Diversity Predicts Ability of Bacterial Consortia to Mitigate a Lethal Wildlife Pathogen

Rachael E. Antwis[1,2], Xavier A. Harrison[3]

1. School of Environment and Life Sciences, University of Salford, Salford, UK

2. Unit for Environmental Sciences and Management, North-West University, Potchefstroom, South Africa

3. Institute of Zoology, Zoological Society of London, Regent's Park, London NW1 4RY

Address for Correspondence:

Dr. Rachael Antwis, University of Salford, Room 336, Peel Building, The Crescent, Salford, M5 4WT, UK

01612954641

r.e.antwis@salford.ac.uk

Running Title:

Diversity predicts bacterial anti-fungal activity

Conflict of Interest:

The authors declare no conflict of interest.

ABSTRACT

Symbiotic bacterial communities can protect their hosts from infection by pathogens. Treatment of wild individuals with protective bacteria can combat the spread of emerging infectious diseases (EIDs), but it is unclear whether the degree of bacterially-mediated host protection is uniform across multiple isolates of globally-distributed pathogens. Here we use the lethal fungal pathogen *Batrachochytrium dendrobatidis (Bd)* as a model to investigate the traits predicting broad-scale *in vitro* inhibitory capabilities of both individual bacteria and multiple-bacterial consortia. We show that inhibition of multiple pathogen isolates is rare, with no clear phylogenetic signal at the genus level. Bacterial consortia offer stronger protection against *B. dendrobatidis* compared to single isolates, but critically this was only true for consortia containing multiple genera, and this pattern was not uniform across all *B. dendrobatidis* isolates. These novel insights have important implications for the effective design of bacterial probiotics to mitigate EIDs.

INTRODUCTION

The last 50 years have seen the emergence of several hypervirulent wildlife pathogens with broad host ranges (Tompkins et al 2015). These emerging infectious disease (EIDs) have decimated wildlife populations globally, and are a major contributor to the so-called current "biodiversity crisis" (e.g. Skerrat et al 2007; McCallum 2012). Both climate change (Cohen et al 2017) and the global trade in animals (Tompkins et al 2015) are contributing to the increased spread of EIDs, and broad-scale, effective treatments and/or prophylaxis for these pathogens in the wild are often lacking (Sleeman 2013; Garner et al 2016). Developing such treatments is often complicated by broad variation in genetic and phenotypic traits such as virulence exhibited by these pathogens (e.g. de Jong & Hien 2006; Schock et al 2010; Farrer et al 2011). Successful mitigation of EIDs in the wild demands that preventative or curative therapies demonstrate broad activity over as many genetic variants of the pathogen as possible, and developing mitigation strategies that satisfy this criterion remains a major outstanding research goal.

Most EIDs are attributed to fungal pathogens, including *Pseudogymnoascus destructans* that causes white nose syndrome in bats, and *Batrachochytrium spp.*, which causes chytridiomycosis in amphibians (Fisher et al 2012). *Batrachochytrium dendrobatidis* comprises multiple, deeply diverged lineages, and is capable of rapid evolution (Farrer et al 2011; 2013). Endemic lineages of *B. dendrobatidis* have been identified, including *Bd*CAPE (South Africa), *Bd*CH (Switzerland), *Bd*Brazil (Brazil) and a lineage from Japan (Goka et al 2009; Farrer et al 2011; Schloegel et al 2012; Rosenblum et al 2013; Rodriguez et al 2014), although there are cases where these have spread to other regions and are implicated in population declines in those regions (e.g. *Bd*CAPE in Mallorcan midwife toads, *Alytes muletensis;* Doddington et al 2013). The globally distributed and hypervirulent global panzootic lineage (*Bd*GPL) is the genetic lineage of *B. dendrobatidis* associated with phenomenal mass mortalities and rapid population declines of amphibians around the world, and is a major driver of the current "amphibian extinction crisis" (Fisher et al 2009; Farrer et

65 al 2011; Olson et al 2012). Isolates within this lineage exhibit enormous and unpredictable
66 variation in virulence, even within a single host species exposed under laboratory conditions
67 (Farrer et al 2011; Farrer et al 2013). There is currently no cure for this disease in the wild
68 (reviewed in Garner et al 2016), and given that amphibian communities may be host to
69 multiple *Bd*GPL genotypes (Morgan et al 2007; Rodriguez et al 2014), and the continuous
70 global movement of humans and wildlife that can transport the fungus, any prophylactic or
71 curative treatment needs to effective against multiple *B. dendrobatidis* genotypes and
72 isolates.

73 Bacterial probiotics represent a promising tool to combat major emerging fungal pathogens in
74 the wild, including *Pseudogymnoascus destructans* (Hoyt et al 2015), *B. dendrobatidis*, and
75 the closely related *B. salamandrivorans* (Martel et al 2013; 2014). Of these, probiotic
76 research is currently most advanced for *B. dendrobatidis* (reviewed in Bletz et al 2013 and
77 Rebollar et al 2016). Laboratory and field studies have shown host-associated bacterial
78 communities (hereafter referred to as the 'microbiome') protect amphibians from *B.*
79 *dendrobatidis* infection, and that it is possible to artificially augment the microbiome with
80 'probiotic' bacteria to improve survivorship in response to the pathogen (Bletz et al 2013; Jani
81 & Briggs 2014; Becker et al 2015; Walke et al 2015).

82 To date, most *in vitro Bd*GPL challenge experiments have tested the ability of candidate
83 probiotics to limit the growth just a single isolate of *Bd*GPL. This is problematic, because the
84 inhibitory capabilities of individual bacteria are not uniform across the variation presented by
85 *Bd*GPL (Antwis et al 2015). Previous work has found no evidence of a phylogenetic signal in
86 the ability of bacterial genera to inhibit a singular BdGPL isolate (Becker et al 2015), but a
87 major gap in our understanding concerns whether some bacterial genera are more likely to
88 show broad-spectrum inhibition across a range of *Bd*GPLs, allowing a more focussed search
89 for effective amphibian probiotics. Furthermore, both *in vivo* amphibian probiotic trials and i*n*
90 *vitro* challenges focus on the application of a singular bacterial isolate to arrest the growth of
91 *B. dendrobatidis*, yet the importance of a complex and diverse microbiome for resilience to

infection has been repeatedly demonstrated across a range of host taxa (e.g. Dillon et al 2005; Matos et al 2005; Van Elsas et al 2012; Eisenhauer at el 2013). A novel alternative strategy involves a 'bacterial consortium' approach to probiotics, whereby multiple inhibitory bacterial isolates are applied simultaneously. Multi-species consortia can increase the inhibition of *Bd*GPL (Piova-Scott et al 2017), and so may offer greater inhibitory capabilities across a wider range of *B. dendrobatidis* isolates, but the generality of this pattern across multiple pathogen variants remains untested. Addressing the shortfall in our understanding is critical for developing effective tools for the mitigation of EIDs in the wild.

Here we extend previous work to quantify the ability of metabolites from both individual bacteria and co-cultured bacterial consortia to demonstrate broad-scale inhibition across a panel of *B. dendrobatidis* isolates. First, we test 58 bacterial isolates from 10 genera for inhibition against a suite of 10 different *Bd*GPL isolates to quantify i) variation among bacterial genera in ability to demonstrate broad-spectrum *Bd*GPL inhibition and ii) variation among *Bd*GPL isolates is susceptibility to inhibition. Second, we quantify the relative efficacy of using single bacterial isolates or bacterial consortia to modify *B. dendrobatidis* growth rates *in vitro*. Specifically, we investigate iii) whether consortia yield stronger inhibition than single bacteria across three *B. dendrobatidis* isolates from two lineages (*Bd*GPL and *Bd*CAPE); and iv) whether the diversity of a bacterial consortium (number of member genera) affects inhibitory capabilities.

