Upper gastrointestinal tract disease and probiotics

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Diseases of the oropharynx, oesophagus, stomach and duodenum are common. This review discusses the microflora of the upper gastrointestinal tract with particular reference to lactic acid bacteria and the effect of acid suppression. Probiotics can survive in these sites and evidence is presented for potential roles in disease prevention and treatment, particularly with regards to peptic ulcer disease, *Helicobacter pylori* infection and gastric cancer.

Introduction

Diseases of the upper gastrointestinal tract including the oropharynx, oesophagus, stomach and duodenum are a common cause of human mortality and morbidity. These diseases may be iatrogenic or may occur as primary processes. Alterations in the microflora at these sites may be partly or wholly responsible for development of disease.

This review discusses the microflora of the upper gastrointestinal tract, with particular reference to the lactic acid bacteria group (LAB). The role of probiotics in the prevention and treatment of diseases will be reviewed.

Microbiology of the upper gastrointestinal tract

The normal microflora of the mouth, stomach and duodenum are a rich ecosystem of enormous complexity containing a large number of species of bacteria[1]. The oesophagus and mouth have similar bacterial populations. In the fasting state the stomach and duodenum contain very few micro-organisms which are mainly derived from the oral cavity and throat. The total population and species show dramatic variations along the gastrointestinal tract with the highest concentrations in the colon. The microflora of normal gastric juice is shown in Table 1 as observed in 322 samples of gastric juice from normal subjects. As is evident, the spectrum of organisms grown from the gastric juice is similar to that which normally inhabits the mouth, pharynx and oesophagus.

During fasting the gastric juice contains only small numbers of bacteria and yeasts usually only about 10^2 to 10^5 /ml. The predominant bacterial groups found in the stomach and duodenum *include Streptococci, Lactobacillus sp, Veillonella sp* and *Clostridium perfringens*.

After a meal the bacterial counts in gastric juice increase 100 to 1000-fold[2,3]. This increase in transient with return to baseline levels within 1 to 2 hours as a result of a decrease in gastric juice volume and pH as well as the effects of gastric motility.

A wide variation of bacterial types occurs among individuals, however the number of species and population of bacteria are relatively stable in healthy adults. Within the upper gastrointestinal tract the normal established "resident" bacterial microflora may be altered by bacteria introduced into the body as a normal part of food ("transient" microflora) or as contaminants ("accidental" microflora). In the upper gastrointestinal tract these transient bacteria have a much greater effect on the resident microflora because of the lower numbers of the latter being present.

Table 1. Microflora of normal gastric juice
% of normal subjects with bacterial organisms*

Organism	% of Patients
Staphylococcus epidermidis	61
Streptococcus mitis	59
Yeasts (*Candida albicans* and others)	53
Lactobacillus spp.	50
Streptococcus salivarius	50
Neisseria spp.	37
Micrococcus spp.	35
Corynebacterium spp.	33
Staphylococcus auerus	16

*modified from reference 5

In spite of its stability the intestinal microflora can vary enormously in the stomach and duodenum dependent on host factors such as level of gastric acid secretion[2,3], bile salts, and mucous in the intestinal wall. In addition medications, diet, infections, age, stress and climate can also alter the microflora[4]. The contents of microflora may

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also be influenced by bacterial interaction such as antagonism or symbiosis. Adaptation of intestinal microflora can occur to most substances that enter the intestines from the oral tract or via the biliary system. This adaptation occurs within several days with the ability of intestinal microflora to metabolise these substances. Gastric acid inhibits the growth of micro-organisms, with the stomach of patients having no acid showing an increased number of bacteria[2,5]. In these subjects counts of bacteria of between 10^6 to 10^7/ ml. have been observed. In subjects with no gastric acid (achlorhydria) the flora is similar to that found in the colon with 50% or more of patients having coliforms, Bacteroides or other colonic type[2,6]. After gastric resective surgery, which is associated with a decrease in gastric acid, a change in the bacterial counts are also observed with 10,000 fold higher levels noted in some subjects. In addition to the higher bacterial counts an increase in coliforms is also observed[6,7].

