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ORIGINAL ARTICLE

Glucomannan hydrolysate (GMH) inhibition of *Candida albicans* growth in the presence of *Lactobacillus* and *Lactococcus* species

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Abstract

Konjac glucomannan hydrolysate (GMH) was compared with inulin and glucose for its capacity to support the growth of probiotic bacteria but inhibit the growth of *Candida albicans* *in vitro*. The growth of lactic acid bacteria (LAB) was studied under aerobic, anaerobic and 5% CO₂ conditions where the GMH progressively supported the growth of LAB as a function of concentration (0.1, 0.5, 1.0 and 2.0% w/v). In mixed cultures, GMH promoted the growth of LAB (even at concentrations as low as 0.1%) and consequently increased inhibition of *C. albicans* under anaerobic conditions, 5% CO₂ or aerobic conditions. Inhibition of *C. albicans* growth was generally higher than that with glucose or inulin and, of the LAB strains, *Lactobacillus jensenii* exhibited most inhibition of the pathogen. The stimulatory effects of low concentrations of GMH on LAB and inhibition of *C. albicans* make these prebiotic and probiotic combinations a potential prophylactic or therapeutic agent for vaginal episodes.

Key words: glucomannan hydrolysate (GMH), probiotic isolates, prebiotic, biocontrol, *Candida albicans*, bacterial vaginosis

Introduction

Lactobacilli are the dominant microbiota of the vagina. Common lactobacilli isolated from the vagina include *L. jensenii*, *L. iners*, *L. crispatus*, *L. gasseri*, *L. cellobiosus* and *L. fermentum* (1). Other species include *L. brevis*, *L. salivarius*, *L. acidophilus* and *L. plantarum* (2,3). A number of lactobacilli have the capacity to suppress the growth of opportunistic pathogens such as *Candida albicans*, which may asymptotically colonize the vagina in low numbers (4,5). However, when the vaginal microbiota is diminished, for example as a result of antibiotic therapy, opportunist disease can occur (6). *C. albicans* causes candidiasis, and while this can be successfully treated with antifungal drugs, chronic infection and frequent re-occurrences are common (7).

Administration of lactobacilli has been considered as a possible vaginal therapy to prevent infection – especially once any yeasts have been eradicated (8,9) – while having no deleterious effects on the host. Additional benefits include the fact that

lactobacilli are generally easily and cheaply cultured to produce large numbers and are generally recognized as safe – even as ‘probiotic’ food supplements. For vaginal therapy, it would be beneficial for the patient that the probiotic persisted by colonizing the epithelium (10).

With regards to treatment or prevention of vaginal disease using a probiotic applied locally in the form of a pessary, cream, douche or spray, the application of lactobacilli directly to the vagina has been correlated with a reduction in yeast vaginitis (11). In addition, oral administration of probiotic *L. fermentum* (reclassified as *L. reuteri*) RC14 combined with *L. rhamnosus* GR-1 has resulted in significant beneficial changes in the vaginal microbiota of patients; increasing the numbers of lactobacilli but decreasing yeasts and coliforms (9).

In the vaginal tract, anaerobic, microaerophilic or aerobic conditions may exist (1,12). Therefore a probiotic organism should, ideally, be capable of establishing growth under any of these atmospheric conditions. The atmospheric conditions (anaerobic or microaerophilic) are critically important. While

the majority of vaginal lactobacilli are regarded as being microaerophilic, anaerobic species may be isolated from up to 45% of women (1,13,14). The pH of the vaginal tract at ~4 is also an important factor in the healthy vaginal ecosystem (15). Lactobacilli play a significant physiological role in the maintenance of the ecological balance of the vaginal ecosystem.

According to Gibson and Roberfroid (16), prebiotics may be defined as 'A non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves the host health'. Glucomannans promote the growth, metabolism and antimicrobial properties of probiotic microorganisms (17) – supporting the potential application of GMH for vaginal therapy.

In the present study, the ability of a novel prebiotic consisting of konjac glucomannan hydrolysate (GMH) (17) to promote the growth of probiotic lactic acid bacteria (LAB) *in vitro* in the presence of aerobic, 5% CO₂ or anaerobic conditions was examined. The LAB included *L. reuteri* RC14 and *L. rhamnosus* GR-1, strains which have previously been shown to be capable of colonizing the vagina (9) with positive health implications. Additionally, the synbiotic ability of GMH to inhibit *C. albicans* growth *in vitro* was studied. The overall objective was to characterize this novel biotherapeutic agent 'alone' or in conjunction with probiotic bacteria for the potential treatment and prevention of the vaginal infections with *C. albicans*.