115 **METHODS**

116 *Phylogeny screening*

117 *In vitro* challenges were conducted for 58 bacteria isolated from wild *Agalychnis spp.* frogs in
118 Belize (Antwis et al 2015) to screen for inhibitory capabilities against 10 *Bd*GPL isolates
119 (Table 1, Figure 1) using an *in vitro* spectrophotometer assay method adapted from Bell et al.
120 (2013), Woodhams et al (2014) and Becker et al (2015). Bacteria belonged to 10 genera,
121 with 3-11 bacterial isolates per genus (Table S1). Bacteria were grown by adding 50ul of
122 frozen stock bacteria (stored in 30% glycerol, 70% tryptone solution at -80°C) to 15ml of 1%
123 tryptone, and incubating at 18°C for 36 hours until visibly turbid (three cultures per bacterial
124 isolate). Bacterial densities were counted using a haemocytometer and adjusted to ~500,000
125 cells/ml. Bacteria were then filtered through a 0.22um sterile filter (Millipore, Ireland) to
126 remove live cells, leaving only bacterial metabolites in solution, which were combined across
127 cultures for a given bacterial isolate, and then kept on ice until *B. dendrobatidis* challenges
128 were conducted. *Bd*GPL (Table 1) isolates were grown in 1% tryptone broth until maximum
129 zoospore production was observed (~3-4 days; ~1 x 10^6 zoospores ml^{-1}). As with bacteria,
130 three flasks per *B. dendrobatidis* isolate were grown and then combined prior to challenges
131 to limit flask-effect. Zoospores were separated from sporangia by filtering through 20um
132 sterile filters (Millipore, Ireland). To conduct the spectrophotometer assays, 50ul of bacterial
133 metabolites and 50ul of *B. dendrobatidis* suspension were pipetted into 96-well plates. Each
134 *B. dendrobatidis*-bacteria combination was run with three repeats. Positive controls were
135 included using 50ul 1% tryptone instead of bacterial metabolites. Negative controls were
136 included using 50ul sterile water and 50ul of heat-treated *B. dendrobatidis* for each isolate.
137 Plate readings were taken every 24 hours for four days using a 492nm filter.

138

139 For each measurement, data were transformed using the equation Ln(OD/(1-OD)), and a
140 regression analysis was used to gain the slope values for each sample over time. Slopes of
141 triplicate replicates for each Bd/Bacteria combination were averaged, and total *B.*

142 *dendrobatidis* inhibition was calculated using the formula: Inhibition (%) = [1-(slope of
143 sample/slope of control)] x 100. A positive value represents inhibition of *B. dendrobatidis*
144 growth, and a negative value indicates enhanced growth of *B. dendrobatidis*.

145

146 *Bacterial consortium challenges*

147 Three bacteria were then selected from each of four genera (*Acinetobacteria,*
148 *Chryseobacterium, Serratia, Stenotrophomonas*) based on their inhibition profiles; poor to
149 medium inhibitors were selected to determine whether combining these bacteria would
150 improve their inhibitory capabilities. Bacteria were grown individually until turbid, adjusted to
151 ~500,000 cells/ml, and added to fresh tryptone either individually (strains A, B and C of each
152 genus separately), or as a triple (strains A, B and C of each genus together to form single-
153 genus mixes, or a random combination of strains across genera to form multi-genus (Table
154 2)). To combine bacteria, a total of 3ml of bacteria were added to 12ml of fresh 1% tryptone
155 broth and left to grow together for 18 hours. The volume of each bacterium added depended
156 on whether the consortium contained one or three bacteria, and the volume was split evenly
157 between the number of bacteria added to each group. Following this, bacteria-*B.*
158 *dendrobatidis* challenges were conducted using the same methods as described above
159 against three *B. dendrobatidis* isolates (Table 1). Average inhibition percentages for each
160 consortium-*B. dendrobatidis* combination were calculated as previously described.

161

162 *Statistical Analysis*

163 All statistical analyses were conducted in the software R v.3.3.2 (R Core Team 2016). R
164 scripts for all analyses presented in this manuscript are provided as a R Markdown document
165 in supplementary information.

166

167 *Phylogeny Data:* To quantify differences among genera in proportion of *Bd*GPL isolates
168 inhibited (inhibition score >0), we fitted a Binomial GLM with the proportion of the 10 *Bd*GPL
169 isolates each bacterial isolate inhibited as the response, and genus as a fixed effect. We
170 used the quasibinomial error structure as the model was overdispersed (dispersion 6.4), and
171 tested the model containing a genus term with the reduced intercept-only model using a
172 likelihood ratio test.

173 To quantify differences among genera in the *degree* of inhibition (size of inhibition score), we
174 fitted a hierarchical model in the R package *MCMCglmm* (Hadfield 2010) with the individual
175 inhibition scores of each bacterial isolate (n=58) for each *Bd*GPL isolate (n=10; total n = 580)
176 as a Gaussian response. We fitted both *Bd*GPL isolate, and bacterial strain ID nested within
177 bacterial genus as random effects. We use uninformative, parameter-expanded priors for the
178 random effects as detailed in Hadfield (2010). We ran models for a total of 100,000 iterations
179 following a burn-in of 10,000 iterations and using a thinning interval of 50. Posterior model
180 checks indicated no significant autocorrelation within chains (all values < 0.05) and adequate
181 convergence using the Geweke diagnostic (Geweke 1992). Inspection of model residuals
182 from the frequentist analogue of this model fitted in *lme4* (Bates et al 2015) revealed
183 normally-distributed residuals and no evidence of heteroscedasticity. Rerunning models with
184 stronger priors has no effect on model results.

185 To calculate % variance in inhibition explained by *Bd*GPL isolate, bacterial genus, and
186 bacterial strain respectively, we extracted the variance components from the variance-
187 covariance matrix of the model above. We expressed the variance of a component *V* as a
188 percentage of the total variance calculated as ($V_{BdGPL} + V_{genus} + V_{strain} + V_{residual}$). We
189 calculated both mean and 95% credible intervals using the posterior samples from the
190 model. To construct Figs. 1 and 2, we extracted the marginal means and 95% credible
191 intervals for each bacterial strain and *Bd*GPL isolate, respectively. That is, the bacterial strain
192 modes are marginalised with respect to *Bd*GPL and vice versa, to quantify whether the
193 *average* scores for each *Bd*GPL or bacterial isolate are significantly different from zero.

194 *Consortium Data:* To calculate the relative mean inhibition of single-genus (SG) vs multi-
195 genus (MG) consortia, we fitted a mixed model in *MCMCglmm* with inhibition as a Gaussian
196 response, consortium type as a 2-level factor, and a random effect of *B. dendrobatidis* and
197 using uninformative priors. To calculate whether consortia exhibited stronger inhibition than
198 the mean of their individual isolates, we constructed a binary variable with outcome 1 if a
199 consortium's inhibition was greater than the single isolate mean and 0 if lower. We fitted this
200 as a response in a binary GLMM with consortium type as a fixed effect, *B. dendrobatidis* as a
201 random effect and using uninformative priors. Neither model exhibited signs of
202 autocorrelation and Geweke statistics for both models indicated convergence.

203

204 *Simulated Consortium Trials*: To probe the relative effectiveness of single bacteria, SG
205 consortia and MG consortia (hereafter 'probiotic types') for modifying the growth rates of *B.*
206 *dendrobatidis*, we ran three sets of simulations, each comprising 1000 iterations. For each
207 set of simulations, we calculated i) the proportion of times a MG consortium yielded higher
208 inhibition than a SG consortium; ii) the proportion of times a MG consortium yielded higher
209 inhibition than a single bacterial isolate; iii) the probability that a MG, SG or single bacterial
210 isolate would yield at least 50% inhibition, which we class as strong inhibition. We derived
211 95% confidence intervals for each test statistic by performing 10,000 bootstrap samples with
212 replacement from the test distributions. The three simulations were as follows:

213 (1) Averaged Over All *B. dendrobatidis* isolates: For each iteration, we randomly selected a
214 *B. dendrobatidis* isolate, and then randomly selected both a SG and a MG consortium. A
215 Single bacterial isolate score was then selected randomly from one of the members of the
216 MG consortium.

217 (2) *B. dendrobatidis* specific scores: To investigate the potential for the effectiveness of
218 consortia to differ depending on *B. dendrobatidis* isolate, we repeated the simulations as in
219 (1) but performed 1000 simulations for *each B. dendrobatidis* isolate. (3) Sequential *B.*
220 *dendrobatidis* exposure: Finally, we examined the ability of the three probiotic types to inhibit

221 two *B. dendrobatidis* isolates in series by randomly selecting two of the three *B.*
222 *dendrobatidis* isolates. For each iteration, we selected a random MG and SG consortium,
223 followed by a randomly-selected single isolate member from the MG consortium. Individual
224 inhibition scores for these three groups were then extracted for both selected *B.*
225 dendrobatidis isolates (i.e. probiotic ID was kept consistent over both pathogen isolates). We
226 calculated the probability that the MG consortium would yield superior inhibition to the SG
227 consortium and single bacterial isolate across both *B. dendrobatidis* isolates, and the
228 probability that all three probiotic types would yield >50% inhibition.

