From lab to law: emerging applications, potential benefits, evolving regulatory framework and challenges for engineered probiotics

Supplementary File 1

Numerous cross-bred Vitis varieties of grapes, also known as PIWI (from the German Pilzwiderstandsfähige Rebsorten, or "fungus-resistant grape varieties"), exhibit genetic resistance to fungal diseases such as powdery mildew (*Erysiphe necator*) and downy mildew (*Plasmopara viticola*). These varieties derive from interspecies crosses between *Vitis vinifera* (the typical table and wine grape) and other species of the *Vitis* genus (*Vitis rotundifolia*, *Vitis rupestris*, *Vitis romanetii*, *Vitis riparia*, *Vitis amurensis*, etc.) that possess specific resistance loci. The loci conferring fungal resistance have been identified through genomic studies and marker-assisted selection. "Pyramiding" resistance, or the insertion of multiple resistance loci into a single variety, is a modern strategy for producing long-lasting and qualitatively valid cultivars. Some Italian and international varieties that incorporate these resistance genes include the Solaris (resistant to powdery mildew and downy mildew, blood of *V. amurensis*), Regent (Ren 3, Rpv 3, from *V. rupestris*), Fleurtai, Soreli, Sauvignon Kretos (Italian crosses with multiple resistances), Cabernet Volos, Merlot Khorus, and Julius (red grape, with pyramid resistances). These varieties are increasingly popular in sustainable viticulture, as they allow for a drastic reduction in the use of pesticides. *Run* and *Rpv* are not canonical names of chromosomal loci in the strict sense of classical genetics, but are functional acronyms used in grapevine genetics to identify genomic regions, or QTL (Quantitative Trait Loci) associated with resistance to specific pathogens. Run stands for "Resistance to *Uncinula necator*" (former name of the powdery mildew pathogen, now known as *Erysiphe necator*). *Run* genes (e.g., *Run1, Run2.1, Run2.2*) have also been identified in American species such as *Vitis rotundifolia* (Muscadinia) and are associated with strong resistance to powdery mildew. *Rpv* stands for "Resistance to Plasmopara viticola" (*Plasmopara viticola* is the fungus responsible for downy mildew). Rpv genes (e.g., *Rpv1, Rpv3, Rpv10*, etc.) have been mapped in several *Vitis* species (such as *V. rupestris*, *V. amurensis*, *V. riparia*) and are used to select resistant varieties. These acronyms indicate chromosomal regions where resistance genes have been located through molecular genetic studies and marker-assisted selection. They do not refer to a single gene, but to complexes of genes or quantitative traits that contribute to resistance. For example: *Run1* is

located on chromosome 12 in grapevines and confers almost complete resistance to powdery mildew; *Rpv3* is one of the most studied and is located on chromosome 18, often present in PIWI varieties such as Regent. Examples are given in the table of the Highlight Box 2. These acronyms have become a standard in grapevine breeding because they allow multiple resistances to be pyramided into a single variety. For example, a cultivar with *Run1* + *Rpv3* + *Rpv10* will have combined resistance to powdery mildew and downy mildew, drastically reducing the use of pesticides.

Supplementary File 2

QTL	Pathogen	Chromosome	Origin
Run1	Powdery Mildew	12	*Vitis rotundifolia*
Run2.1	Powdery Mildew	18	*Vitis rotundifolia*
Run2.2	Powdery Mildew	18	*Vitis rotundifolia*
Rpv1	Downy Mildew	12	*Vitis rotundifolia*
Rpv2	Downy Mildew	18	*Vitis rotundifolia*
Rpv3	Downy Mildew	18	*Vitis rupestris*
Rpv10	Downy Mildew	9	*Vitis amurensis*
Rpv12	Downy Mildew	14	*Vitis amurensis*
Rpv14	Downy Mildew	5	*Vitis cinerea*

Note: Some loci, such as Run2.1 and Run2.2, are closely linked and located in the same region of chromosome 18 but are considered distinct due to their different modes of resistance expression.

Supplementary File 3

Delivery strategy	Representative study / system	Host chassis	Payload / effector	Release mechanism	Lytic module	Main application
Secretory	Engineered *L. plantarum* WCFS1 expressing PelAh	*Lactiplantibacillus plantarum* WCFS1	PelAh antibiofilm hydrolase	Extracellular secretion of recombinant enzyme	Not applicable	Disruption of *Pseudomonas aeruginosa* biofilms
Secretory	Engineered MG1363-pMG36e-GLP-1	*Lactococcus lactis* subsp. *cremoris* MG1363	GLP-1	Secretion of recombinant human peptide	Not applicable	Neuroprotection in AD / PD-related models
Secretory	Engineered ATCC 27092-ACE2	*Lacticaseibacillus paracasei* ATCC 27092	CTB-ACE2 fusion protein	Secretion followed by mucosal uptake / transmucosal transport	Not applicable	Diabetic retinopathy-related protection
Lytic	Saeidi et al., 2011 sense-kill-lyse system	Engineered *Escherichia coli*	Pyocin S5	Quorum-sensing-triggered intracellular production followed by programmed cell lysis	E7 lysis protein	Targeted killing of *P. aeruginosa*
Lytic	Engineered EcN targeting *P. aeruginosa*	*Escherichia coli* Nissle 1917	Pyocin S5 + dispersin B	Pathogen-sensing intracellular production followed by lysis-mediated release	E7 lysis protein	Treatment / prevention of *P. aeruginosa* gut infection
Lytic	SLIC tumor-immunotherapy platform	*Escherichia coli* Nissle 1917	Anti-PD-L1 and anti-CTLA-4 nanobodies	Quorum-synchronized intratumoral lysis	ϕX174E	Local tumor immunotherapy

Representative secretory versus lytic payload-delivery strategies in engineered probiotics and related bacterial therapeutic systems. The table compares representative systems according to host chassis, cargo, release mode, lytic module, and main application. Secretory examples include engineered *Lactiplantibacillus plantarum* WCFS1 expressing PelAh [51], *Lactococcus lactis* subsp. *cremoris* MG1363-pMG36e-GLP-1 [83], and *Lacticaseibacillus paracasei* ATCC 27092-ACE2 [86]. Lytic examples include the 2011 “sense–kill–lyse” system described by Saeidi et al. [108], engineered *Escherichia coli* Nissle 1917 targeting *Pseudomonas aeruginosa* [95], and the SLIC tumor-immunotherapy platform [97].

Supplementary File 4

Bt cotton is a genetically modified cotton variety containing genes from *Bacillus thuringiensis*. These genes enable the plant to produce a protein (Cry protein) that is lethal to specific insects, such as *Helicoverpa armigera* (bollworm), *Spodoptera* spp. (nocturnal insects), and tobacco budworm. When insects chew the leaves or bolls of Bt cotton, they ingest the Bt protein, which destroys their gut cells, leading to their death. There are numerous agronomic advantages to using Bt cotton, including reduced use of chemical insecticides, increased yields due to reduced losses from infestations, reduced environmental impact, and integrated plant protection.