

In vitro Assessment of the Host Response against *Enterococcus faecalis* Used in Probiotic Preparations

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Abstract

Along with other lactic acid bacteria, enterococci are used in food products and as health promoting agents. The safety of these products must be ensured, because they contain potentially pathogenic microorganisms. Here we present an *in vitro* opsonophagocytic assay that closely mimics the protective human immune response to *Enterococcus faecalis* and *Enterococcus faecium*. A collection of closely related *E. faecalis* isolates used as probiotics showed different susceptibilities to opsonic killing, suggesting that some of these isolates possess a capsule while other do not. This information may be helpful in assessing the safety of a given bacterial isolate used and could detect likely enterococcal candidates for probiotic preparations.

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Introduction

Enterococcus faecalis is part of the normal gastrointestinal flora in humans [1]. Normally, this species does not cause infections in otherwise healthy persons [2]. However, enterococci can cause serious and sometimes life-threatening systemic infections, especially in immunocompromised patients [3].

Enterococci and several other species of lactic acid bacteria are used for starter cultures in dairy products and are also marketed as medical probiotic preparations to enhance the host immune response [4–7]. In these applications, the safety of the bacteria used is critical. Strains with applications in food products should not contain known or putative virulence factors or antibiotic resistance traits [8, 9].

The protective host immune response to systemic bacterial pathogens involves specific opsonic antibodies, the complement system, and granulocytes [10]. The susceptibility of bacteria to the host immune system varies between species as well as between specific isolates of a given single species. Several bacterial factors, especially the presence of a capsule, contribute to the serum resistance of a given strain [11]. With encapsulated pathogens, the presence of all three components (i.e. specific antibodies directed

against the capsular polysaccharide antigens, complement, and granulocytes) is necessary to elicit an effective and protective immune response [12].

We have developed an opsonophagocytic *in vitro* assay for *E. faecalis* and *Enterococcus faecium* which can be used to assess the resistance of a specific strain to the host immune response [10, 13]. Here we present additional data which assist in differentiating the role of the different components of the immune response operative from that of a specific strain.

Materials and Methods

Bacterial strain S1/X (DSM 16440) is an *E. faecalis* isolate used as medical probiotic (Symbioflor 1[®], Symbiopharm, Herborn, Germany). EFS S1-01, EFS S1-02, EFS S1-05, and EFS S1-07 are several of the separate clones that constitute Symbioflor 1[®]. They have been determined to be clonally related by RAPD and MLST (unpublished observation).

The opsonophagocytic assay has been performed as described elsewhere [10]. Polymorphonuclear leukocytes (PMN) were prepared from fresh human blood collected from healthy adult volunteers. The serum used as complement source was taken from healthy volunteers, immediately frozen and stored at –80 °C until use. The percent killing was calculated as described elsewhere [10] using t-test and ANOVA with Tukey's post test (Prism3, Graph-Pad Software Inc.).

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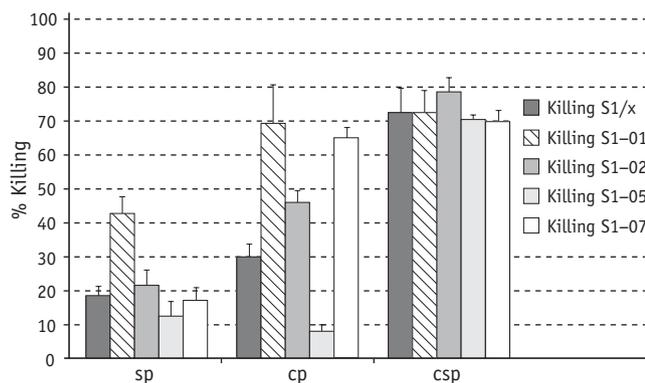


Figure 1. Opsonic killing of the mixture of enterococcal isolates (S1/X) and the single isolates S1-01, S1-02, S1-05, and S1-07 with normal rabbit serum plus PMNs (sp); complement plus PMNs (cp); and complement plus normal rabbit serum plus PMNs (csp). Error bars represent the standard deviation.