229
230

231 ## RESULTS

232 ### Phylogenetic Signals of *Bd*GPL Inhibition

233 We assayed the ability of 58 bacterial isolates from 10 genera to modify the growth rates of
234 10 *Bd*GPL isolates. Mean inhibition scores ranged from100% (complete inhibition of growth)
235 to -225% (strong facilitation of growth). At the genus level, there was no significant variation
236 among genera in mean proportion of *Bd*GPL isolates inhibited (Binomial GLM; χ^2_9 = 6.2,
237 p=0.72; Table 3). Six isolates from five genera showed at least weak inhibition across all 10
238 *Bd*GPLs, whilst seven isolates from five genera facilitated the growth of all 10 isolates
239 (Supplementary Table S1).

240

241 Variance component analysis revealed considerably more variation in inhibition scores
242 among bacterial strains *within* genera than among genera themselves (Fig. 1). Bacterial
243 strain ID explained 51% [95% credible interval (CRI) 37-63%] of the variation in *Bd*GPL
244 inhibiton scores compared to just 1.3% [0.09-6%] for bacterial genus. *Bd*GPL isolate
245 explained 15.6% [4.8-30%] of the variation in inhibition scores and highlighted two isolates
246 whose marginal effect sizes were significantly negative (JEL423 and AUL2), and one isolate
247 with a significantly positive marginalised inhibition score (08MG04; Fig. 2). JEL423 and AUL2
248 therefore exhibit strongly enhanced growth in the presence of bacterial metabolites, whereas
249 08MG04 is particularly susceptible to inhibition of growth. The remaining seven *Bd*GPL
250 isolates demonstrated no evidence of systematic susceptibility to inhibition of their growth
251 rates across the bacteria tested (Fig. 2).

252

253 ### Multi-Isolate Consortia as Tools for Pathogen Mitigation

254 Consortia containing isolates from Multi-Genus (MG) exhibited significantly higher mean
255 inhibition scores compared to Single-Genus (SG) consortia when marginalising with respect
256 to *B. dendrobatidis* isolate (MG consortia mean inhibition: 36.88%; SG consortia mean:

257 16.9%; 95% CRI of difference 4.12 – 36.52%, p_{MCMC} = 0.02; Fig. 3). If the ability of a
258 consortium to inhibit *B. dendrobatidis* was simply an additive function of the inhibitory
259 capabilities of the individual bacteria it comprised, we would expect the consortium's
260 inhibition score to be equal to the mean of the individual inhibition scores, weighted by
261 relative abundance. Inhibition scores of consortia greater than the mean of individual isolate
262 scores are indicative of synergistic effects, whereby the combined pool of metabolites from
263 multiple bacteria inhibits *B. dendrobatidis* more strongly than the individual isolates. MG
264 consortia had a 61% probability of demonstrating stronger inhibition than the mean of their
265 single composite bacterial isolates, which was significantly higher than the corresponding
266 probability for SG isolates (26.6%, Mean difference 39.4% [95% Credible Interval 11.2-
267 65.1%], p_{MCMC} = 0.01).

268

269 *in silico Probiotic Trials*: Of the 1000 simulated probiotic trials, naïve application of a MG
270 consortium yielded higher *B. dendrobatidis* inhibition in 69.4% of cases [95% CI 66.5-72.3%]
271 compared to SG consortia (null expectation 50%, p_{RAND}<0.001). Moreover, MG consortia had
272 a 38.1% [35.1 – 41.1%] probability of yielding inhibition greater than 50% (strong inhibition),
273 compared to only 13.9% [11.8 – 16.1%] probability for SG consortia. MG consortia
274 outperformed the single isolate in 61% [58-64%] of cases (null expectation 50%,
275 p_{RAND}<0.001). However, by averaging over all *B. dendrobatidis* isolates, these results
276 masked substantial variation among *B. dendrobatidis* isolates in the relative efficacy of MG
277 versus SG consortia. We repeated the above simulations separately for each *B.*
278 *dendrobatidis* isolate, and found that MG consortia were superior to SG consortia and single
279 bacterial isolates for only two *B. dendrobatidis* isolates (*Bd*GPL MODS28 and *Bd*CAPE
280 TF5a1), and performed slightly worse than SG consortia for *Bd*GPL SFBC019 (Fig. 4A).
281 Moreover, although MG consortia have the greatest probability of yielding >50% inhibition for
282 *Bd*GPL MODS28 and *Bd*CAPE TF5a1, this was not the case for *Bd*GPL SFBC019, where
283 SG consortia had a marginally higher probability of delivering strong inhibition (Fig. 4B).

Finally*, we* tested the ability of both MG and SG consortia to inhibit the growth of two different *B. dendrobatidis* isolates in series, as individuals in a single location may be exposed to multiple variants of a pathogen (Goka et al 2009; Schloegel et al 2012; Rodriguez et al 2014; Jenkinson et al 2016), or strong spatial structure of the pathogen and high host dispersal may expose individuals to multiple pathogen variants consecutively. For a given trial, the modelling outcomes were; i) MG consortia inhibited both *B. dendrobatidis* isolates more strongly than SG consortia; ii) SG consortia inhibited both *B. dendrobatidis* isolates more strongly than MG consortia; iii) MG inhibited the first *B. dendrobatidis* isolate more strongly than SG consortia, but not the second; iv) MG inhibited the second *B. dendrobatidis* isolate more strongly than SG consortia, but not the first. Applying the same MG consortium to two *B. dendrobatidis* isolates in series achieved stronger inhibition than SG consortia in 49.4% [46.3 – 52.5%] of cases (i.e. modelling outcome i; null expectation 25% [0.5^2], $p_{RAND}<0.001$). This compared to only 7.9% [6.4-9.6%] of cases where SG consortia exhibited superior inhibition for both *B. dendrobatidis* isolates (i.e. modelling outcome iv). MG consortia provided superior inhibition for only one of the *B. dendrobatidis* isolates in the remaining 43% of cases (mean 20.3% and 22.4% of simulations with superior inhibition for the first and second isolate respectively). MG consortia exhibited strong inhibition (>50%) for both isolates in 14.7% [12.5-17%] of cases, compared to zero cases where SG isolates did so. Applying a single bacterial isolate instead of a SG or MG consortium resulted in strong inhibition for both *B. dendrobatidis* isolates in only 4% [2.9-5.3%] of cases (Fig. 4C).

306 **DISCUSSION**

307 The principal objectives of this study were two-fold: i) to determine the magnitude, if any, of
308 phylogenetic signal in the ability of certain genera of bacteria to inhibit a broad range of
309 *Bd*GPL isolates; and ii) to examine the relative effectiveness of single bacteria and bacterial
310 consortia to inhibit several isolates of *B. dendrobatidis*. We found no evidence of variation
311 among bacterial genera in their ability to exhibit broad-range inhibition across multiple
312 *Bd*GPL isolates. Furthermore, our data suggested consortia provide superior *B.*
313 *dendrobatidis* inhibition than individual bacteria, but critically this pattern is not uniform
314 across pathogen isolates, and is contingent on consortium taxonomic diversity. Our results
315 have important implications for our understanding of the factors determining *in vivo*
316 resistance to infection in the wild, and provide novel insights into effective strategies for
317 designing probiotic therapies to mitigate lethal cutaneous infections.

