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Short Communication

Susceptibility of lactic acid bacteria, bifidobacteria and other bacteria of intestinal origin to chemotherapeutic agents

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ABSTRACT

Chemotherapy is a cornerstone of cancer treatment but it can have serious side effects, such as intestinal mucositis. This work reports the susceptibility/resistance profiles of 34 species of lactic acid bacteria (LAB), bifidobacteria and other intestinal bacteria from different collections to various chemotherapeutic agents (CAs) currently used in cancer treatments in an attempt to identify microorganisms that could prevent or treat mucositis symptoms. The highest concentrations of the CAs tested were equal to or higher than those reached in plasma during anticancer treatments. All 34 species proved to be resistant at the highest concentrations assayed [minimum inhibitory concentrations (MICs) > 128 μg/mL] to capecitabine, cyclophosphamide, docetaxel, erlotinib, gefitinib, irinotecan and paclitaxel. For doxorubicin, 5-fluorouracil, gemcitabine and, especially, afatinib and pemetrexed, interspecies variation in the MIC was observed. In further work to assess the interspecies and intraspecies variability, MICs of the CAs pemetrexed and afatinib were determined for 32 strains belonging to four *Bifidobacterium* spp. of intestinal origin. For pemetrexed, a bimodal MIC curve was obtained (modes <2–8 μg/mL and >256 μg/mL), whilst a normal unimodal curve was obtained for afatinib (mode 128 μg/mL). Altogether, these results suggest that the majority of CAs should not, by themselves, perturb the microbial populations of the gut microbiota (but considering that they could be transformed *in vivo* into more toxic compounds). However, LAB and bifidobacteria, which are key players in the intestinal microbial balance of the healthy state, might be particularly inhibited by CAs such as gemcitabine or doxorubicin.

1. Introduction

Cancer remains a major cause of death worldwide, with lung, breast, prostate and colorectal cancer among the most commonly diagnosed forms [1]. Chemotherapy is an important part of the cancer treatment arsenal. Different classes of chemotherapeutic agent (CA), such as taxoids, DNA-alkylating agents, antimetabolites, etc., are available, which attack cancer cells via different mechanisms. Although they may be effective, they fail to distinguish between normal and neoplastic cells and their use commonly entails a variety of side effects [2]. One of the most debilitating is gastrointestinal mucositis, i.e. atrophy and ulceration of the gut mucosa caused by CAs attacking and hampering the renewal of basal epithelial cells [2].

Depending on the dose and type of agent used, between 40 and 100% of patients develop this condition [3,4]. Bacteraemia, malnutrition and other conditions are usually associated with this problem, which can significantly reduce the quality of life of patients undergoing chemotherapy [2]. Symptoms can become so severe that they require dosages be reduced or even the suspension of treatment, leaving the tumour free to grow [2]. If therapies were available that could prevent or at least reduce the severity of mucositis, not only might the quality of life of patients be improved, it might allow a higher dosage of CAs to be used, contributing to refine cancer outcomes.

CAs can also have a damaging effect on the intestinal microbiota. This microbiota plays a variety of roles, including control of inflammatory processes, reduction of intestinal permeability, maintenance of the integrity of the mucus layer (which enhances resistance towards harmful compounds and stimulates epithelial repair) and the release of immune effector molecules (for a review see Ref. [5]). The use of antibiotics to combat CA-induced bacterial infections during cancer treatment has also been associated with a reduction in the microbial diversity of the gut [6,7].

Lactic acid bacteria (LAB) and bifidobacteria are common inhabitants of the human gastrointestinal tract, where their presence may be important for the maintenance of health [8]. Their

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'generally regarded as safe' status means that different species and strains of LAB and bifidobacteria are commonly used as probiotics in dairy products and dietary supplements [9]. These probiotic strains are selected not only for their health-promoting mechanisms but also for their safety aspects; however, nothing is known with respect to their susceptibility to chemotherapeutics.

The present work examines for the first time the resistance/susceptibility profiles of 34 LAB, bifidobacteria and other intestinal species from culture collections to 12 CAs currently used to combat cancer. In addition, the interspecies and intraspecies variation in the susceptibility of 32 *Bifidobacterium* spp. strains isolated from the human gut to afatinib and pemetrexed were also evaluated.

2. Materials and methods

2.1. Bacterial strains, growth media and culture conditions

The minimum inhibitory concentrations (MICs) of 12 CAs were determined for 34 bacterial species (Table 1), including 23 LAB and bifidobacterial species belonging to the BCCM/LMG Bacterial Collection (Ghent University, Ghent, Belgium), 7 intestinal species belonging to the DSMZ Collection (Leibniz Institute, Potsdam, Germany) and 4 Gram-negative intestinal species from our laboratory collection. The MICs of afatinib and pemetrexed were also determined for a laboratory collection of 32 bifidobacterial strains isolated from the human gastrointestinal tract [10].

Lactococci were grown on M17 agar (Oxoid Ltd., Basingstoke, UK) supplemented with 1% glucose (VWR International, Radnor, PA) at 32 °C for 48 h under aerobic conditions. *Streptococcus thermophilus* was cultured on M17 agar (Oxoid) supplemented with 1% lactose (VWR International) at 37 °C for 48 h in anaerobic chamber (Mac500; Don Whitley Scientific Ltd., Shipley, UK) containing an anoxic atmosphere (10% H_2, 10% CO_2 and 80% N_2). Heterofermentative lactobacilli were recovered on de Man, Rogosa and Sharpe (MRS) agar plates (VWR International) and were incubated for 48 h at 32 °C or 37 °C under aerobic or anaerobic conditions depending on the species. Homofermentative lactobacilli and bifidobacteria were recovered on MRS agar supplemented with 0.25% L-cysteine and were incubated at 37 °C for 48 h under anaerobic conditions. Intestinal anaerobic strains were streaked on the following solid media: *Bacteroides* spp. on Gifu anaerobic medium (GAM) agar (Nissui Pharmaceutical, Tokyo, Japan); *Faecalibacterium prausnitzii* on reinforced clostridial medium (RCM) agar (VWR International); *Blautia obeum* comb. nov. (formerly *Ruminococcus obeum*) and *Blautia coccoides* on 50% RCM/50% brain–heart infusion (BHI) (VWR International) plates; and *Slackia* spp. on GAM agar supplemented with 0.5% arginine.