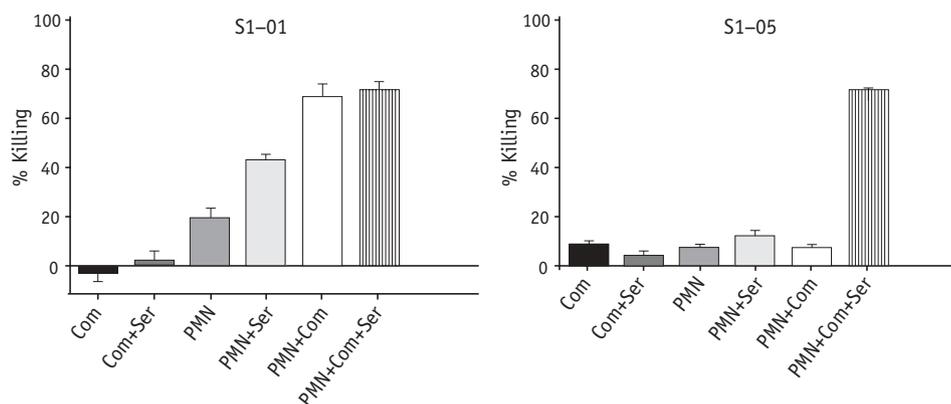


Figure 2. Comparison of two isolates (S1-01 and S1-05) using complement alone (Com); complement plus normal rabbit serum (Com+Ser); PMNs alone (PMN); PMNs plus normal rabbit serum (PMN+Ser); PMNs plus complement (PMN+Com); and PMNs plus complement plus normal rabbit serum (PMN+Com+Ser). Error bars represent the standard error of the mean.

Results and Discussion

Complement alone, or complement with the addition of normal rabbit sera, did not lead to significant killing (i.e. more than 30%) of any of the isolates tested. The opsonic killing of the different bacterial isolates is depicted in figure 1, which shows the susceptibility to PMNs plus normal rabbit serum (sp), PMNs plus complement (cp), and the combination of PMNs, complement and serum (csp). While killing of all isolates was identical (ca. 70%), it was found that when PMNs, complement, and normal rabbit sera were used (csp), there was a clear difference between the isolates as compared to when serum and PMNs or complement and PMNs were used. Using both combinations of components, one isolate was more susceptible (i.e. S1-01) and one isolate was more resistant (i.e. S1-05) to killing.

Figure 2 shows the susceptibility of these two isolates under the various conditions tested.

The isolates tested in this study were genetically related *E. faecalis* isolates used as probiotics. The preparation consists of an aqueous solution of 10^7 cfu/ml of viable bacteria ingested orally. Our data confirm that strains that appear to be similar may in fact exhibit different characteristics regarding their resistance to opsonic killing. The pattern of resistance to killing of isolate S1-05 suggests that this isolate is encapsulated and the capsule is partially protecting it from killing by PMNs. Isolate S1-01 is highly susceptible to killing by serum and PMNs, as well as by complement and PMNs. This pattern indicates that this isolate does not possess a capsule. All isolates tested were equally susceptible to killing when antibodies, complement, and PMNs were used in the opsonophagocytic assay. We employed normal rabbit sera in this setting because we [10, 13] and others [14–16] have observed that they contain low-titered specific antibodies against enterococcal antigens that promote opsonic killing. Killing with specific antibodies against enterococcal capsular polysaccharides is serotype-specific with four different capsular serotypes having been described to date [17].

The present study confirms that the opsonophagocytic assay may be a helpful addition to current tests when assessing the pathogenic potential of enterococci used in food products or in health promoting agents. The format of the assay as presented here (with the separation of the effects obtained by the three components, i.e. complement, serum, and PMNs) may help to better distinguish between the pathogenicity of these isolates.

While all the isolates tested appear to be safe (since all are killed well by the combination of components), one isolate seems to be less virulent than the others.

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