Materials and methods

Glucomannan prebiotic preparation

Konjac glucomannan hydrolysate (GMH) prebiotic (where the mannose:glucose ratio remains as 1.6:1 with a molecular weight (MW) of 1000–6000 Daltons) was supplied by Glycologic Ltd, Glasgow, UK. It was prepared from konjac flour as previously reported (17). Briefly, konjac flour (Luxara 5867, Arthur Branwell & Co. Ltd, Essex, UK) was dispersed (~10% w/v) in deionized water containing 15 mg/ml cellulase (C013P, 3000 U/g, Biocatalysts, Pontypridd, UK) within sealed 250 ml Duran glass screw-neck flasks. Samples were incubated in a gently shaking water bath (30 cycles/min) at 60°C for 1 h. Following incubation, the samples were then placed in a boiling water bath for 15 min to inactivate the enzyme. Next, they were centrifuged at 3000 rpm (1500 g) for 15 min. The supernatant was then freeze-dried to obtain the hydrolysate. A 4% w/v stock solution of GMH was prepared in

peptone water (PW) (10 g of peptone [LP0037, Oxoid, Basingstoke, UK], 5 g NaCl suspended in 1 L of water); the pH was adjusted to 7.0 with 1 M NaOH and the preparation was autoclaved at 121°C for 15 min.

Inulin

A control prebiotic preparation of inulin from dahlia tubers (I-3754, Sigma, UK) was prepared as a 4% w/v stock solution in PW (CM0009, Oxoid, UK) and autoclaved. Inulin is a fructan that is well established as a prebiotic.

Microorganism strains

Strains of (potential) probiotic LAB were used in this study. These included seven lactobacilli (mostly of human origin) and one *Lactococcus* species. This study also included clinical (vaginal) isolates of pathogenic *C. albicans*. The strains chosen were fresh (low passage) clinical isolates recommended by clinicians and therefore considered highly relevant to the study. The strains were either purchased or obtained as gifts from colleagues (Table I).

The lactobacilli cultures were grown on De Man, Rogosa and Sharpe (MRS) agar and in MRS broth (CM0361 and CM0359, respectively, Oxoid, UK). The *C. albicans* isolates were grown on selective diagnostic chromogenic agar (CA220, Chromagar, UK) and in yeast extract peptone dextrose broth (YPD) (1% w/v yeast extract, 2% w/v peptone and 2% w/v dextrose [LP0021, LP0037, and LP0071, respectively; Oxoid, UK], dissolved in water and autoclaved).

Growth of LAB in minimal medium supplemented with GMH

The LAB strains (ADSL 1 to 8, above) were grown as duplicate cultures overnight in 10 ml of MRS broth (as above) at 37°C in 5% CO₂. A standard curve was constructed with preliminary cultures by plotting the optical density (OD) measured at 570 nm against bacterial count (cfu/ml) taken at intervals. With reference to this standard curve, the OD readings for all cultures were converted to cfu/ml and the bacterial count was adjusted by dilution (always by a minimum dilution of 1 in 1000) in PW so that an inoculum provided 150 µl of 2 × 10⁴ cfu/ml. From duplicate cultures, 150 µl was transferred to triplicate sets of wells of sterile microtitre plates (Orange Scientific, Belgium) with the exception of background controls, which received 150 µl of PW. To each set of the non-control wells, 150 µl of PW containing GMH at concentrations of 0, 0.1, 0.5, 1.0 or 2.0% (w/v) was added by pipette. Glucose

Table I. Origin of microorganisms employed in the study.

Microorganism	Source/collection no.	Study code	Additional information
<i>L. plantarum</i>	A. Sutherland*	ADSL1	Isolated from salami
<i>L. gasseri</i>	NCDO ^b 2233 ATCC ^c 33323	ADSL2	Human mouth, gut and faeces
<i>L. acidophilus</i>	NCDO 1748 ATCC 4356	ADSL3	Human vaginal isolate
<i>L. jensenii</i>	ATCC 25258	ADSL4	Human vaginal discharge
<i>L. crispatus</i>	NCIMB ^d 4505	ADSL5	Vaginal isolate of pregnant woman
<i>L. reuteri</i>	RC-14 Urex Biotech Inc. Canada	ADSL6	Human female urogenital tract
<i>L. rhamnosus</i>	GR-1 Urex Biotech Inc.	ADSL7	Human female urogenital tract
<i>Lactococcus lactis</i> ssp. <i>lactis</i>	NCIMB 6681 ATCC 9936	ADSL8	Glasgow Caledonian University
<i>C. albicans</i>	G. Ramage†	ADSC1	Vaginal isolate
<i>C. albicans</i>	G. Ramage†	ADSC2	Vaginal isolate