318

319 *Phylogenetic Signals of* Bd*GPL Inhibition*

320 We detected no phylogenetic signal in the ability of individual bacterial genera to inhibit
321 multiple *Bd*GPL isolates. These data support previous work suggesting the ability to inhibit *B.*
322 *dendrobatidis* is distributed widely over bacterial genera (Antwis et al 2015; Becker et al
323 2015); several isolates demonstrated at least weak inhibition for all 10 *Bd*GPLs but were
324 spread across multiple genera with no clear pattern. That there is clear functional
325 redundancy among genera in this host-protective trait suggests it is not prudent to focus on
326 any one genus in the search for beneficial probiotics (Becker et al 2015), as highly divergent
327 microbial communities can still possess similar functional traits (e.g. Bletz et al 2016). The
328 principal source of variance in inhibition was among bacterial strains, with the number of
329 isolates demonstrating broad-spectrum *facilitation* of *Bd*GPL being roughly equal to the
330 number exhibiting broad-scale *inhibition* of the pathogen. The phenomenon of *Bd*GPL growth
331 facilitation has been described previously for single pathogen isolates (Bell et al 2013;

332 Becker et al 2015), but crucially our results suggest that a bacterial strain's ability to facilitate
333 the growth of *B. dendrobatidis* may extend across a broad suite of pathogen isolates.
334
335 It is unclear *why* some bacterial isolates facilitate *B. dendrobatidis* growth, but one likely
336 explanation is that certain bacterial metabolites can act as growth substrates for fungi
337 (Garbaye 1994; Hardoim et al 2015), or that different bacterial metabolites alter the abiotic
338 environment (e.g. pH) to confer different growth rates (Romanowski et al 2011). Here we
339 have provided some of the first evidence that facilitation of *B. dendrobatidis* growth is not
340 simply a rare phenomenon arising from specific *Bd*GPL/bacterial combinations, but that this
341 is widespread across bacterial isolates, and different *Bd*GPL isolates differ systematically in
342 their growth rates when exposed to bacterial metabolites. That said, all four CORN isolates
343 showed similar levels of inhibition across all bacterial isolates, whereas the two AUL isolates
344 exhibited markedly different inhibition profiles (Figure 2). We identified one *Bd*GPL isolate
345 that was significantly prone to inhibition, and a further two isolates that demonstrated strong
346 resistance to inhibition across the 58 bacterial isolates we tested. That there is variation in
347 this trait among *Bd*GPL isolates is intriguing; if facilitation occurs because *B. dendrobatidis*
348 uses bacterial metabolites for nutrition, it may suggest some *B. dendrobatidis* variants can
349 use those metabolites more efficiently for growth. Data gathered from additional isolates will
350 allow us to formally test this hypothesis by probing whether a *Bd*GPL's susceptibility to
351 inhibition or facilitation correlates with virulence. Previous work has shown no among-isolate
352 variation in susceptibility of *B. dendrobatidis* to an echinocandin antifungal drug (Fisher et al
353 2009), yet our data suggest this pattern is not the same for bacterial metabolites.
354 Recombination among lineages of *Bd*GPL is common (Farrer et al 2011), providing a
355 mechanism whereby metabolic genes favouring enhanced growth may be spread following
356 contact among lineages. Our data have two important implications given the proclivity of *B.*
357 *dendrobatidis* for recombination. First, among-isolate variation in susceptibility to inhibition
358 suggests that the relative efficacy of probiotic or curative therapies in the wild will be modified

359 by local *B. dendrobatidis* genotype. Second, though we tend to treat bacterial inhibition
360 scores as fixed traits, this ignores the ability of genetic recombination among *B.*
361 *dendrobatidis* lineages to modify the relationship between bacterial metabolites and
362 pathogen growth rates. Even the application of probiotics themselves may represent a strong
363 selective pressure favouring genetic variants of *B. dendrobatidis* that lack susceptibility to
364 those probiotics. Although several trials have demonstrated the potential for probiotic
365 prophylaxis against *B. dendrobatidis* (e.g. Harris 2009; Muletz et al 2012; Kueneman et al
366 2016), we still lack the requisite data to measure selection caused by those trials on the
367 pathogen. *In vitro* experimental evolution assays between pathogen and bacteria may prove
368 the most powerful means for detecting such patterns.

369

370 *Consortium-Based Approaches to Combatting Fungal Pathogens*

371 Our results revealed a positive link between the taxonomic richness of a probiotic consortium
372 and its ability to inhibit *B. dendrobatidis* growth, but crucially this relationship was highly
373 dependent on *B. dendrobatidis* isolate. Multi-genus consortia outperformed both single-
374 genus consortia and single bacterial isolates in *B. dendrobatidis* inhibition, and were far more
375 likely to produce strong inhibition of 50% or greater, but only for two of the three pathogen
376 variants.

377

378 The general relationship between inhibition and consortium diversity was in the expected
379 direction; low community relatedness (i.e. high community dissimilarity) and high species
380 richness both increase the resistance of a bacterial community to pathogenic 'invaders' (e.g.
381 Jousset et al 2011; Eisenhauer et al 2012, 2013). Furthermore, previous work has linked
382 higher species diversity of probiotic consortia to increased *B. dendrobatidis* inhibition using a
383 single pathogen isolate (Piova-Scott et al 2017). Superior inhibition from consortia, rather
384 than single isolates, may arise as a by-product of the interference competition over resources

385 created by co-culture (Scheuring & Yu 2012). Thus, even bacteria that are weak inhibitors
386 when grown individually can increase the overall inhibitory power of a consortium by creating
387 a competitive environment that favours greater production of anti-fungal compounds.
388 Functional dissimilarity has been proposed as more important than taxonomic diversity in
389 predicting a community's resilience to invasion (Eisenhauer et al 2013), but may explain why
390 single-genus consortia did not perform as well as multi-genus consortia. In selecting for
391 genetic diversity, we may have been simultaneously selecting for functional diversity not
392 present when co-culturing three members of the same genus.

393

394 That *B. dendrobatidis* isolate can alter the strength of the relationship between consortium
395 diversity and inhibition is a highly novel finding. Our simulated probiotic trials revealed that for
396 two *B. dendrobatidis* isolates, combining bacteria into multi-genus consortia yielded
397 significantly better inhibition than applying one of the member bacteria in isolation. These
398 results provide further support for a synergistic effect of co-culture on inhibition. If multi-
399 genus consortia were no better at inhibition than the mean of their composite members,
400 Monte Carlo integration over all single isolate scores would not have recovered a significant
401 difference between the two groups. Yet, for *Bd*GPL MODS28 and *Bd*CAPE TF5a1, multi-
402 genus consortia yielded by far the highest probability of observing strong inhibition of 50% or
403 more. That this pattern was not conserved for *Bd*GPL SFBC019 is perhaps the most
404 intriguing finding. As for *Bd*GPL variants JEL423 and AUL2 in the phylogenetic trials,
405 SFBC019 was largely resistant to inhibition, with individual bacterial inhibition scores that
406 were often negative. One possible explanation for the lack of efficacy of consortia against
407 SFBC019 is that the when a variant of *B. dendrobatidis* is resistant to inhibition and/or there
408 is little variation in inhibition, co-culture fails to produce any synergistic inhibitory effects. That
409 is, if a pathogen is highly resistant to most bacterial metabolites in the first instance,
410 increases in the relative concentrations of those metabolites through co-culture-mediated
411 competition are unlikely to elicit any significant increases in inhibitory capability. The most

important consequence of this pattern is that for some pathogenic variants, multi-genus consortia are highly unlikely to be able to yield high inhibition in cases where individual isolates have failed to do so. Despite the observed variance in success of consortia across *B. dendrobatidis* isolates, our simulation trials revealed that multi-genus consortia offer the best broad-spectrum protection across multiple *B. dendrobatidis* isolates encountered in series. This finding is important; human-mediated spread of *B. dendrobatidis* through the amphibian trade (Fisher & Garner 2007) means we cannot assume that local populations will be exposed to only one pathogenic variant. Successful mitigation of the pathogen in the wild demands that we employ strategies with the highest broad-spectrum success over multiple pathogen genotypes.

Conclusion

This study adds to a growing body of evidence suggesting that diverse, multi-species consortia may represent powerful disease mitigation tools, offering superior probiotic protection against disease compared to single bacterial isolates. Our work has highlighted that different isolates of a pathogen can modify the strength of inhibition caused by the probiotic, meaning we cannot expect probiotic effectiveness to be uniform across the genetic landscape of the pathogen. Despite the relative merits of multi-genus consortia for mitigating single and multiple *B. dendrobatidis* variants, it remains to be determined how readily these consortia will be able to colonise the host skin *in vivo*. This is crucial for to being able to quantify how applicable inhibition measures derived *in vitro* are to real-world scenarios. Nevertheless, our data highlight the merits of a community-level approach to probiotic mitigation of wildlife disease, which may offer more broad-spectrum host protection in the face of large-scale heterogeneity in pathogen genotype.

ACKNOWLEDGEMENTS

This study was partly funded by a North-West University Postdoctoral Research Fellowship awarded to REA. XAH was funded by an Institute of Zoology Research Fellowship. The authors would like to thank Prof. Richard Preziosi and Dr. Trenton Garner for additional provision of consumables, and Prof. Ché Weldon, Dr. Trenton Garner and Prof. Matthew Fisher for access to *Batrachochytrium dendrobatidis* isolates used in this study.

REFERENCES

Antwis, R.E., Preziosi, R.F., Harrison, X.A., Garner, T.W. (2015). Amphibian symbiotic bacteria do not show a universal ability to inhibit growth of the global panzootic lineage of Batrachochytrium dendrobatidis. Applied and Environmental Microbiology, 81, 3706-3711.