Table 1
Minimum inhibitory concentrations (MICs) of 12 antitumour compounds for the lactic acid bacteria, bifidobacteria and intestinal strains of dominant and representative bacterial groups.

Species	LMG code	MIC (μg/mL)											
		A	DC	E	GF	GM	I	PE	CA	CI	DX	F	PA
Lactococci													
Lc. lactis subsp. *cremoris*	LMG 6987^T	32	>128	>128	>128	16	>128	>128	>128	>128	32	>128	>128
Lc. lactis subsp. *lactis*	LMG 6890^T	32	>128	>128	>128	0.5	>128	>128	>128	>128	4	64	>128
Streptococci													
S. thermophilus	LMG 6896^T	128	>128	>128	>128	>128	>128	>128	>128	>128	8	>128	>128
Homofermentative lactobacilli													
L. acidophilus	LMG 9433^T	64	>128	>128	>128	>128	>128	>128	>128	>128	2	>128	>128
L. delbrueckii subsp. *bulgaricus*	LMG 6901^T	128	>128	>128	>128	0.5	>128	0.125	>128	>128	1	0.25	>128
L. gasseri	LMG 9203^T	>128	>128	>128	>128	>128	>128	>128	>128	>128	8	>128	>128
L. helveticus	LMG 6413^T	128	>128	>128	>128	2	>128	>128	>128	>128	2	>128	>128
L. johnsonii	LMG 9436^T	>128	>128	>128	>128	>128	>128	>128	>128	>128	8	>128	>128
Heterofermentative lactobacilli													
L. brevis	LMG 6906^T	128	>128	>128	>128	>128	>128	>128	>128	>128	4	>128	>128
L. casei	LMG 6904^T	64	>128	>128	>128	16	>128	8	>128	>128	8	>128	>128
L. fermentum	LMG 6902^T	128	>128	>128	>128	16	>128	>128	>128	>128	4	>128	>128
L. paracasei subsp. *paracasei*	LMG 13087^T	64	>128	>128	>128	>128	>128	16	>128	>128	16	>128	>128
L. pentosus	LMG 10755^T	64	>128	>128	>128	0.5	>128	>128	>128	>128	4	8	>128
L. plantarum	LMG 6907^T	128	>128	>128	>128	2	>128	>128	>128	>128	16	>128	>128
L. reuteri	LMG 9213^T	64	>128	>128	>128	>128	>128	≤0.0625	>128	>128	4	128	>128
L. rhamnosus	LMG 6400^T	>128	>128	>128	>128	128	>128	>128	>128	>128	16	>128	>128
L. sakei subsp. *sakei*	LMG 9468^T	32	>128	>128	>128	1	>128	>128	>128	>128	64	128	>128
Bifidobacteria													
B. adolescentis	LMG 10502^T	128	>128	>128	>128	>128	>128	128	>128	>128	1	32	>128
B. animalis subsp. *animalis*	LMG 10508^T	64	>128	>128	>128	>128	>128	≤0.0625	>128	>128	4	>128	>128
B. longum subsp. *longum*	LMG 13197^T	128	>128	>128	>128	>128	>128	>128	>128	>128	4	128	>128
B. pseudolongum subsp. *pseudolongum*	LMG 11571^T	128	>128	>128	>128	>128	>128	≤0.0625	>128	>128	8	128	>128
B. pseudolongum subsp. *globosum*	LMG 11569^T	64	>128	>128	>128	>128	>128	≤0.0625	>128	>128	4	128	>128
B. thermophilum	LMG 21813^T	64	>128	>128	>128	>128	>128	0.5	>128	>128	4	>128	>128
Other intestinal bacteria													
Bacteroides fragilis	DSM 2151^T	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
Bacteroides thetaiotaomicron	DSM 2079^T	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
Blautia coccoides	DSM 935^T	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
Faecalibacterium prausnitzii	DSM 17677	>128	>128	>128	>128	>128	>128	>128	>128	>128	32	>128	>128
Blautia obeum comb. nov.	DSM 25238^T	>128	>128	>128	>128	>128	>128	>128	>128	>128	64	>128	>128
Slackia equolifaciens	DSM 24851^T	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
Slackia isoflavoniconvertens	DSM 22006^T	>128	>128	>128	>128	>128	>128	>128	>128	>128	32	>128	>128
Escherichia coli	A-15	>128	>128	>128	>128	>128	>128	>128	>128	>128	128	>128	>128
Klebsiella pneumoniae	K-78	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
Pseudomonas aeruginosa	PS-25	>128	>128	>128	>128	>128	>128	>128	>128	>128	128	>128	>128
Serratia marcescens	S-54	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128

A, afatinib; DC, docetaxel; E, erlotinib; GF, gefitinib; GM, gemcitabine; I, irinotecan; PE, pemetrexed; CA, capecitabine; CI, cyclophosphamide; DX, doxorubicin; F, 5-fluorouracil; PA, paclitaxel.