NCDO, National Collection of Dairy Organisms, Aberdeen, UK; ATCC, American Type Culture Collection, Manassas, VA, USA;

NCIMB, National Collection of Industrial, Marine and Food Bacteria, Aberdeen, UK.

*Department of Biological Sciences, Glasgow Caledonian University, Glasgow, UK.

†Glasgow University Dental School, Glasgow, UK.

(1.0%) and inulin (1.0%) were also added to separate wells in triplicate as control carbohydrates. The plates were incubated aerobically, in 5% CO₂ (in incubators) or anaerobically in an anaerobic cabinet incubator (Don Whitley, UK) at 37°C overnight. The OD readings were taken until stationary phases for each culture were reached (all within 24 h of culture). The background OD of control wells containing PW or the various carbohydrates was subtracted from the relevant culture wells.

Growth of C. albicans in the presence or absence of LAB strains with or without GMH

The ability of LAB, with or without prebiotic, to inhibit the growth of *C. albicans* (strains ADSC1 and ADSC2) was examined in mixed culture. Two aliquots (10 ml) of each culture for each species of LAB (in MRS broth) and *C. albicans* (ADSC1 and ADSC2 strains in YPD broth) were grown in a 5% CO₂ incubator at 37°C overnight. Using standard growth curves of OD at 570 nm versus microbial count (cfu/ml) as above, cultures were adjusted by dilution (a minimum of 1 in 1000 dilution) in PW so that negligible carbohydrate remained from the original broth. Studies were conducted in duplicate wells of sterile microtitre plates. *C. albicans* (100 µl giving a final count of 1×10^2 cfu/ml) was mixed with 100 µl of eight LAB species (ADSL1 to 8) at a final count of either 1×10^7 , 1×10^5 , 1×10^3 or 0 cfu/ml and 100 µl of either GMH (at 1.0% and 0.1% final concentration), glucose or inulin controls (each at 1% final concentration). Therefore, the effect of LAB along with GMH on *C. albicans* growth could be assessed by comparing growth in the absence of LAB. Sets of microtitre plates were then incubated overnight under three conditions: 5% CO₂, aerobic or anaerobic at 37°C for 24 h. Following incubation, undiluted, 10^{-1} to 10^{-4} serial dilutions of the

mixed cultures were prepared in microtitre plates and 2×10 µl were taken from each dilution and dropped onto agar plates. One plate contained MRS agar with amphotericin B (A9528, Sigma, UK; 10 µg/ml) to count LAB and inhibit *C. albicans* growth, while the other plate comprised Sabouraud dextrose agar (SDA; CM0041, Oxoid, UK) with chloramphenicol (C6455, Sigma, UK; 100 µg/ml) to count *C. albicans* and inhibit LAB growth. Therefore, LAB and *C. albicans* counts could be recorded. The minimum count recoverable of *C. albicans* was 50 cfu/ml.

Statistical analysis

All data for statistical significance testing between groups were first normalized by log transformations and were then subjected to analysis of variance (ANOVA) using GraphPad Prism 5.0 (GraphPad Software Inc., CA, USA). Differences were considered as statistically significant at $p < 0.05$.

Results

Selection of minimal growth medium

In preliminary growth studies no LAB species (ADSL1 to 8) or *C. albicans* isolates (ADSC1 to 2) grew in PW. However, they all grew well in PW when it was supplemented with 1% glucose. This shows (as expected) that the LAB and *C. albicans* need a metabolizable carbohydrate in the media. Hence, 1% glucose was added to the PW and then considered suitable as a 'minimal medium' for determining the effects of GMH on the growth of LAB and *C. albicans*. *C. albicans* cultures within all carbohydrate systems were examined microscopically for pseudohyphal formation or clumping where none were found.