Bates, D., Maechler, M., Bolker, B., Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software, 67, 1-48. doi:10.18637/jss.v067.i01.

Becker, M.H., Walke, J.B., Murrill, L., Woodhams, D.C., Reinert, L.K., Rollins-Smith, L.A., et al. (2015). Phylogenetic distribution of symbiotic bacteria from Panamanian amphibians that inhibit growth of the lethal fungal pathogen Batrachochytrium dendrobatidis. Molecular Ecology, 24, 1628-1641.

Bell, S.C., Alford, R.A., Garland, S., Padilla, G., Thomas, A.D. (2013). Screening bacterial metabolites for inhibitory effects against Batrachochytrium dendrobatidis using a spectrophotometric assay. Diseases of Aquatic Organisms, 103, 77-85.

Bletz, M.C., Loudon, A.H., Becker, M.H., Bell, S.C., Woodhams, D.C., Minbiole, K.P. et al. (2013). Mitigating amphibian chytridiomycosis with bioaugmentation: characteristics of effective probiotics and strategies for their selection and use. Ecology letters, 16, 807-20.

Bletz, M.C., Goedbloed, D.J., Sanchez, E., Reinhardt, T., Tebbe, C.C., Bhuju, S., et al. (2016). Amphibian gut microbiota shifts differentially in community structure but converges on habitat-specific predicted functions. Nature Communications, 7.

Cohen, J. M., Venesky, M. D., Sauer, E. L., Civitello, D. J., McMahon, T. A., Roznik, E. A. et al. (2017). The thermal mismatch hypothesis explains host susceptibility to an emerging infectious disease. Ecology Letters, 20, 184–193. doi:10.1111/ele.12720

de Jong, M.D., Hien, T.T. (2006). Avian influenza A (H5N1). Journal of Clinical Virology, 35, 2-13.

Dillon, R.J., Vennard, C.T., Buckling, A., Charnley, A.K. (2005). Diversity of locust gut bacteria protects against pathogen invasion. Ecology Letters, 8, 1291-8.

Doddington, B.J., Bosch, J., Oliver, J.A., Grassly, N.C., Garcia, G., Schmidt, B.R., et al. (2013), Context-dependent amphibian host population response to an invading pathogen. Ecology, 94, 1795-1804.

Eisenhauer, N., Scheu, S., Jousset, A. (2012). Bacterial diversity stabilizes community productivity. PloS one, 7, e34517.

Eisenhauer, N., Schulz, W., Scheu, S., Jousset, A., Pfrender, M. (2013). Niche dimensionality links biodiversity and invasibility of microbial communities. Functional Ecology, 27, 282-8.

Farrer, R.A., Weinert, L.A., Bielby, J., Garner, T.W., Balloux, F., Clare, F., et al. (2011) Multiple emergences of genetically diverse amphibian-infecting chytrids include a globalized

hypervirulent recombinant lineage. Proceedings of the National Academy of Sciences of the United States of America, 108, 18732-6.

Farrer, R.A., Henk, D.A., Garner, T.W., Balloux, F., Woodhams, D.C., Fisher, M.C. (2013). Chromosomal copy number variation, selection and uneven rates of recombination reveal cryptic genome diversity linked to pathogenicity. PLoS Genetics, 9, p. e1003703.

Fisher, M.C., Garner, T.W.J. (2007) The relationship between the emergence of Batrachochytrium dendrobatidis, the international trade in amphibians and introduced amphibian species. Fungal Biology Reviews, 21, 2-9.

Fisher, M.C., Bosch, J., Yin, Z., Stead, D.A., Walker, J., Selway, L., et al. (2009) Proteomic and phenotypic profiling of the amphibian pathogen Batrachochytrium dendrobatidis shows that genotype is linked to virulence. Molecular ecology, 18, 415-29.

Fisher, M.C., Henk, D.A., Briggs, C.J., Brownstein, J.S., Madoff, L.C., McCraw, S.L., et al. (2012). Emerging fungal threats to animal, plant and ecosystem health. Nature Reviews, 484, 186-194.

Garbaye, J. (1994). Helper bacteria: a new dimension to the mycorrhizal symbiosis. New Phytologist, 128, 197-210.

Garner, T.W., Schmidt, B.R., Martel, A., Pasmans, F., Muths, E., Cunningham, A.A., et al. (2016). Mitigating amphibian chytridiomycoses in nature. Phil. Trans. R. Soc. B, 371, 20160207.

Geweke, J. (1992) Evaluating the accuracy of sampling-based approaches to calculating posterior moments. In *Bayesian Statistics 4* (ed JM Bernado, JO Berger, AP Dawid and AFM Smith). Clarendon Press, Oxford, UK.

Goka, K., Yokoyama, J., Une, Y., Kuroki, T., Suzuki, K., Nakahara, M., et al. (2009). Amphibian chytridiomycosis in Japan: distribution, haplotypes and possible route of entry into Japan. Molecular Ecology, 18, 4757-74.

Hadfield, J.D. (2010). MCMC Methods for Multi-Response Generalized Linear Mixed Models: The MCMCglmm R Package. Journal of Statistical Software, 33(2), 1-22. URL: http://www.jstatsoft.org/v33/i02/.

Hardoim, P.R., van Overbeek, L.S., Berg, G., Pirttilä, A.M., Compant, S., Campisano, A., et al. (2015). The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. Microbiology and Molecular Biology Reviews, 79, 293-320. doi:10.1128/MMBR.00050-14.

Harris, R.N., Lauer, A., Simon, M.A., Banning, J.L., Alford, R.A. (2009). Addition of antifungal skin bacteria to salamanders ameliorates the effects of chytridiomycosis. Diseases of Aquatic Organisms, 83, 11-16.

Hoyt, J.R., Cheng, T.L., Langwig, K.E., Hee, M.M., Frick, W.F., Kilpatrick, A.M. (2015). Bacteria isolated from bats inhibit the growth of Pseudogymnoascus destructans, the causative agent of white-nose syndrome. PLoS One, 10, e0121329.

Jani, A.J., Briggs, C.J. (2014). The pathogen Batrachochytrium dendrobatidis disturbs the frog skin microbiome during a natural epidemic and experimental infection. Proceedings of the National Academy of Sciences of the United States of America, 111, E5049-58.

Jenkinson, T.S., Betancourt Román, C.M., Lambertini, C., Valencia-Aguilar, A., Rodriguez, D., Nunes-de-Almeida, C.H., et al. (2016) Amphibian-killing chytrid in Brazil comprises both locally endemic and globally expanding populations. Molecular Ecology, 25, 2978–2996.

Jousset, A., Schmid, B., Scheu, S., Eisenhauer, N. (2011). Genotypic richness and dissimilarity opposingly affect ecosystem functioning. Ecology Letters, 14, 537-45.

Kueneman, J.G., Woodhams, D.C., Harris, R., Archer, H.M., Knight, R., McKenzie, V.J., (2016) Probiotic treatment restores protection against lethal fungal infection lost during amphibian captivity. Proc. R. Soc. B, 283, 1839.

Martel, A., Spitzen-van der Sluijs, A., Blooi, M., Bert, W., Ducatelle, R., Fisher, M.C., et al. (2013). Batrachochytrium salamandrivorans sp. nov. causes lethal chytridiomycosis in amphibians. Proceedings of the National Academy of Sciences, 110, 15325-15329.

Martel, A., Blooi, M., Adriaensen, C., Van Rooij, P., Beukema, W., Fisher, M.C., et al. (2014). Recent introduction of a chytrid fungus endangers Western Palearctic salamanders. Science, 346, 630-631.

Matos, A., Kerkhof, L., Garland, J.L. (2005). Effects of microbial community diversity on the survival of Pseudomonas aeruginosa in the wheat rhizosphere. Microbial Ecology, 49, 257-64.

McCallum, H. (2012) Disease and the dynamics of extinction. Philos Trans R Soc Lond B Biol Sci 367: 2828–2839. doi: 10.1098/rstb.2012.0224. pmid:22966138

Morgan, J.A., Vredenburg, V.T., Rachowicz, L.J., Knapp, R.A., Stice, M.J., Tunstall, T., et al. (2007). Population genetics of the frog-killing fungus *Batrachochytrium dendrobatidis*. Proceedings of the National Academy of Sciences of the United States of America, 104, 13845-50.

Muletz, C.R., Myers, J.M., Domangue, R.J., Herrick, J.B., Harris, R.N. (2012). Soil bioaugmentation with amphibian cutaneous bacteria protects amphibian hosts from infection by Batrachochytrium dendrobatidis. Biological Conservation, 152, 119-126.

