Phibro ETHANOL™

EXPERIENCE PERFORMANCE

# Antibiotic Interactions:  Antagonism vs. Synergy

*Addressing Industry Questions*

**OCTOBER 05, 2021**

# OVERVIEW & AGENDA

**Industry Questions and Technical Review**

**Phibro Data and Third-Party References**

**Factors Impacting Lab Studies and Antimicrobial Efficacy**

- Choosing an Organism for Laboratory Studies
- VM Products in Laboratory Studies

# Industry Questions

- **Do virginiamycin and penicillin work together in synergy or are they antagonistic?**
  - Are the industry's historic practices wrong or damaging?
  - Does a plant need to rotate antibiotics or can you use a combinatory product long-term?

- **Technical Journal Article Review**
  - Yeh P, Tschumi AI, Kishony R., Functional classification by properties of their pairwise interactions. (slides 4-7)

# Referenced Technical Journal

Yeh P, Tschumi AI, Kishony R., Functional classification by properties of their pairwise interactions. Nat 38: 489-494

Article in Nature Genetics – May 2006
DOI: 10.1038/ng1755- Source
PubMed

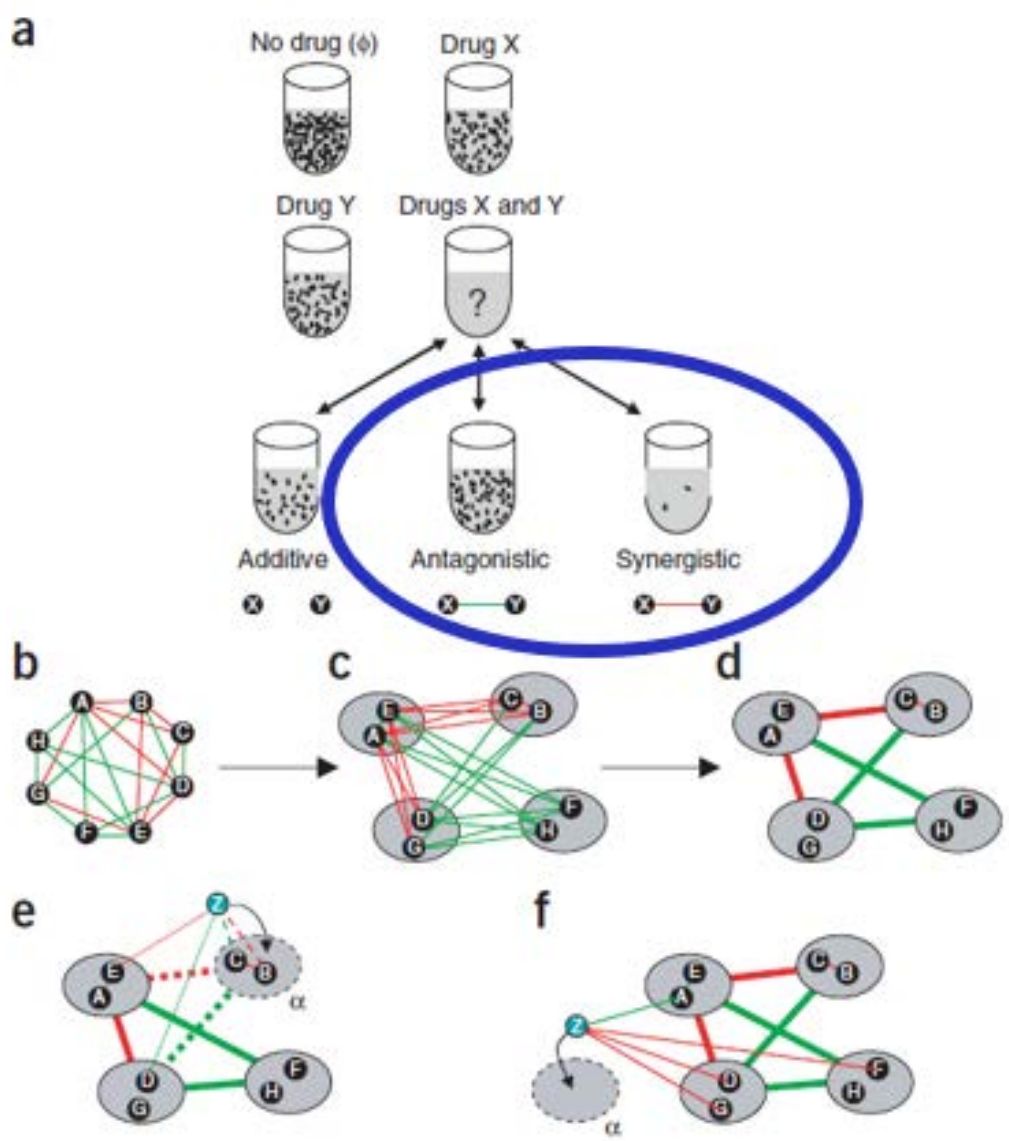



**Figure 1** Clustering of individual drugs into functional classes solely on the basis of properties of their mutual interaction network. (a) Schematic illustration of additive, synergistic and antagonistic interactions between drugs X and Y by measurements of bacterial growth under the following conditions: no drugs, drug X only, drug Y only, and both drugs X and Y. (b–d) A network (b) of synergistic interactions (red lines) and antagonistic interactions (green lines) between drugs (black circles) can be clustered into functional classes that interact with each other monochromatically (that is, with purely synergistic or purely antagonistic interactions between any two

# Abbreviations from Technical Journal

Table 1 List of all drugs used in the study, abbreviation, dose used and mechanism of action