Growth of LAB in minimal medium supplemented with GMH

The mean highest OD (maximum growth), obtained from the stationary phase, in LAB cultures under the three atmospheric conditions tested in PW supplemented with various carbohydrates is shown in Table II. Most (six of eight) of the LAB species tested grew best in 5% CO₂, with the other two species (*L. gasseri* and *L. jensenii*) growing well but not optimally in 5% CO₂.

The *L. gasseri* grew marginally best aerobically and *L. jensenii* grew marginally best anaerobically, but both grew well in all atmospheres. *L. reuteri* and *L. crispatus* grew poorly overall but not aerobically, while *L. acidophilus* achieved its highest OD aerobically but gave more consistent growth in 5% CO₂.

In general, the GMH progressively supported the growth of the LAB (under any atmospheric conditions) as the concentration increased from 0.1% up to 2.0%. *L. crispatus* was the exception, where under anaerobic conditions it did not grow well in the presence of the GMH at any concentration, despite growth in glucose- and inulin-supplemented broth.

The growth data (OD values) for all species of LAB under all atmospheric conditions were combined and analysed (one-way ANOVA). A comparison of the growth of LAB in GMH, inulin or glucose showed that for 1.0 or 2.0% GMH, the growth of LAB was significantly ($p < 0.001$) more than for 1.0% glucose or inulin. For 0.5% GMH, there was no significant difference in LAB growth when compared to 1.0% glucose. However, it produced significantly ($p < 0.05$) more LAB growth than 1.0% inulin. The 0.1% GMH showed no significant difference in LAB growth compared to 1.0% glucose or inulin.

Growth of *C. albicans* in the presence or absence of LAB strains with or without GMH

The inhibition of growth of both *C. albicans* strains, ADSC1 and ADSC2, at a starting count of 1×10^2 cfu/ml was tested in the presence of all species of LAB at counts of 1×10^7 , 1×10^5 , 1×10^3 or 0 cfu/ml and in the presence of GMH with glucose and inulin as controls under aerobic, 5% CO₂ and anaerobic atmospheric conditions. *C. albicans* ADSC1 grew well (Table III) in the absence of LAB. This occurred in the presence of any carbohydrate, although 0.1% GMH and 1.0% inulin led to significantly ($p < 0.05$ and $p < 0.01$, respectively) lower growth of *C. albicans* than the control carbohydrate, 1.0% glucose.

The growth inhibition of *C. albicans* ADSC1 is summarized in Table IV. Incidences of the inhibition of growth were noted as either $\pm$, + or ++, where $\pm$ was greater than or equal to a 1 log reduction in *C. albicans* compared to growth in the presence of the same carbohydrate but without the LAB species being present; + was greater than or equal to a 2 log reduction in growth and ++ was complete inhibition of growth (limit of detection was 50 cfu/ml). Generally, growth inhibition of *C. albicans* ADSC1 increased progressively as the LAB count increased (Table IV). From Table IV it can be seen that when counts of the various species of LAB were 1×10^7 cfu/ml, the GMH prebiotic at 1.0% gave the most incidences of inhibition of ADSC1. This was followed by GMH at 0.1% and then inulin at 1.0%. This was particularly noticeable where inhibition of > 2 log is considered (where 1.0% and 0.1% GMH prebiotic gave 19 and 14 incidences of inhibition of ADSC1, compared with 6 for glucose and 12 for inulin). At counts of 1×10^5 to 1×10^3 of

Table II. Maximum optical density (OD) growth of LAB cultures under various atmospheric conditions in media supplemented with carbohydrates.