Olson, D.H., Aanensen, D.M., Ronnenberg, K.L., Powell, C.I., Walker, S.F., Bielby, J., et al. (2013). Mapping the global emergence of Batrachochytrium dendrobatidis, the amphibian chytrid fungus. PloS one, 8, e56802.

Piovia-Scott, J., Rejmanek, D., Woodhams, D. C., Worth, S. J., Kenny, H., McKenzie, V., et al. (2017). Greater Species Richness of Bacterial Skin Symbionts Better Suppresses the Amphibian Fungal Pathogen Batrachochytrium Dendrobatidis. Microbiology Ecology, DOI 10.1007/s00248-016-0916-4.

R Core Team. (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rebollar, E.A., Antwis, R.E., Becker, M.H., Belden, L.K., Bletz, M.C., Brucker, R.M., et al. (2016). Using "Omics" and Integrated Multi-Omics Approaches to Guide Probiotic Selection to Mitigate Chytridiomycosis and Other Emerging Infectious Diseases. Frontiers in Microbiology, 7, 68.

Rodriguez, D., Becker, C. G., Pupin, N.C., Haddad, C.F.B., Zamudio, K. R. (2014). Long-term endemism of two highly divergent lineages of the amphibian-killing fungus in the Atlantic Forest of Brazil. Molecular Ecology, 23, 774-87.

Romanowski, K., Zaborin, A., Fernandez, H., Poroyko, V., Valuckaite, V., Gerdes, S., et al. (2011). Prevention of siderophore- mediated gut-derived sepsis due to P. aeruginosa can be achieved without iron provision by maintaining local phosphate abundance: role of pH. BMC Microbiology, 11: 212

Rosenblum, E.B., James, T.Y., Zamudio, K.R., Poorten, T.J., Ilut, D., Rodriguez, D., et al. (2013). Complex history of the amphibian-killing chytrid fungus revealed with genome resequencing data. Proceedings of the National Academy of Sciences of the United States of America, 110, 9385-90.

Scheuring, I., Yu, D.W. (2012). How to assemble a beneficial microbiome in three easy steps. Ecology Letters, 15, 1300-7.

Schock, D.M., Bollinger, T.K., Collins, J.P. (2009). Mortality rates differ among amphibian populations exposed to three strains of a lethal ranavirus. EcoHealth, 6, 438-48.

Schloegel, L.M., Toledo, L.F., Longcore, J.E., Greenspan, S.E., Vieira, C.A., Lee, M., et al. (2012) Novel, panzootic and hybrid genotypes of amphibian chytridiomycosis associated with the bullfrog trade. Molecular Ecology, 21, 5162-77.

Skerratt, L.F., Berger, L., Speare, R., Cashins, S., McDonald, K.R., et al. (2007) Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. Ecohealth 4: 125–134.

Sleeman, J.M. (2013). Has the time come for big science in wildlife health? EcoHealth, 10, 335-338.

Tompkins, D.M., Carver, S., Jones, M.E., Krkošek, M., Skerratt, L.F. (2015). Emerging infectious diseases of wildlife: a critical perspective. Trends in Parasitology, 31, 149-159.

van Elsas, J.D., Chiurazzi, M., Mallon, C.A., Elhottova, D., Kristufek, V., Salles, J.F. (2012). Microbial diversity determines the invasion of soil by a bacterial pathogen. Proceedings of the National Academy of Sciences of the United States of America, 109, 1159-64.

Walke, J.B., Becker, M.H., Loftus, S.C., House, L.L., Teotonio, T.L., Minbiole, K.P. et al. (2015). Community Structure and Function of Amphibian Skin Microbes: An Experiment with Bullfrogs Exposed to a Chytrid Fungus. PloS one, 10, e0139848.

Woodhams, D.C., Brandt, H., Baumgartner, S., Kielgast, J., Küpfer, E., Tobler, U., et al. (2014). Interacting symbionts and immunity in the amphibian skin mucosome predict disease risk and probiotic effectiveness. *PLoS One*, *9*(4), p.e96375.

650 **Table 1**

651 *Batrachochytrium dendrobatidis* isolates used in the study.

652

653

654 **Table 2**

655 Composition of multi-genus consortia used in the study. Single-genus consortia comprised all
656 three bacterial isolates (A, B and C) for a given genus (*Acinetobacter, Chryseobacterium,*
657 *Serratia, Stentrophomonas*).

658

659

660

661 **Table 3**

662 Mean Proportion of 10 *Bd*GPL isolates for which at least weak inhibitory capability was
663 observed, averaged over all bacterial isolates in a genus. 95% CI: 95% confidence intervals
664 from an overdispersion-corrected Binomial GLM.

665

666

667

668

669

670 **Figure 1. Inhibition scores of 58 bacterial strains from 10 genera when tested against**

671 **10 *Bd*GPL isolates.**

672 Estimates are derived from a Bayesian mixed effects model with bacterial isolate nested

673 within genus, and *Bd*GPL isolate fitted as random effects. Points are conditional modes of

674 the individual isolate random effects, marginalised with respect to *Bd*GPL isolate. Error bars

675 are 95% credible intervals.

676

677

678

679

680 **Figure 2. Inhibition scores of 10 *Bd*GPL isolates**

681 Estimates are derived from a Bayesian mixed effects model with bacterial isolate nested
682 within genus, and *Bd*GPL isolate fitted as random effects. Points are conditional modes of
683 the individual *Bd*GPL isolate random effects, marginalised with respect to bacterial isolate.
684 Error bars are 95% credible intervals.

685

686

687

688 **Figure 3.** Inhibition scores for Single-Genus and Multi-Genus Consortia across three *B.*

689 *dendrobatidis* isolates (*Bd*GPL MODS28.1, *Bd*GPL SFBC019 and *Bd*CAPE TF5a1).

690 Points have been jittered for display purposes.

691

692

693

694 **Figure 4. Simulation results examining the relative efficacy of different probiotic**

695 **strategies**

696 (A) the probability of Multi-Genus Consortia (MGC) yielding higher inhibition compared to

697 Single-Genus Consortia (SGC) or a Single Bacterial Isolate (Single); (B) the probability of

698 MGC, SGC or Single bacteria yielding inhibition > 50% when applied to each of three *B.*

699 *dendrobatidis* isolates; (C) The probability of an individual consortium type yielding >50%

700 inhibition when applied to two randomly chosen *B. dendrobatidis* isolates in series.

701

702

Isolate	Lineage	Geographic origin	Host species	Collector	Year	Phylogeny screening
MG04	GPL	Silver Mine, Western Cape, South Africa	*Amietia fuscigula*	Trenton Garner	2010	X
CORN2.2	GPL	Penhale Farm, Cornwall, UK	*Ichthyosaurus alpestris*	Trenton Garner	2012	X
CORN2.3	GPL	Penhale Farm, Cornwall, UK	*Ichthyosaurus alpestris*	Trenton Garner	2012	X
CORN3.1	GPL	Penhale Farm, Cornwall, UK	*Ichthyosaurus alpestris*	Trenton Garner	2012	X
CORN3.2	GPL	Penhale Farm, Cornwall, UK	*Ichthyosaurus alpestris*	Trenton Garner	2012	X
AUL1.2	GPL	Lac d'Aule, France	*Alytes obstetricans*	Matthew Fisher	2010	X
AUL2	GPL	Lac d'Aule, France	*Alytes obstetricans*	Matthew Fisher	2010	X
IA2011	GPL	Ibon Acherito, Spain	*Alytes obstetricans*	Matthew Fisher	2011	X
MODS 28.1	GPL	Mont Olia, Sardinia	*Discoglossus sardus*	Trenton Garner	2010	X
JEL423	GPL	Guabal, Panama	*Agalychnis lemur*	Joyce Longcore	2004	X
SFBC019	GPL	Sellafield, Cumbria, UK	*Epidalea calamita*	Peter Minting	2010	
TF5a1	CAPE	Torrent des Ferrerets, Mallorca	*Alytes muletensis*	Matthew Fisher	2007	

Consortium challenges
X
X
X

Inter-genera mix 1	**Inter-genera mix 2**	**Inter-genera mix 3**	**Inter-genera mix 4**
Chryseobacterium B	*Acinetobacter C*	*Acinetobacter A*	*Acinetobacter B*
Serratia B	*Serratia C*	*Chryseobacterium A*	*Chryseobacterium C*
Stentrophomonas B	*Stentrophomonas C*	*Serratia A*	*Stentrophomonas A*