| Drug | Drug abbreviation | Dose ($mg\ ml^{-1}$) | Main mechanism(s) of action | Mechanism abbreviation |
|---|---|---|---|---|
| Chloramphenicol | CHL | 1 | Protein synthesis, 50S | R |
| Clindamycin | CLI | 4 | Protein synthesis, 50S | R |
| Erythromycin | ERY | 4 | Protein synthesis, 50S | R |
| Spiramycin | SPR | 20 | Protein synthesis, 50S | R |
| Fusidic acid | FUS | 40 | Protein synthesis, 50S | R |
| Amikacin | AMK | 20 | Aminoglycoside, protein synthesis, 30S | A |
| Tobramycin | TOB | 0.9 | Aminoglycoside, protein synthesis, 30S | A |
| Streptomycin | STR | 5 | Aminoglycoside, protein synthesis, 30S | A |
| Tetracycline | TET | 2 | Protein synthesis, 30S | R |
| Doxycycline hyclate | DOX | 1 | Protein synthesis, 30S | R |
| Spectinomycin | SPX | 9 | Protein synthesis, 30S | R |
| Piperacillin | PIP | 0.8 | Cell wall | W |
| Ampicillin | AMP | 5 | Cell wall | W |
| Cefoxitin | FOX | 0.8 | Cell wall | W |
| Nalidixic acid | NAL | 2 | DNA gyrase | D |
| Lomefloxacin | LOM | 0.07 | DNA gyrase | D |
| Ciprofloxacin | CPR | 0.006 | DNA gyrase | D |
| Bleomycin | BLM | 5 | Nucleic acid, anticancer drug | B |
| Sulfamonomethoxine | SLF | 0.1 | Folic acid biosynthesis | F |
| Trimethoprim | TMP | 0.5 | Folic acid biosynthesis | F |
| Nitrofurantoin | NIT | 0.3 | Multiple mechanisms | M |

Since virginiamycin and penicillin are not specifically listed, we will look at other drugs with the same mode of action; annotated with a “R” for VM and a “W” for penicillin

# Referenced Technical Journal

Based on this study, the likely type of interaction between Virginiamycin and Penicillin blends would be a synergistic effect.

