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Letter to the editor

Functional insights on probiotics activity in the gut from metagenomic data

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To the editor

Recently, a novel way was described for selection of antibiotic resistance genes in the microbiome of the gut, in which probiotic bacteria are involved. Classical, direct selection of resistance against a particular antibiotic is straightforward, and this can be accompanied by cross-resistance, when one resistant mechanism applies to more than one antimicrobial. Co-selection occurs when multiple antibiotic resistance genes (ARGs) are simultaneously transferred between bacteria in a single transfer event. To these three selection mechanisms that result in antimicrobial resistance, we can now add a fourth mechanism. It was shown that propagation of bacteria that carried ARGs had occurred in volunteers who had first been treated with a combination of antibiotics (ciprofloxacin and metronidazole) and then took a cocktail of probiotic bacteria (Bio-25, Ambrosia SupHerb Ltd., Netanya, Israël – containing 11 different species) (Montassier *et al.*, 2021). In a small number of individuals, this resulted in proliferation of certain bacterial species in their gut that carried vancomycin resistance genes. The authors showed that these vancomycin resistance genes did not stem from the probiotic bacteria that had been administered. Whether these genes resulted in overt vancomycin resistance was not assessed, but the observations identify a novel mechanism by which ARGs can be enriched in a microbiome: the sequential application of antibiotics and probiotics. Importantly, and a bit worrisome, the selected ARGs were not active against the applied antibiotics. The authors warn for this unwanted effect of probiotics (Montassier *et al.*, 2021).

Do these observations actually pose a reason for concern? Or do they describe an important mechanism how probiotics can exert their beneficial health effects? The data indicate that their presence in the gut can result in a shift in commensal bacterial populations, resulting in expansion of species that are beneficial to the host.

The described enrichment of ARG-carrying bacteria was observed following metagenomic sequencing of endoscopic samples. The effect was only seen in individuals who the authors described as 'permissive', meaning the probiotic bacteria colonised their gut. The effect was only observed in the microbiome of their lower gastrointestinal tract, and only in lumen samples but not in mucosal endoscopic samples or in faeces. The effect was not observed in individuals who the authors described as 'resistant', meaning the probiotic bacteria didn't multiply in their gut. Following the treatment, the 'permissive' individuals had larger numbers of AGR-carrying *Blautia* species and *Clostridium* species (*Clostridium citroniae*, *Clostridium leptum*) in their gut, and by the use of mouse experiments it was confirmed that *Blautia coccoides*, *Blautia hominis* and *Blautia producta* had all increased as a result of the sequential antibiotic and probiotic treatment (Montassier *et al.*, 2021).

The beneficial effects of these species, and conversely, correlations between their absence or lower abundance and diseased states, have been known for some time. Decreased levels of *Blautia* species have been associated with liver diseases (Bajaj *et al.*, 2012; Kakiyama *et al.*, 2013), colorectal cancer (Chen *et al.*, 2012) and paediatric type 1 diabetes (Murri *et al.*, 2013). Abundance of intestinal *Blautia* species associates negatively with graft-versus-host disease (Jenq *et al.*, 2015). *B. coccoides* was shown to inhibit nuclear factor kappa beta (NF-κB) activity in CaCo-2 cells (Lakhdari *et al.*, 2011), and did not evoke an inflammatory response in mononuclear cells *in vitro* (Tuovinen *et al.*, 2013). This species is often less abundant in the elderly (Rondanelli *et al.*, 2015). A triggered pro-inflammatory

response in HT-29 cells was decreased in presence of *B. producta* (Pathmanathan *et al.*, 2020). Abundance of *C. citroniae* correlated strongly and negatively with waist circumference in adults with metabolic syndrome (Qin *et al.*, 2021). *C. leptum* was found in lower abundance in ankylosing spondylitis patients (Cardoneanu *et al.*, 2021) and in patients suffering from depression (Mason *et al.*, 2020), although this species was reported as enriched in a pro-inflammatory diet study group (Zheng *et al.*, 2020) and in Systemic Lupus Erythematosus (Chen *et al.*, 2021). Whether *Romboutsia timonensis*, which had also increased in numbers, is contributing to health or correlates with disease is not clear at present.

Many commensal *Blautia* and *Clostridium* species have anti-inflammatory effects, while the latter are important butyrate producers; the beneficial effects of this short-chain fatty acid (SCFA) are well-known and this does not need further elaboration. A shift in the gut microbiome that favours these organisms would likely have beneficial health effects.

The description of the enrichment of these commensal bacteria by Montassier and colleagues was focussed on the antibiotic resistance genes present in these organisms; their proliferation resulted in a detected expansion of the intestinal 'resistome' (Montassier *et al.*, 2021). However, the clinical relevance of this reported expansion would most likely be limited. Only when vancomycin were to be given to individuals with higher abundance of vancomycin-resistant genes in their resistome might this be relevant, provided the genes are expressed, resulting in clinical resistance. However, these resistance genes were already present in the indigenous microbiota. Therefore, intake of vancomycin would select for any residing population of resistant bacteria, independent of previous enrichment, which limits the clinical relevance of possible pre-enrichment of such ARGs by probiotic treatment.

The focus on presence of ARG can be misleading. For instance, *B. producta* can, together with other commensal bacteria, actually restore colonisation resistance against vancomycin-resistant enterococci (Caballero *et al.*, 2017), by means of expressing particular lantibiotics (Kim *et al.*, 2019). Irrespective of a bacterial species containing certain ARG, the overall effect of their presence can be positive or negative to the health of the host. Given the characteristics of the organisms that were propagating in the responsive individuals, positive rather than negative health effects can be expected.

The interesting metagenomic work by Montassier and colleagues thus provides elegant evidence for the mechanism by which probiotic bacteria may exert their effect: they modify the gut microbiome in such a manner that allows for the proliferation of commensal bacteria that are beneficial to the host's health. This contrasts with the conclusions drawn by the authors who point out 'opposing person-specific and antibiotic-dependent effects of probiotics on the resistome' (Montassier *et al.*, 2021). That the observations were limited to 'permissive' individuals strongly point towards a contribution by the probiotic bacteria, who mostly reside in the lumen and do not typically adhere to the mucosa. The latter may explain why the effect was not seen for mucosal samples.

The functional mechanisms by which probiotics exert their health effects have been elucidated over the past decades. Whereas in pre-antibiotic times probiotics were selected to display antigenistic effects towards pathogens to fight off infectious diseases, later their anti-inflammatory activity was recognised and characterised. Now it is realised that probiotic bacteria can also be powerful modulators of the intestinal microbiome. This potency was neatly observed in this latest study, which had focussed on ARGs but actually demonstrated one of the major mechanistics of probiotics, namely modulation of the microbial community in the gut.

Conflict of interest

The author consults companies producing probiotic products, but there is no connection with the company producing Bio-25.

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