LAB species	Mean* maximum OD in culture								
	Aerobic			5% CO ₂			Anaerobic		
	Glucose (1.0%)	Inulin (1.0%)	GMH (%) 0.1, 0.5, 1.0, 2.0	Glucose (1.0%)	Inulin (1.0%)	GMH (%) 0.1, 0.5, 1.0, 2.0	Glucose (1.0%)	Inulin (1.0%)	GMH (%) 0.1, 0.5, 1.0, 2.0
<i>L. gasseri</i>	0.62	0.10	0.15, 0.40, 0.60, 0.80	0.28	0.12	0.19, 0.48, 0.52, 0.68	0.15	0.02	0.09, 0.29, 0.44, 0.79
<i>L. plantarum</i>	0.22	0.10	0.20, 0.30, 0.35, 0.45	0.18	0.15	0.22, 0.34, 0.48, 0.67	0.14	0.16	0.15, 0.15, 0.20, 0.28
<i>L. jensenii</i>	0.29	0.13	0.20, 0.03, 0.30, 0.50	0.45	0.29	0.43, 0.45, 0.54, 0.80	0.40	0.42	0.41, 0.60, 0.68, 1.05
<i>L. rhamnosus</i>	0.10	0.00	0.05, 0.30, 0.40, 0.60	0.28	0.10	0.15, 0.40, 0.45, 0.61	0.27	0.19	0.18, 0.33, 0.40, 0.52
<i>L. reuteri</i>	0.00	0.00	0.00, 0.00, 0.00, 0.00	0.20	0.06	0.09, 0.15, 0.16, 0.25	0.17	0.17	0.07, 0.19, 0.29, 0.07
<i>L. crispatus</i>	0.00	0.00	0.00, 0.00, 0.00, 0.00	0.05	0.01	0.05, 0.10, 0.22, 0.27	0.43	0.40	0.10, 0.14, 0.13, 0.14
<i>L. acidophilus</i>	0.15	0.11	0.10, 0.05, 0.70, 1.00	0.15	0.14	0.21, 0.35, 0.50, 0.65	0.18	0.17	0.17, 0.16, 0.21, 0.33
<i>Lactococcus lactis</i>	0.29	0.16	0.20, 0.40, 0.45, 0.60	0.15	0.15	0.24, 0.33, 0.45, 0.63	0.18	0.16	0.15, 0.25, 0.38, 0.48

GMH, glucomannan hydrolysate.

*Mean of duplicate cultures tested in triplicate.

Table III. Mean growth (cfu/ml)* of *C. albicans* ADSC1 in peptone water supplemented with different carbohydrates and in the absence of LAB.

Atmospheric condition†	Carbohydrate			
	1.0% glucose	1.0% inulin	0.1% GMH	1.0% GMH
Aerobic	5.8×10^6	7.6×10^5	9.0×10^5	3.9×10^6
5% CO ₂	2.6×10^7	2.9×10^6	3.9×10^6	1.6×10^7
Anaerobic	4.6×10^5	1.4×10^5	1.6×10^5	5.0×10^5

*Mean data from studies conducted under aerobic, 5% CO₂ or anaerobic growth conditions.

†Inulin and 0.1% GMH gave significantly ($p < 0.01$ and $p < 0.05$, respectively) less growth than glucose (control) under the three atmospheric conditions tested.

the various LAB species, GMH at 0.1% supported the most incidences of inhibition, followed by inulin and GMH at 1.0%. Glucose was poorer at aiding inhibition of ADSC1 by LAB species. Overall, 0.1% GMH gave the most incidences of inhibition (41),

although inulin and 1.0% GMH gave similar incidences (39 and 35 incidences, respectively) while glucose was poorest (30 incidences). The 0.1% GMH was better at inhibiting *C. albicans* than 1.0% GMH. This is possibly because *C. albicans*

Table IV. Mean inhibition of *C. albicans* ADSC1 growth in mixed culture with probiotic LAB compared to growth without LAB.