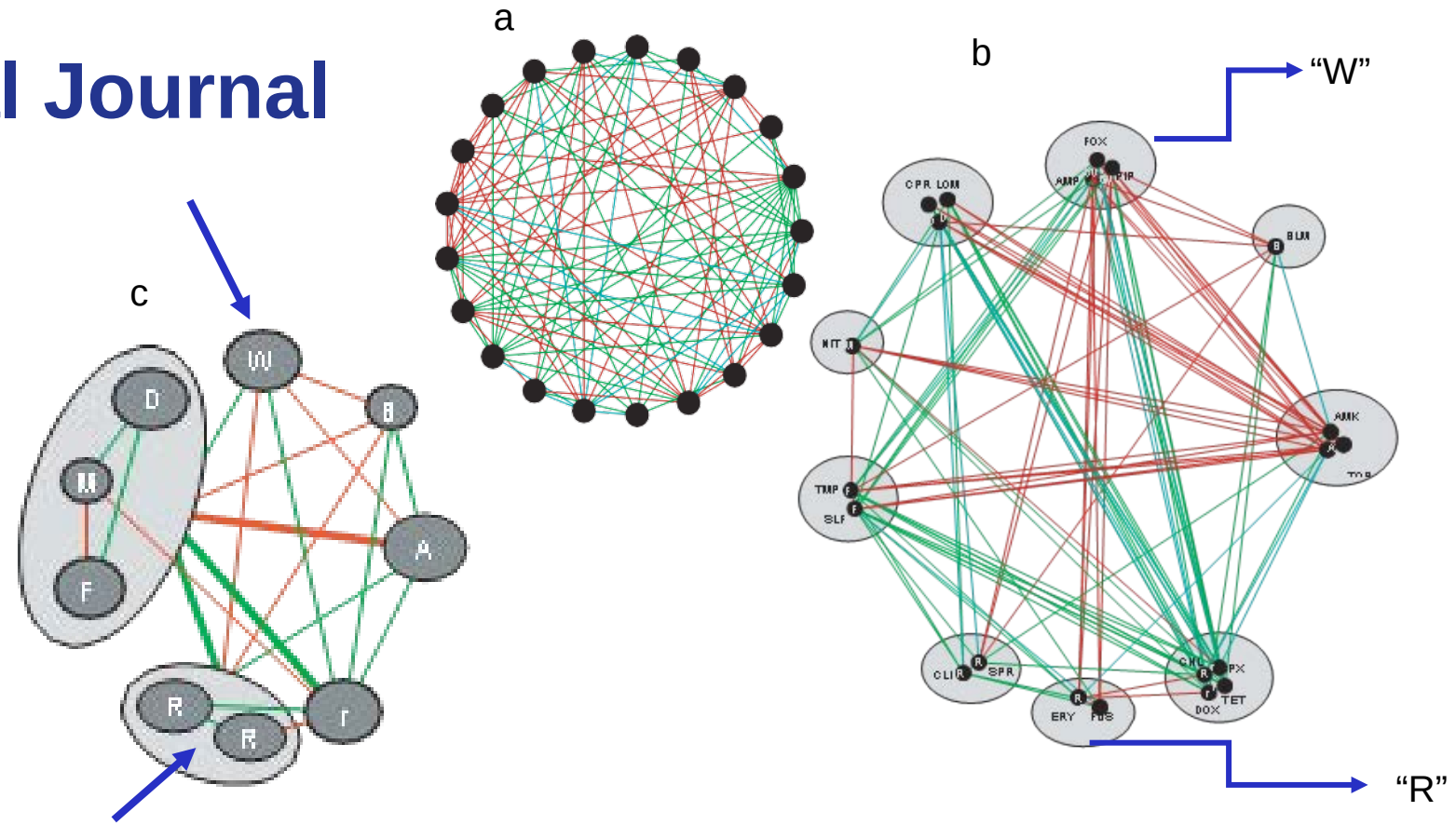
