ação antiaderente parece ser uma forma promissora de reduzir a probabilidade das *E. coli* de estabelecer quadros infecciosos diarréicos (Morrow et al., 2005; Sharon, 2006; Silva et al., 2011).

Dentre as moléculas potencialmente empregadas para reduzir a adesão de *E. coli* na parede intestinal, destacam-se os oligossacarídeos dietéticos notadamente, os mananoligossacarídeos (MOS), que atuam por meio de exclusão competitiva ao ligarem-se seletivamente as fimbrias tipo 1, impedindo a aderência à mucosa promovendo a eliminação do agente conjuntamente com o bolo fecal. MOS são suplementos dietéticos, classificados como prebióticos, compostos principalmente de carboidratos derivados da parede celular externa de levedura*Saccharomycescerevisiae* e amplamente disponíveis no Brasil, visto que são subprodutos oriundos da indústria sucroalcooleira(Heinrichs et al., 2003; Miguel et al., 2004).

Até o momento, a maioria dos estudos *in vitro* avaliou de forma qualitativa o potencial aglutinante do MOS frente a diferentes agentes patogênicos entéricos, incluindo *E. coli* (Borowsky et al., 2009). A imprevisibilidade caracteriza o desenvolvimento da pesquisa qualitativa, já que na avaliação subjetiva é comum existirem variações nas análises entre os diferentes envolvidos, pois apresentam conhecimentos parciais e limitados. Portanto, o objetivo deste estudo foi padronizar uma técnica quantitativa para avaliar a capacidade in vitro de aglutinação de diferentes mananoligossacarídeos (MOS) à cepa de Escherichia coli isoladas de casos de diarreia infantil, de crianças de 0 a 5 anos de vida.

2. Material e métodos

As linhagens de *E. coli* utilizadas na pesquisa foram cedidas por laboratórios de diagnóstico privados e públicos dos municípios de Presidente Prudente e Tupã, São Paulo, Brasil, onde foram mantidas em estoque, congeladas à -70°C até o momento do uso. Todas as amostras obtidas durante o ano de 2014-2015 foram reisoladas em meios bioquímicos para confirmação de *E. coli* e sorogrupadas através de reações sorológicas comanti-soros direcionados contra antígenos somáticos (O). Os sorogrupos e sua freqüência encontrados nesse estudo estão descritos na tabela 1.

Para avaliar a expressão da fimbriais tipo 1 procedeu-se o ensaio de microhemaglutinação em placa com suspensões de hemácias de cobaio a 1% em presença e na ausência de D-manose 1%(Awad-Masalmeh et al., 1982; Duguid et al., 1979). O sangue de cobaio foi obtido por punção cardíaca e depositado em frascos contendo citrato de sódio 3,2%. A seguir, procedeu-se três lavagens consecutivas em tampão fosfato-salina (PBS, pH 7,4), centrifugando a suspensão de hemácias a 1500 xGpor 10 minutos. A suspensão final foi suplementada com D-manose previamente esterilizada por filtração na concentração final de 1%.

Para os ensaios de microhemaglutinação, inicialmente, colônias bacterianas presentes nos meios de conservação, TSB com glicerol a 2%, foram reisoladas em ágar MacConkey para avaliar pureza e viabilidade. A seguir, as colônias foram submetidas a 10 passagens sucessivas em 10 mL de caldo tripticase de soja (TSB) a cada 72 horas para ativação da expressão de fímbrias tipo 1 (Spring et al., 2000). Após este procedimento, as linhagens foram inoculadas em Ágar CFA (Colonization Factor Antigen) e incubadas em estufa a 37°C por 24 horas. Os isolados obtidos no meio CFA foram utilizados para preparo de inóculos em tampão fosfato (PBS) correspondentes a $1,2x10^9$ células/mL (escala de MacFarland, tubo 4)

Os testes de microhemaglutinação foram conduzidos em microplacas com 96 poços em forma de "U" mantidas sobre gelo. A partir dos inóculos obtidos nos procedimentos anteriores realizou-se diluições seriadas correspondentes as proporções de 1:2 até 1:256, em solução de tampão fosfato, com volume final de 50 µL por poço. As diluições foram realizadas em duplicata (duas fileiras), sendo que nos poços de uma das fileiras foi adicionado 50 µL de suspensões de hemácias de cobaio a 1% suplementadas com 1% de manose e em outra, a mesma suspensão sem presença de manose. As placas foram refrigeradas a 5°C e as leituras realizadas após 2 horas de exposição, com auxílio de lupa microscópica. Foram consideradas positivas, as amostras bacterianas que apresentaram título aglutinante igual ou superior a 1:4. Linhagens que apresentaram aglutinação inibida pela D-manose foram consideradas como portadoras das fímbrias do Tipo 1. A amostra de EPEC E2348/69 do sorotipo O127:H6 foi empregada como controle positivo do teste e como controle negativo a cepa K12. Os resultados foram avaliados na forma de título (Adegbola and Old, 1983).