Probiotic	Atmosphere	10 ⁷ cfu/ml probiotic LAB				10 ⁵ cfu/ml probiotic LAB				10 ³ cfu/ml probiotic LAB			
		Glucose (1.0%)	Inulin (1.0%)	GMH (1.0%)	GMH (0.1%)	Glucose (1.0%)	Inulin (1.0%)	GMH (1.0%)	GMH (0.1%)	Glucose (1.0%)	Inulin (1.0%)	GMH (1.0%)	GMH (0.1%)
<i>L. crispatus</i>	Aerobic	±	±	±	—	—	—	—	—	—	—	—	—
	5% CO ₂	±	—	—	—	—	±	—	±	—	—	—	—
	Anaerobic	±	—	—	—	—	—	—	—	—	—	—	—
<i>L. gasseri</i>	Aerobic	+	—	+	±	—	—	±	±	—	—	—	—
	5% CO ₂	±	±	+	±	—	±	±	±	—	—	—	—
	Anaerobic	++	+	+	++	++	++	—	++	—	±	—	±
<i>L. jensenii</i>	Aerobic	—	—	—	—	—	+	—	±	±	±	—	+
	5% CO ₂	+	+	+	±	±	+	±	+	±	+	±	+
	Anaerobic	++	++	++	++	++	++	++	++	±	±	+	+
<i>Lactococcus lactis</i>	Aerobic	—	±	+	+	—	±	±	±	—	—	—	—
	5% CO ₂	±	+	+	+	±	±	—	±	—	—	—	—
	Anaerobic	±	++	++	++	—	—	—	—	—	—	—	—
<i>L. plantarum</i>	Aerobic	—	+	+	+	—	—	±	—	—	—	—	—
	5% CO ₂	±	+	+	+	—	±	±	±	—	—	—	—
	Anaerobic	±	++	++	++	—	±	±	+	—	—	—	±
<i>L. reuteri</i>	Aerobic	±	+	+	+	±	±	—	—	—	—	—	—
	5% CO ₂	—	—	—	—	—	—	—	—	—	—	—	—
	Anaerobic	+	±	+	+	±	—	±	±	—	—	—	—
<i>L. rhamnosus</i>	Aerobic	—	±	+	±	—	±	+	±	—	—	—	—
	5% CO ₂	+	±	+	±	±	±	±	—	—	—	—	—
	Anaerobic	±	++	+	++	±	+	—	++	—	±	—	±
<i>L. acidophilus</i>	Aerobic	±	±	+	+	±	—	±	—	—	—	—	—
	5% CO ₂	—	+	+	+	—	—	±	±	—	—	—	—
	Anaerobic	±	+	++	++	—	±	±	±	—	—	—	—
Total ±		12	7	1	5	7	10	12	11	3	4	1	3
Total +		4	8	15	8	0	3	0	2	0	1	1	3
Total ++		2	4	4	6	2	2	1	3	0	0	0	0
Total ±, + and ++		18	19	20	19	9	15	13	16	3	5	2	6

±, Greater than or equal to a 1 log reduction in *C. albicans* ADSC1 compared to growth in the presence of the same carbohydrate without the probiotic organism being present; +, greater than or equal to a 2 log reduction in *C. albicans* ADSC1 compared to growth in the presence of the same carbohydrate without the probiotic organism being present; ++, complete inhibition of *C. albicans* ADSC1 growth (limit of detection was 50 cfu/ml).

did not grow well in 0.1% compared with 1.0% GMH (Table III), whereas the LAB species grew increasingly better as GMH concentration increased (Table II).

Considering the LAB species separately, *L. jensenii* gave most incidences (i.e. 29) of inhibition of ADSC1 with *L. rhamnosus* and *L. gasseri* next (22 and 21 incidences, respectively). When considering inhibition of ADSC1 under various atmospheric conditions the LAB caused inhibition at all conditions with anaerobic, 5% CO₂ and aerobic conditions supporting 57, 48 and 41 incidences of inhibition, respectively. Although most bacteria grew best in single culture at 5% CO₂ (Table II), in mixed culture, *C. albicans* may grow more slowly under anaerobic conditions, which may allow the bacteria to inhibit better in this atmosphere.

It was found that LAB in mixed culture (data not shown) grew to greater than 5×10^6 cfu/ml after 24 h from any inoculum count (1×10^3 , 1×10^5 or 1×10^7 cfu/ml), except for *L. crispatus* and *L. reuteri*, which gave counts between 1×10^5 and 5×10^6 cfu/ml. These two organisms have previously been shown to be slow growing in cultures (Table II).

The same studies as above were repeated with the *C. albicans* strain ADSC2. In all major criteria, the results were the same as for studies on inhibition of strain ADSC1 (data not shown). Again, most incidences of inhibition occurred when LAB were at 1×10^7 cfu/ml. Glucose was poorer than either 1.0% or 0.1% GMH at supporting inhibition. The GMH at 1.0% was better at creating growth inhibition at higher bacterial concentrations, but overall 0.1% GMH was best. Inulin, although better than glucose, did not support inhibition of ADSC2 growth as well as the GMH at 1.0% or 0.1% concentrations. Again, *L. jensenii* was found to be the most inhibitory of all bacterial strains, giving 30 incidences of inhibition. Also, as with studies on strain ADSC1, inhibition occurred under all atmospheric conditions tested with 66, 41 and 32 incidences under anaerobic, 5% CO₂ and aerobic conditions, respectively.

Discussion

Most acute vaginal (infection) episodes may be treated with antimicrobial agents. However, chronic infections and frequent recurrences are a common phenomenon with candidiasis (7). This in turn results in more resistant microbial strains developing to put patients at risk from pathogen overgrowth (7). On the other hand, a large population of microflora usually constitutes the vaginal ecosystem (18). Lactobacilli are the dominant bacteria in a healthy

subject (19). Due to the numerous advantages of colonization by lactobacilli (the main factor for prevention of vaginal thrush), there are clear clinical advantages where these might be used as biotherapeutic agents in the treatment and prevention of vaginal infections.