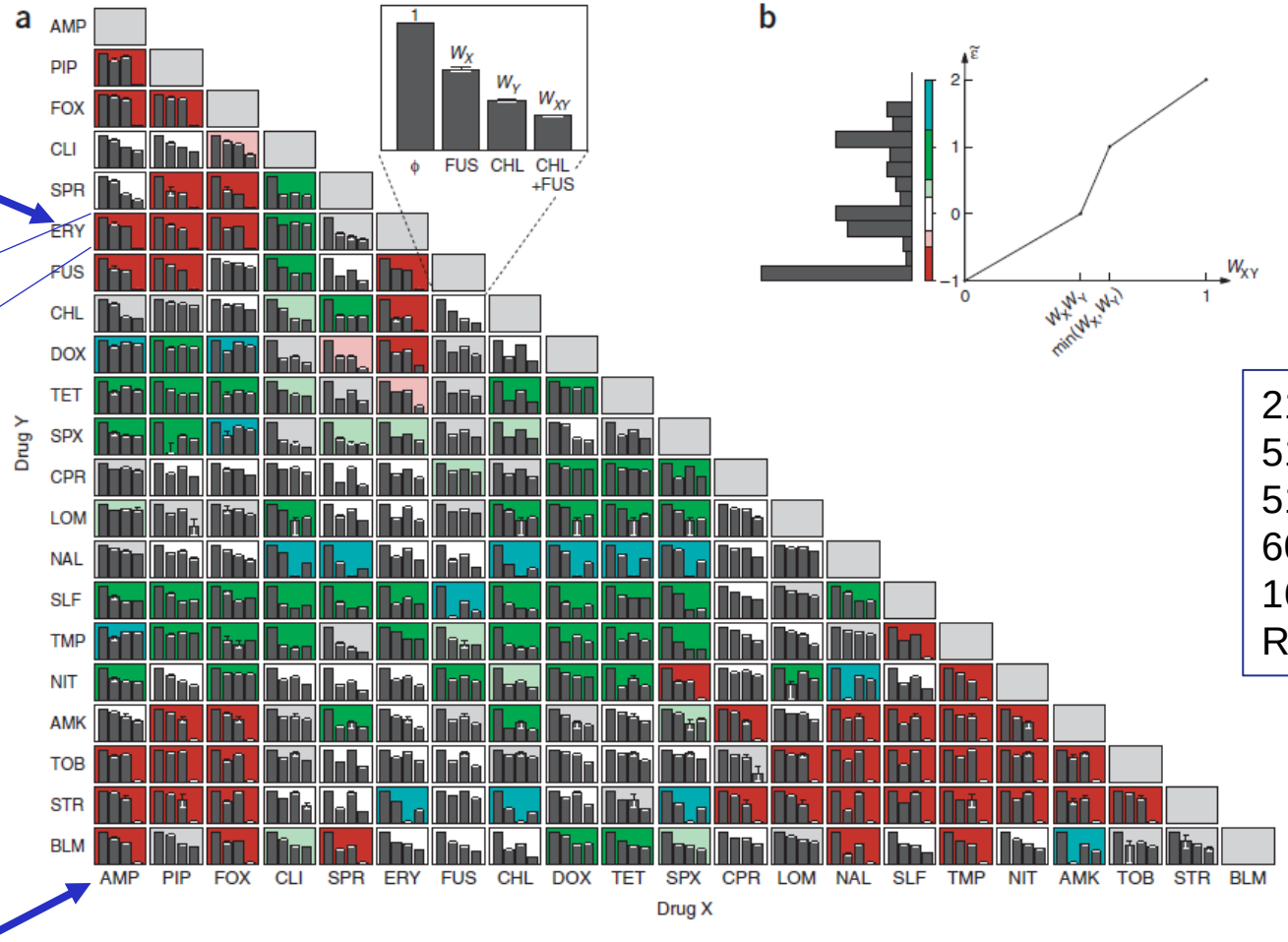