Para realização dos ensaios de aglutinação de oligossacarídeos utilizou-se três marcas comerciais de MOS, extraídos de *S. cerevisae*, sendo os grupos 1, 2 e 3 (Tabela 2). Inicialmente foram preparadas suspensões de MOS a 0,1% em PBS esterilizadas por autoclavação a 121^{o}C por 15 min. A seguir, 1 mL de cada suspensão foi misturada com 1mL da suspensão bacteriana preparada conforme etapa anterior, em tubos de vidro de 5 mL de capacidade. Após incubação por 30 minutos a 5°C, a suspensão foi transferida para uma seringa de 5mL de capacidade e filtrada através de um microfiltro com poros de 0,8 µm de diâmetro. O filtrado obtido foi diluído em PBS nas frações 10^{-1} a 10^{-8}. De cada diluição, retirou-se 0,1 ml para semeadura superficial em placas de Petri contendo Ágar cromogênico *E. coli*. Diluições puras de *E. coli* sem adição de MOS foram utilizadas como controle positivo e amostras de MOS sem *E. coli* como controle negativo. Após 24 de incubação, placas com 30 a 300 UFC foram separadas e as colônias enumeradas. Os valores aferidos foram expressos em Unidades Formadoras de Colônia por mL de filtrado (UFC/mL).

Para avaliação microscópica da capacidade aglutinante do MOS, inicialmente preparou-se suspensões na concentração de 0,1% em PBS, com cada marca comercial. A seguir, depositou-se 50 µl da suspensão obtida em orifícios de microplacas de 96 poços. Em cada poço, depositou-se 50 µl de suspensão de bactérias, preparada conforme etapa anterior. Após incubação por 60 minutos a 5°C, 20 µL de cada suspensão foi depositado em lâmina para observação microscópica do padrão aglutinante,

em aumento de 40X. As análises foram realizadas em triplicata. Amostras de MOS a 0,1%, sem suspensão bacteriana foram utilizadas como controle negativo. Foram consideradas como aglutinantes, amostras com agregados de MOS visivelmente grosseiros e de tamanho grande, em comparação a amostra controle (Borowsky et al., 2009). Para confirmar a atividade aglutinante, alíquotas das suspensões de MOS + *E. coli* foram depositadas em lâminas e coradas pelo método de Gram. A capacidade aglutinante da amostra foi confirmada após observar-se bastonetes Gram negativos aderidos aos grumos.

Para estimar a contagem bacteriana total (CBT) por 0,1 ml de fluído filtrado, calculou-se a média das contagens do conjunto de três placas e multiplicou-se o valor pela recíproca da diluição correspondente. As médias de CBTs sofreram transformação logarítmica na base 10 para normalização dos dados. Pressupostos de homogeneidade de variâncias e normalidade de dados foram validados, respectivamente, pelo teste de Levene e Shapiro-Wilk. Variáveis homocedásticas foram comparadas por análise de variância em uma via (ANOVA *one-way*), com contrastes pelo método de Tukey. Variáveis heterocedásticas foram comparadas com ANOVA *one-way* com aplicação da correção de Welche contrastes pelo método de Games-Howell. As correlações entre CBTs, Títulos hemaglutinantes, diâmetros e áreas dos glóbulos foram avaliados pelo teste de correlação de Pearson. Todas as análises foram conduzidas no software R, considerando-se probabilidade de erro tipo 1<5%(R Development Core Team, 2016).

Tabela 1

Sorogrupos e frequência de *E. coli* utilizadas no estudo.

Sorogrupo	Frequência
O26	1(3,3%)
O55	5 (16,7%)
O86	5 (16,7%)
O111	3 (10,0%)
O114	2(6,7%)
O125	5 (16,7%)
O126	2(6,7%)
O142	4 (13,3%)
O158	3(10,0%)
Total	30 (100%)

Tabela 2

Composição dos diferentes MOS.