A number of powerful acid suppressant drugs are now available with a statistically significant relationship between the gastric luminal pH and the number of organisms in gastric juice in patients taking these agents[5]. As the pH of gastric juice increases a plateau is reached at about pH 5 to 6 in the median bacterial counts which peak at between 10^6 to 10^8/ ml. It has been suggested that the concentration of carcinogenic N-nitroso compounds are increased in gastric juice after antisecretory drugs. Some authors however have found either no change or a decrease *in vivo* nitrosation as intragastric pH rises[5].

Lactic acid bacteria (LAB) including *Lactobacillus, Lactococcus, Pediococcus, Leuconostoc* and *Bifidobacterium* are found throughout the gastrointestinal tract. The predominant population of lactic acid bacteria in the upper gastrointestinal tract are *Lactobacillus* species. Lactobacilli may colonise the mucosal surface of the duodenum as well as the stomach. For this to occur they must possess certain properties including adhesion, competitive exclusion ability and bacterial inhibitor production.

Probiotics in the upper gastrointestinal tract

Probiotics, that is live microbial food supplements beneficially affecting the host by improving its microbial balance, can survive passage through the stomach and duodenum. Both *Bifidobacterium* species and *Lactobacillus* species are capable of transiting the oesophagus, stomach and duodenum in normal subjects. Gastric acid does affect LAB. However, survival of bifidobacteria in fermented milk products occurs *in vitro* and *in vivo* for up to 3 hours at a pH of 3[8]. Similarly lactobacilli can survive similar acidic conditions of around pH 4 for several weeks *in vitro*.

Diseases of the upper gastrointestinal tract

Diseases of the oesophagus, stomach and duodenum occur in a high proportion of adults with the lifetime incidence of peptic ulcer disease of 10%, gastro-oesophageal reflux disease of 25%, indigestion of unknown cause (non-ulcer dyspepsia) of 20% in subjects living in the Western world. Gastric cancer varies considerably on a geographic basis with rates of between 4 to 80 per 100,000 of the population per year.

The important problem of peptic ulcer disease is now known to be attributed to a bacterial pathogen, *H. pylori*, in conjunction with gastric acid and ulcerogenic drugs. *H. pylori* is a gram negative which colonises the gastric mucosa and upper duodenum and causes long-term histological inflammation in all infected subjects[8]. The bacterium is found within gastric mucus and on the surface of gastric epithelial cells. This organism is transmitted between humans via oral/oral or faecal/oral spread and once colonisation occurs it results in an inflammatory process which is sustained for life.

The accompanying inflammation of the stomach may remain stable or along with other bacterial and environmental factors, gastric or duodenal ulcer disease, or less commonly gastric malignancy (carcinoma and certain forms of lymphoma) will develop. *H. pylori* infects between 30 to 80% of the world's adult population with the prevalence higher in low socio-economic groups, institutionalised individuals, and amongst family members of infected subjects. The high prevalence of this disease and its associated pathology reveals that this is the most common infectious disease worldwide and its control would result in marked diminution in mortality and morbidity from diseases of the upper gastrointestinal tract.

Current therapies to eradicate this infection relate to the use of antimicrobial agents in combination with a bismuth compound or a potent acid suppressing agent[8]. These agents are effective in 80-95% of subjects however side effects, particularly related to nausea, abdominal pain and diarrhoea, effect up to 15% of subjects.

Gastric cancer is a cause of high mortality in individuals who develop the disease with the distribution worldwide varying considerably[9]. *H. pylori* is classified by the WHO as a biological carcinogen causing gastric cancer. A strong correlation between the prevalence *of H. pylori* in the community and mortality from gastric cancer is observed. Moreover, a high prevalence of *H. pylori* infection occurs in subjects with the disease. In experimental animals co-infection with *Helicobacter* results in increased susceptibility to chemical carcinogens inducing gastric cancer. Long-term infection with *H. pylori*, particularly that which is acquired prior to adolescence, appears to increase the susceptibility to develop gastric cancer. Gastric mucosal changes of atrophy and intestinal metaplasia predispose to the development of malignancy and are a function of the duration of infection. Other factors including dietary agents, chemical carcinogens (nitrites and N-nitroso compounds) and hypoacidity all appear to be co-factors in development of cancer.