Genus	Number Isolates	Mean Proportion *BdGPL* Inhibition	95% CI
Acinetobacter	6	0.33	0.1-0.65
hryseobacteriu	8	0.5	0.24-0.76
Citrobacter	3	0.67	0.24-0.95
Comamonas	4	0.7	0.32-0.95
Enterobacter	11	0.54	0.31-0.75
Microbacterium	4	0.4	0.1-0.77
Sanguibacter	3	0.63	0.21-0.94
Serratia	6	0.47	0.19-0.76
Staphylococcus	4	0.73	0.34-0.96
enotrophomon	9	0.49	0.25-0.73

Figure 1

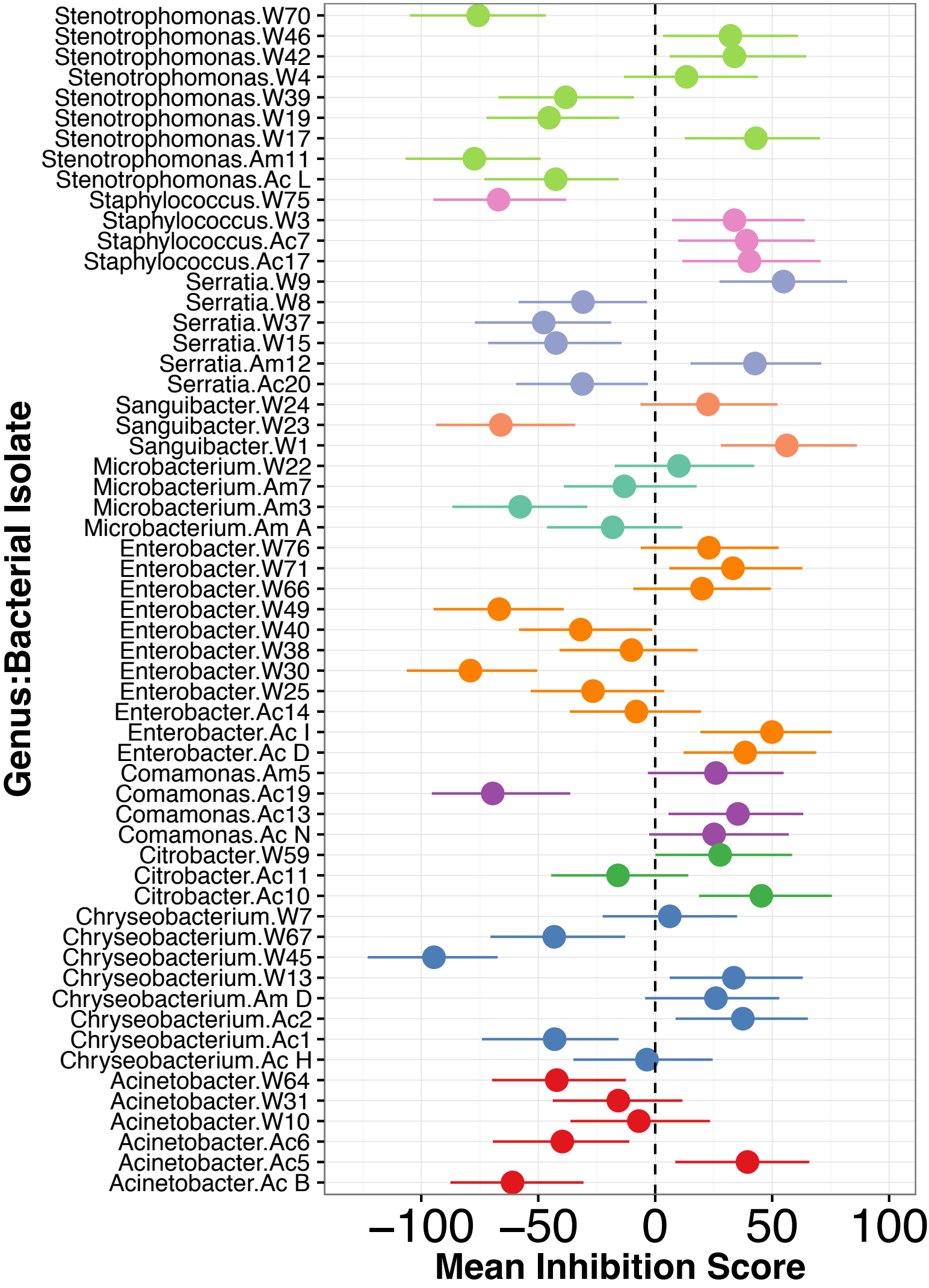

Genus:Bacterial Isolate
Stenotrophomonas.W70
Stenotrophomonas.W46
Stenotrophomonas.W42
Stenotrophomonas.W4
Stenotrophomonas.W39
Stenotrophomonas.W19
Stenotrophomonas.W17
Stenotrophomonas.Am11
Stenotrophomonas.Ac L
Staphylococcus.W75
Staphylococcus.W3
Staphylococcus.Ac7
Staphylococcus.Ac17
Serratia.W9
Serratia.W8
Serratia.W37
Serratia.W15
Serratia.Am12
Serratia.Ac20
Sanguibacter.W24
Sanguibacter.W23
Sanguibacter.W1
Microbacterium.W22
Microbacterium.Am7
Microbacterium.Am3
Microbacterium.Am A
Enterobacter.W76
Enterobacter.W71
Enterobacter.W66
Enterobacter.W49
Enterobacter.W40
Enterobacter.W38
Enterobacter.W30
Enterobacter.W25
Enterobacter.Ac14
Enterobacter.Ac I
Enterobacter.Ac D
Comamonas.Am5
Comamonas.Ac19
Comamonas.Ac13
Comamonas.Ac N
Citrobacter.W59
Citrobacter.Ac11
Citrobacter.Ac10
Chryseobacterium.W7
Chryseobacterium.W67
Chryseobacterium.W45
Chryseobacterium.W13
Chryseobacterium.Am D
Chryseobacterium.Ac2
Chryseobacterium.Ac1
Chryseobacterium.Ac H
Acinetobacter.W64
Acinetobacter.W31
Acinetobacter.W10
Acinetobacter.Ac6
Acinetobacter.Ac5
Acinetobacter.Ac B
−100
−50
0
50
100
Mean Inhibition Score