MOS	COMPOSIÇÃO	REFERENCIA
1	Derivado da parede celular da levedura *Saccharomyces cerevisiae*, cepa 1026	Collet, 2000
2	Fração ativa da mananoproteína, derivada de um mananoligossacarídeo.	Alltech, 2010
3	Aditivo prebiótico rico em ß-glucanos e mananoligossacarídeos, derivado de uma cepa especialmente selecionada de levedura *Saccharomyces cerevisiae*	Icc, 2010

3. Resultados e Discussão

Das 30 cepas avaliadas nos ensaios de microhemaglutinação, 25 (83,3%) expressaram capacidade hemaglutinante com títulos variando entre 1:4 e 1:16 (média geométrica = 5,9 ± 0,88). Na tabela 3 estão sumarizados os resultados das CBTs, Área e Perímetro dos glóbulos de aglutinação, expressos como média ± desvio padrões para as 25 cepas selecionadas. Na tabela 4 estão descritos os resultados da análise de correlação.

Os dados da tabela 3 sugerem que o MOS apresentou ação efetiva na aglutinação das bactérias, visto que as estimativas de CBTs das cepas tratadas com MOS foram inferiores as contagens da amostra pura. Os glóbulos de MOS com as bactérias aderidas possivelmente ficaram retidas nos poros presentes no interior dos filtros. Estes resultados indicam que a técnica de filtragem pode representar uma alternativa interessante quando comparada aos métodos de quantificação por escores após visualização microscópica, que apresentam certo grau de subjetividade (Borowsky et al., 2009).

Os resultados observados na tabela 3, também sugerem que o grau de aglutinação pode variar segundo a composição do MOS, visto que o produto 1 apresentou CBTs superiores aos demais, denotando que os glóbulos aglutinados não foram capazes de reter bactérias nos poros dos filtros de forma similar aos produtos 2 e 3. As diferenças na capacidade aglutinante podem ser devido a processos industriais de obtenção industrial das frações da parede das leveduras, bem como a composição dos produtos (Alltech, 2010; Icc, 2010; Collet, 2000).

As diferenças entre contagens observadas para diferentes produtos (tabela 3) sugerem que a formulação e granulometria influenciam no processo de aglutinação. Este resultado é reforçado pelas comparações entre diâmetro e área, que se mostraram estatisticamente diferentes para cada produto, o que pode ser devido a sua composição ou origem das cepas de leveduras, *Saccharomyces cerevisiae*, utilizadas ou até mesmo pelos diferentes processos de produção do mananoligossacarídeo e sua ação (tabela 4) (Sakita et al., 2014). Esta hipótese, entretanto, não é ratificada pela análise de correlação, que não foi capaz de detectar relação existente entre tamanho dos glóbulos e CBTs (tabela 4). Desta

forma, outros quesitos devem ser levados em conta na capacidade aglutinante dos diferentes produtos a base de MOS, incluindo a capacidade da cepa teste em expressar fímbrias tipo 1.

Tabela 3

Médias e desvio-padrões das contagens bacterianas totais, área e perímetro dos glóbulos de aglutinação.

Produto	Log (CBT)	Área (nm^2)	Perímetro (nm)
Puro	9,27(A) ± 0,22	NA*	NA*
1	9,04 (B) ± 0,27	39236,26 (A) ± 7535,52	1007,92 (A) ± 230,55
2	7,48 (C) ± 0,54	47579,15 (B) ± 8034,37	1151,14 (B) ± 277,56
3	7,42 (C) ± 0,50	57088,87 (C) ± 5485,03	1330,49 (C) ± 190,21

Letras diferentes na mesma coluna indicam diferenças significativas ($p<0,05$).* NA= não avaliado.