Probiotics in prevention and treatment of upper gastrointestinal tract disease

Probiotic bacteria have important properties that would make them potentially useful in the treatment and prevention of upper gastrointestinal tract disease. These include the ability to adhere to human intestinal mucosa, the provision of temporary and potentially permanent colonisation of the gastrointestinal tract, the production of

antimicrobial agents resulting in inhibition of pathogen growth, as well as the tolerance to acid and bile. In addition LAB and fermented milk products may possess anti-mutagenic and anti-carcinogenic properties.[10] Probiotic bacteria containing these properties are known to exist and include a number of *Lactobacillus* species.

Lactic acid bacteria produce a number of major fermentation products including lactic acid, acetic acid, as well as hydrogen peroxide, bacteriocins and other metabolites as described by Mishra and Lambert[11]. Recent studies have shown that *Lactobacillus acidophilus* and other lactic acid bacteria will inhibit the growth of *H. pylori in vitro*[12,13]. Lactic acid, acetic and hydrochloric acid inhibit *H. pylori* growth *in vitro* (Fig 1). The concentrations of lactic acid produced by strains of LAB tested ranged from 50 to 156 mmol/l and correlated with *H. pylori* inhibition (Table 2). Six strains of *Lactobacillus acidophilus* and one strain of *Lactobacillus casei* subsp and *rhamnosus* inhibited *H. pylori* growth where as *Bifidobacterium bifidus*, *Pediococcus pentosaceus* and *Lactobacillus bulgaricus* did not[13].

Table 2. Inhibition of *Helicobacter pylori* NCTC 11637 by probiotic culture supernatant fluid in an agar well diffusion assay

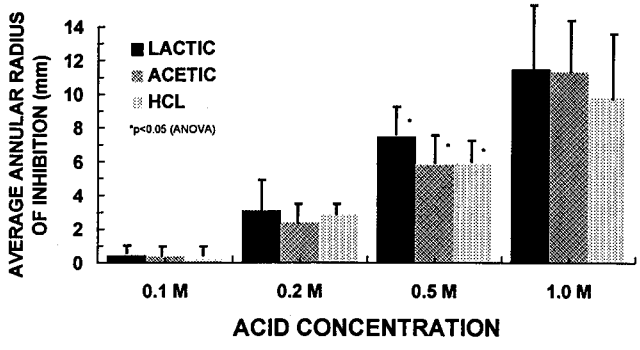
Organism	CSCC No.*	pH	L-Lactic acid (mmol/ l°)	*H. pylori* NCTC 11637**
Lactobacillus acidophilus	2400	3.9	70	0 ± 0
L. acidophilus	2401	3.9	95	1.5 ± 0.5
L. acidophilus	2403	3.9	133	2.0 ± 0.6
L. acidophilus	2404	4.0	91	2.5 ± 0.6
L. acidophilus	2405	4.1	87	2.1 ± 0.5
L. acidophilus	2406	4.0	86	1.1 ± 0.4
L. acidophilus	2409	4.1	85	0 ± 0
L. acidophilus	2422	4.1	121	1.9 ±0.5
L. casei	2622	3.8	156	2.1±0.4
L. bulgaricus	2515	3.8	50	0 ± 0
Pediococcus pentosaceus	2304	4.9	37	0 ± 0
Bifidobacterium bifidus	1900	5.6	12	0 ± 0
3% lactic acid		2.3	445	3.5 ± 0.2

*CSCC = CSIRO Starter Culture Collection ** Average annular radius of inhibition of nine assays ± S.D. (mm)
***Modified from reference 13

Other components produced by LAB may have *in vitro* anti-Helicobacter properties. Included in these are bacteriocins and antibiotic like substances[14,15]. The bacteriocin nisin, potentiated by EDTA or citrate has *in vitro* activity against *H. pylori in vitro*[15].

Recent reports have reviewed the activities of other milk components including lactoferrin. Probiotics may be given in milk based products, whey, proteins and casei.[16,17] Lactoferrin is a glycoprotein found in mammalian milk and is known to possess activity against a variety of gram negative bacteria. When tested *in vitro* against *Helicobacter pylori* at concentrations up to 2 mg/ml inhibition was noted[16]. A peptic digest of lactoferrin appeared to be relatively inactive. Other components of milk protein which maybe administered along with probiotics have been found to exert anti-Helicobacter effect *in vitro*[17].

Figure 1. Effect of acid type on inhibition of *H. pylori* NCTC 11637 by agar well diffusion assay.