A number of workers (3,8,9,20,21) have discussed the use of probiotics for the prevention of vaginal infections. However, very little evidence, if any, has been reported for the beneficial application of prebiotics for such episodes. Wagner et al. (22) studied the effect of probiotic bacteria on candidiasis in immunodeficient mice. These authors reported that the probiotics produced biotherapeutic effects by inhibition of *C. albicans* growth, stimulation of the mucosal and systemic immune systems and possibly by nutritional and competitive means. They concluded that *Bifidobacterium animalis* was the most biotherapeutic by stimulating the host resistance to candidiasis.

Commercially available vaginal therapeutic products on the market relevant to this study are either based on probiotics alone or on drugs that affect the fungal cell membrane. These drugs have several disadvantages, such as providing only temporary relief from symptoms like itching. They may also have adverse effects on normal bacteria that are commonly present in the vagina. The occurrence of resistant strains of pathogens (12) has also been detected in patients repeatedly taking these drugs. The introduction of probiotics alone to the vagina may not result in the re-establishment of beneficial colonization (23,24). This is one possibility why probiotic treatments for thrush have not yet proven to be unequivocally successful (7). However, using prebiotics or synbiotics (prebiotic plus probiotic) for such treatments in the form of capsules, pessaries or creams is likely to stimulate the growth of LAB that are naturally available in the vagina or which are introduced along with the prebiotic. This would enable lactobacilli to colonize the organ more efficiently and vaginitis is less likely to re-occur.

In the present study, the GMH supported growth of the LAB strains tested (under any atmospheric condition in which they could grow) as the concentration rose from 0.1% up to 2.0%. In mixed culture of LAB and *C. albicans*, it was observed that inhibition of growth of *C. albicans* occurred progressively as the LAB count increased (Table IV). This implies that large numbers of probiotic LAB are needed to inhibit *C. albicans* in the vagina and urinary tract. In healthy individuals this may occur with resident flora. In others with low counts of natural flora, the LAB could be readily introduced at counts exceeding 1×10^7 cfu/ml. Each of these

LAB species would benefit from further studies such as attachment to human epithelial cells and measurement of hydrogen peroxide and biosurfactant production to determine optimal utilization. It has been reported that LAB delivered orally at $>10^8$ cfu/ml can reduce vaginal colonization by pathogenic bacteria and yeasts (4,9,21).

Growth inhibition of *C. albicans* by GMH was compared to inulin. Inulin did not cause inhibition of yeast growth as well as the GMH at 1.0% or 0.1%. *L. jensenii* was found to be the most inhibitory strain, giving 30 incidences of inhibition. It is worth noting that *L. jensenii* is one of the most frequently isolated species from the vagina and as the strain studied is a human isolate it is likely to colonize the vagina well. *L. rhamnosus* and *L. gasseri* are also commonly isolated from the vagina and the isolates tested are of human origin and/or already shown to colonize the vagina well (4,21).

When considering inhibition of ADSC1 under different atmospheric conditions, the LAB caused inhibition in all conditions, with anaerobic, 5% CO₂ and aerobic conditions supporting 57, 48 and 41 incidences of inhibition, respectively. This is an interesting finding, as although the vagina is anaerobic, movement towards microaerophilic and even aerobic conditions have been reported in the abnormal vagina, especially where the normal flora has diminished (1). *Lactococcus lactis* ssp. *lactis* (a species often used as a cheese starter) and an *L. plantarum* strain isolated from salami both grew well in the presence of GMH and could inhibit *C. albicans* growth in mixed culture. Previous results have indicated that GMH, in combination with LAB, inhibited growth of pathogenic microorganisms (including *C. albicans*) even when glucose was present. Although glucose can function as a substrate for *C. albicans*, it may be readily absorbed from the vagina and hence may not provide a source of energy for the microorganisms (25). These organisms, in combination with GMH could, therefore, potentially produce functional foods for orally delivered therapy similar to the approach advocated by Reid et al. (9).

Overall these data indicate that it would be worth examining further the inhibitory activities of the GMH and selected probiotic LAB with a view to employing them as prophylactic or therapeutic synbiotics in vaginal infection.

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