Figure 2

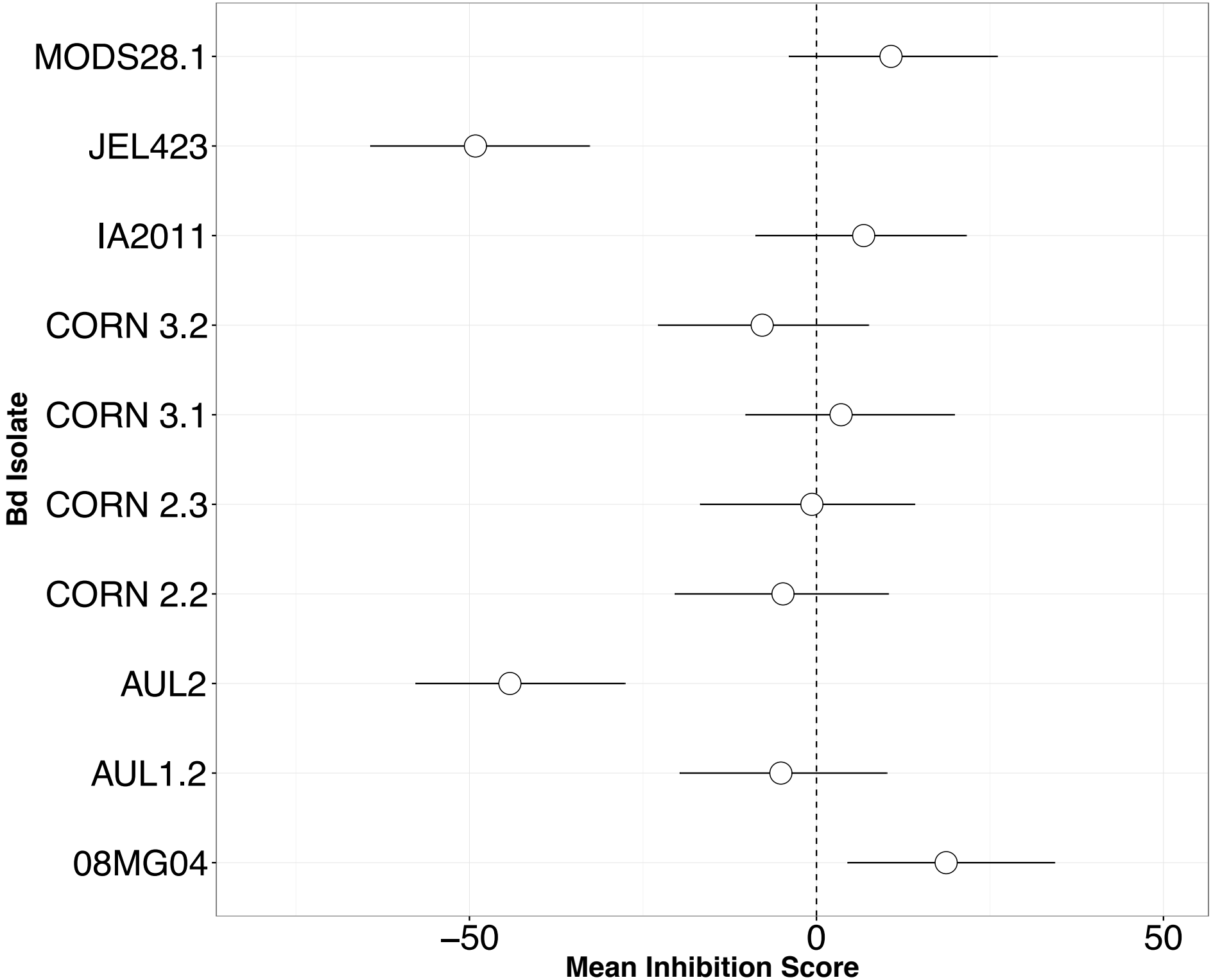

MODS28.1
JEL423
IA2011
CORN 3.2
CORN 3.1
CORN 2.3
CORN 2.2
AUL2
AUL1.2
08MG04
Bd Isolate
−50
0
50
Mean Inhibition Score

Figure 3

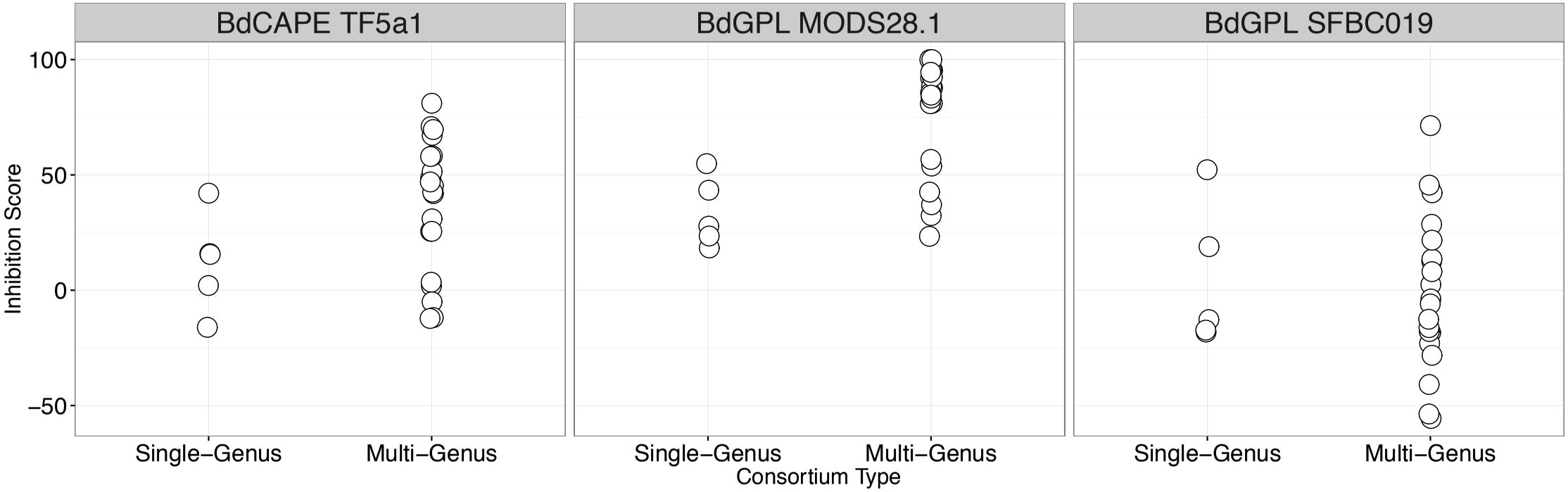
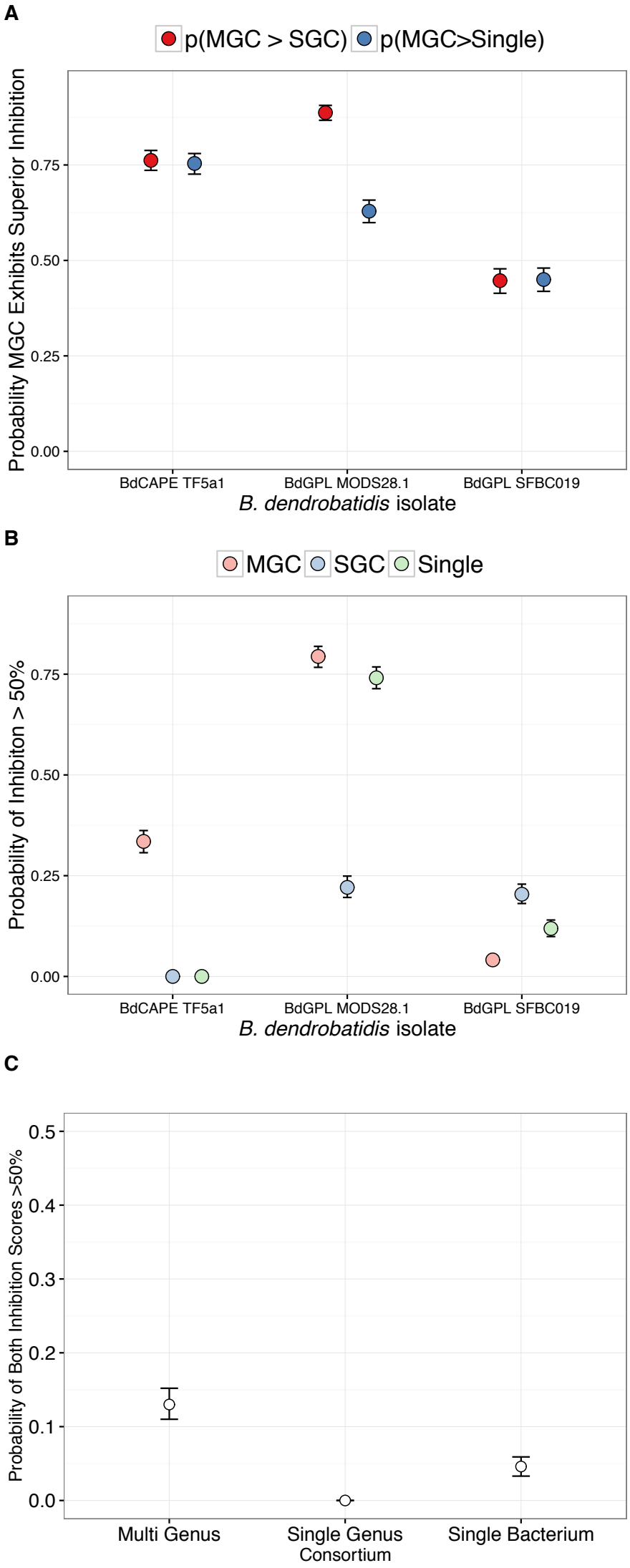

BdCAPE TF5a1
BdGPL MODS28.1
BdGPL SFBC019
Inhibition Score
100
50
0
−50
Single−Genus
Multi−Genus
Consortium Type

Figure 4


A
p(MGC > SGC)
p(MGC>Single)
Probability MGC Exhibits Superior Inhibition
0.00
0.25
0.50
0.75
BdCAPE TF5a1
BdGPL MODS28.1
BdGPL SFBC019
B. dendrobatidis isolate
B
MGC
SGC
Single
Probability of Inhibiton > 50%
0.00
0.25
0.50
0.75
BdCAPE TF5a1
BdGPL MODS28.1
BdGPL SFBC019
B. dendrobatidis isolate
C
Probability of Both Inhibition Scores >50%
0.0
0.1
0.2
0.3
0.4
0.5
Multi Genus
Single Genus Consortium
Single Bacterium