A pH study by Michetti and colleagues[18], has recently been undertaken in a randomised, double-blind, controlled clinical trial incorporating a whey-based *Lactobacillus acidophilus* (strain LA1) culture supernatant along with either a potent acid suppressor in the form of omeprazole (with anti-*H. pylori* properties) or placebo. Twenty volunteers were randomised in this study and treated for a 14 day period. A breath test assessing *H. pylori* status revealed a significant fall in the *H. pylori* colonisation following the LA1 culture supernatant therapy. Of interest is the finding that this effect was sustained over a period of 6 weeks post-treatment. This study suggests that culture supernatant of a Lactobacillus is a potential useful adjuvant for *H. pylori* treatment.

Other components of milk may also be useful in protection of the gastric mucosa against different ulcerogenic agents. Milk phospholipids may protect the animal[19] and human[20] gastric mucosa against damage by exogenous ulcerogenic agents. Milk also has been shown to contain substantial amounts of prostaglandins which in animal experiments also protect against stress-induced gastric ulceration.

Thus, there is accumulating evidence that a number of LAB have *in vitro* and *in vivo* activity against *H. pylori* infection. Moreover the effect of LAB in the prevention of antibiotic induced diarrhoea when given as an adjunct to therapy for *H. pylori* may be important. Currently up to 15% of subjects develop side effects associated with antimicrobial therapy used to treat *H. pylori* . Fermented milk products in the form of yoghurts may also have additional potential benefits in suppressing *H. pylori* as demonstrated *in vitro*.

Gastric malignancy is caused by *H. pylori* and other forms of gastritis and a number of environmental factors including smoking, vitamin C deficiency and mutagen and N-nitroso compound formation from food[10]. Fresh vegetables, dairy foods, vitamin C, vitamin A, carotene and selenium protect against the development of cancer[21]. The role of milk based probiotic agents in the development

of malignancy has however not been reviewed as an independent preventive factor. The theoretical benefits of LAB in the breakdown of chemical carcinogens[22] in decreasing undesirable bacterial enzymes (including nitroreductase) implicated in carcinogenesis, as well as altering the gastric mucosal permeability and structure are potential mechanisms of action for prevention of malignancy. Antimutagenic properties of milk fermented with *Lactobacillus bulgaricus* and *Streptococcus thermophilus* have been demonstrated by Bodana and Rao[23]. In addition, strains of lactobacilli and *E. coli* have been shown to be active in degrading nitrosamines suggesting a potential role of intestinal flora in degrading gastric procarcinogens[24].

Healthy human volunteers fed *Lactobacillus acidophilus* strains NCFM and N-2 had a significant decrease in the activity of three luminal bacterial enzymes-B glucuronidase, nitroreductase and azoreductase[25]. These enzymes may release carcinogens into the stomach and intestine. In spite of the *in vitro* and *in vivo* data no long term preventive studies evaluating fermented dairy products containing LAB have been conducted.

Summary and conclusions

In summary, evidence is accumulating that lactic acid bacteria may have some role in the management of upper gastrointestinal tract disease. The anti-*Helicobacter* effects of these bacteria as well as milk components, as demonstrated *in vitro*, lends support for further evaluation of these agents. Moreover, the benefits in prevention of side effects from antimicrobial agents when used to eradicate this infection may be important.

The management of infections of the oropharynx and oesophagus in subjects immuno-compromised requires aggressive treatment often including toxic antiviral and antifungal agents. Concomitant administration to both prevent and as adjunctive therapy for established infection using probiotics may be of potential benefit in the future.

Although cancer of the stomach is common in certain geographic areas of the world, the role of lactic acid bacteria and probiotic organisms is unclear. The theoretical benefits of a regular intake of probiotics including a decrease in pathogenic bacteria, including *Helicobacter*, alterations in immune function, and a decrease of potential carcinogens, are now becoming clearer. Further evaluation in longitudinal studies of probiotics, particularly in milk based products, are required to define their beneficial effects.

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上消化道疾病和原生菌（ Probiotic ）

摘要

口咽部、食道、胃和十二指腸疾病是常見病，這篇綜述討論上消化道菌群，特別關於乳酸杆菌和酸的抑制作用。原生菌（ Probiotic ）能在這些部位存活，有証據顯示其在疾病預防和治療上的潛在作用，特別是關於消化性潰瘍病，幽門螺杆菌（ Helicobacter pylori ）感染和胃癌。

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