Figure 4 Unsupervised classification of the antibiotic network into monochromatically interacting classes of drugs with similar mechanisms of action. (a) The unclustered network of drug-drug interactions with synergistic (red), antagonistic buffering (green) and antagonistic suppression (blue) links. (b) Prism algorithm classification of drugs into monochromatically interacting functional classes. This unsupervised clustering shows good agreement with known functional mechanism of the drugs (single letter inside each node; see Table 1). Bleomycin (BLM), which is believed to affect DNA synthesis, although its mechanism is not well understood, cannot be clustered monochromatically with any other class. The multifunctional drug nitrofurantoin (NIT) shows non-monochromatic interactions. (c) System-level interactions between the drug classes defined in b. Larger ellipses show higher-level classification of DNA gyrase inhibitors (D) with inhibitors of biosynthesis of DNA precursors (F) and classification of the two subclasses of drugs involved in the inhibition of protein synthesis via the 50S ribosomal subunit (R).

# Referenced Technical Journal


4th bar in chart shows synergy



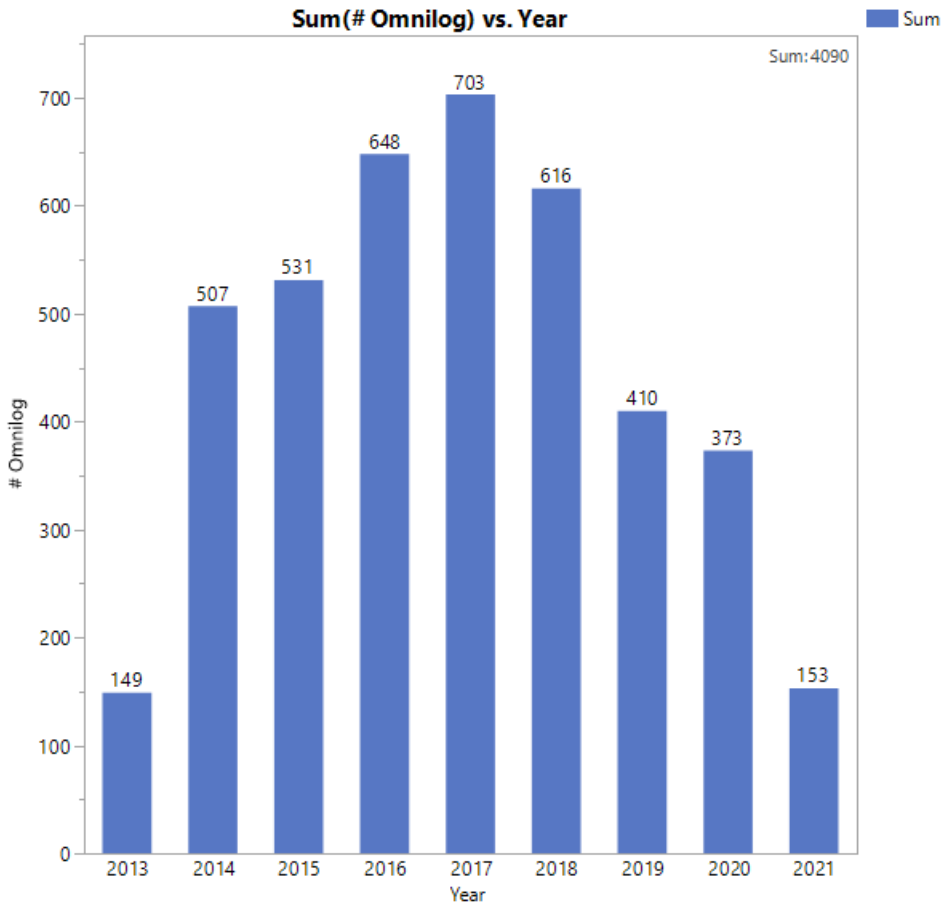

210 Combinations:
51 Red/Lt. Red
51 White
60 Green/Lt. Green
16 Blue
Remainder Gray

**Figure 3** Systematic measurements of pairwise interactions between antibiotics. (**a**) Growth measurements and classification of interaction for all pairwise combinations of drugs X and Y (see **Table 1** for drug abbreviations). Within each panel, the bars represent measured growth rates for, from left to right: no drugs (ϕ), drug X only, drug Y only and the combination of the two drugs X and Y (see inset). Error bars represent variability in replicate measurements (see Methods). The background color of each graph designates the form of epistasis according to the scale in **b**: synergistic (red: $\tilde{\varepsilon}_{max} < -0.5$; pink: $-0.5 < \tilde{\varepsilon}_{max} < -0.25$), antagonistic buffering (green: $0.5 < \tilde{\varepsilon}_{min} < 1.15$; light green: $0.25 < \tilde{\varepsilon}_{min} < 0.5$), antagonistic suppression (blue: $\tilde{\varepsilon}_{min} > 1.15$) or additive (white: $-0.25 < \tilde{\varepsilon}_{max} < 0.5$ and $-0.5 < \tilde{\varepsilon}_{min} < 0.25$). Cases that do not fall into any of these categories are labeled inconclusive (gray background). $\tilde{\varepsilon}_{min}$ and $\tilde{\varepsilon}_{max}$ define our confidence interval for $\tilde{\varepsilon}$ (Methods). (**b**) Graphic representation defining the epistasis interaction scale $\tilde{\varepsilon}$ ($W_{XY}$, $W_X$, $W_Y$) as a function of the normalized growth rate under the double-drug combination ($W_{XY}$) and the two single drugs ($W_X$, $W_Y$)[16]. The histogram of $\tilde{\varepsilon}$ over all drug pairs (at left) shows a trimodal distribution of interactions, with antagonistic, additive and synergistic modes.