Observou-se correlação significativa e negativa entre a hemaglutinação sensível à manose e aglutinação de MOS para os produtos 1 e 2 (tabela 4), o que sugere que intensidade da aglutinação por bactérias pode estar relacionada a expressão de fímbrias do tipo 1. Resultados de outras pesquisas sugerem que a adsorção das bactérias pelo MOS *in vivo* poderia impedir a adesão à parede intestinal após a ligação previa com MOS. Esta hipótese foi confirmada por Oyofo et al., (1989a)que testaram o efeito de diferentes açúcares sobre a aderência de bactérias à células epiteliais *in vitro* e relataram inibição da aderência por manose em mais de 90% das amostras seguida por uma diminuição da colonização cecal de *S. typhimurium* em aves. Em outros estudos, Oyofo et al., (1989b, 1989c)e Line et al., (1997) obtiveram resultados similares quando relataram a diminuição da colonização de *S. typhimurium* com *Saccharomyces boulardii* em aves. Os resultados também foram reforçados pelo estudo de Spring et al., (2000), que após realizaram três ensaios em pintinhos,verificaram que a adição de MOS a ração reduziu a concentração cecal de *S. typhimurium* cepa 29E nesses animais.

Tabela 4

Correlações entre título hemaglutinante (TH), CBTs, perímetro (D) e área (A) dos glóbulos de aglutinação.

Correlação	r	IC-95%	p
TH x CBT			
Pura	-0.3322	-0.64 a 0.07	0.538
1	-0.1293	-0.50 a 0.28	0.038*
2	-0.417	-0.70 a -0.03	0.024*
3	-0.4497	-0.72 a -0.07	0.824
TH x A			
1	-0.0467	-0.43 a 0.35	0.8245
2	0.2798	-0.13 a 0.61	0.1755
3	-0.0323	-0.42 a 0.37	0.878
TH x D			
1	-0.0351	-0.42 a 0.37	0.8677
2	0.0395	-0.36 a 0.43	0.8512
3	0.1322	-0.28 a 0.50	0.5288
CBT x A			
1	0.1281	-0.28 a 0.50	0.5417
2	0.1087	-0.30 a 0.48	0.6051
3	-0.0806	-0.46 a 0.32	0.7019
CBT x D			
1	0.2909	-0.12 a 0.62	0.1582
2	0.1672	-0.24 a 0.53	0.4243
3	0.0034	-0.39 a 0.40	0.9873

r = coeficiente de correlação de Pearson; IC-95% = intervalo de confiança para estimativa do coeficiente de correlação de Pearson; p =significância estatística para hipótese de que os r é estatisticamente diferente de zero; * p <0,05.

A aglutinação de prebiótico, produto a base de MOS, é um fenômeno observado em diversas espécies bacterianas, incluindo *E. coli*, *Salmonella* .e *Campylobacter sp.*, conforme estudos conduzidos por Spring et al., (2000) e Borowsky et al., (2009). Embora existem várias adesinas presentes na superfície celular destes agentes, a fímbria tipo1 é a única que contribui efetivamente para a colonização do trato intestinal (Althouse et al., 2003; Bäumler et al., 1996; Boyen et al., 2008). Apesar disso, mudança no fenótipo aglutinante para o não aglutinante pode ocorrer em algumas cepas (Althouse et al., 2003), conforme demonstraram Mirelman et al., (1980) e Müller et al., (1991). Nestes estudos verificaram que a expressão do gene fim A em linhagens de S*almonella* depende de condições do meio onde o agente infecciosos se encontra. Estas relacionadas podem estar relacionadas à temperatura, osmolaridade e sais biliares,que correspondem ao ambiente presente no intestino

(Swenson et al., 1991). A expressão foi relacionada a proteínas reguladoras, além de proteínas fim-específicas (Tinker et al., 2001; Tinker and Clegg, 2000) foi associado desta forma, a regulação é susceptível a genes sensíveis aos estímulos ambientais e ao estado fisiológico da célula (Althouse et al., 2003;Bäumler et al., 1997).

O uso de filtros para mensurar o poder aglutinante de diferentes produtos a base de MOS pode apresentar limitações importantes. Os grumos de aglutinados produzidos podem sofrer pressão na filtragem e se romperem permitindo a passagem de bactérias para o meio de cultura, visto se tratarem de estruturas frágeis. Desta forma, as culturas podem subestimar a quantidade de bactérias retidas nos filtros. Outra limitação se refere à possibilidade dos grumos aglutinados sofrerem deformações nas lâminas durante a observação microscópica, diminuindo a precisão das mensurações do perímetro e da área dos mesmos.

Conclui-se que a técnica de filtragem e cultura pode ser utilizada para avaliar diferentes graus de aglutinação de produtos a base de MOS e a quantidade de bactérias retidas nos filtros parece estar relacionada mais com a expressão de fímbrias tipo 1manano-sensíveis do que com os tamanhos dos glóbulos produzidos.

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