# Phibro Data and Third-Party References

*Drug Interactions Studied since 2012; Data Collection Ensuing 2013-mid 2021 (Phibro Laboratory)*

***Never** encountered a case of antagonistic performance between VM and Pen*




**Total number of discreet samples analyzed = 4090**

**(2013 - 2021)**

**Includes a minimum of 5 antimicrobial combinations per sample**

**5 X 4090 = >20,450 evaluations**

# Phibro Data Example of Antibiotic Performance

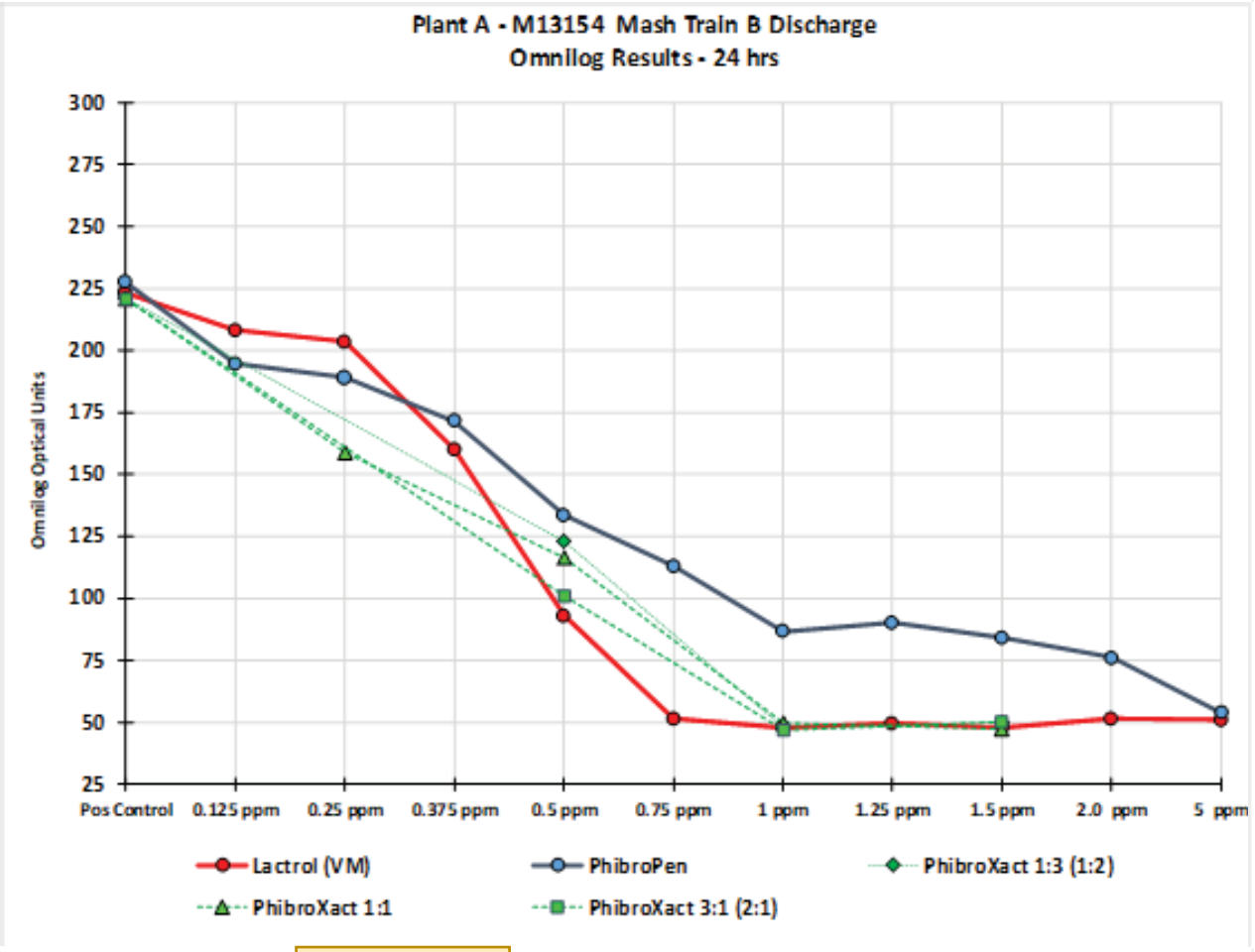



*Figure 1 Additive effect of blends of VM and PEN*

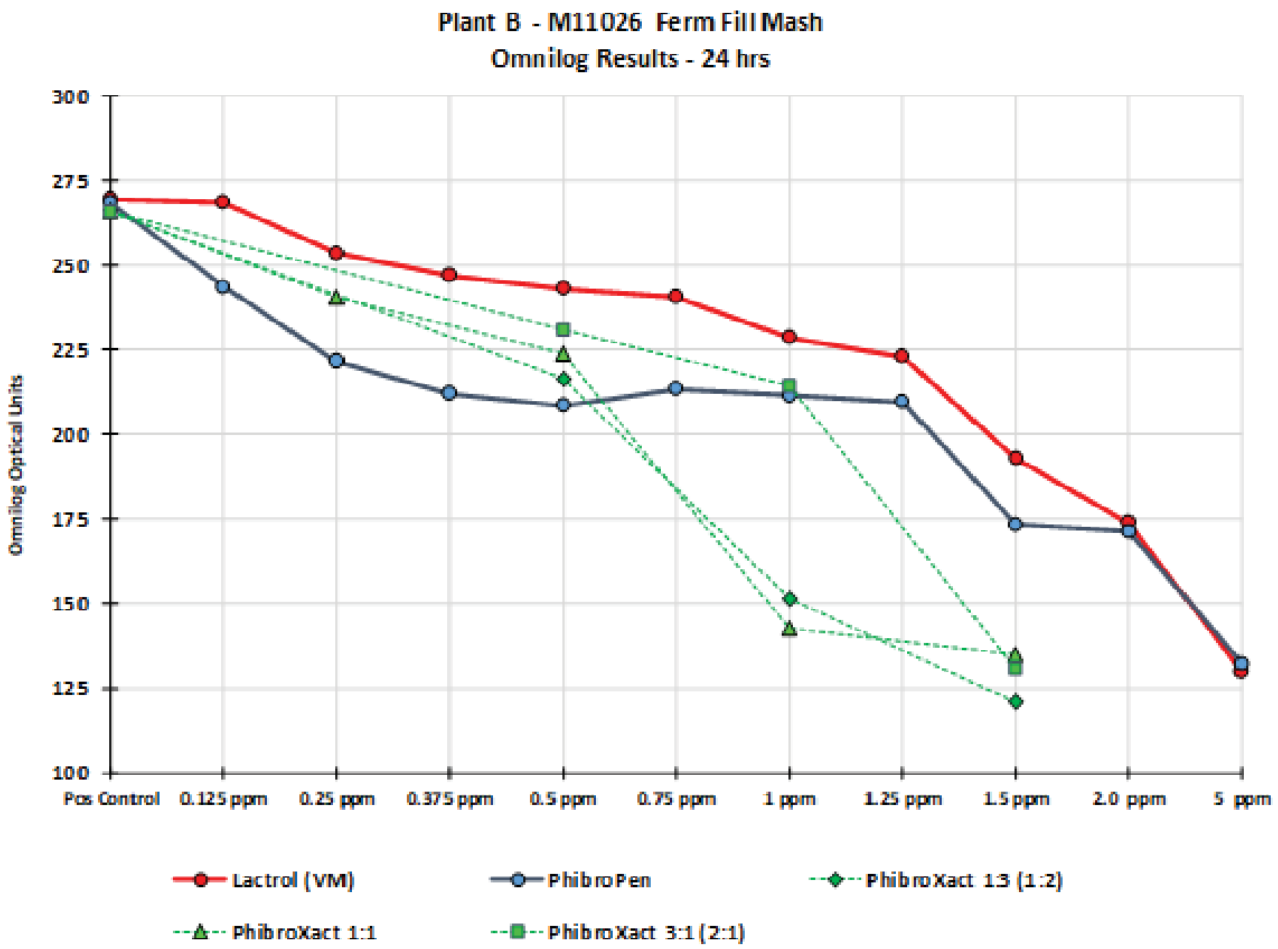
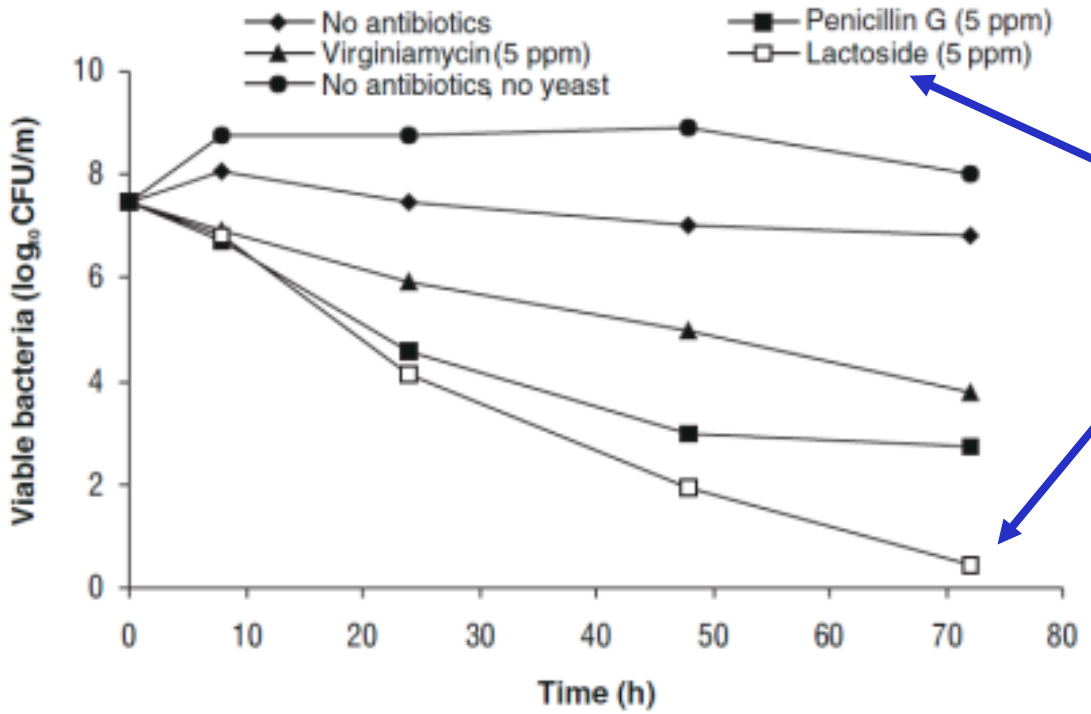



*Figure 2 Possible synergistic combination of VM and PEN*

# Industry Supporting Data – Third-Party Reference

*Study Reported in* The Alcohol Textbook, 4th Edition






Combinatory Product

**Figure 6.** Survival of *L. paracasei* in the fermentation of corn mash (30% DS, pH 5.6) at 31.1°C (88°F) in the presence or absence of various antibiotics. Values are means of duplicate fermentations that had a CV of <8%.

*The Alcohol Textbook, 4th Edition*

# Alternate Third-Party References

**Tyers M, Wright GD. Drug combinations: a strategy to extend the life of antibiotics in the 21st century. Nature reviews. Microbiology. 2019. 17(3):141-155.**

- "Other commercially available antibiotic drug combinations include topical agents such as bacitracin and polymyxin B (Polysporin®; sometimes with the addition of gramicidin) and Neosporin®, which combines neomycin, bacitracin and gramicidin. These combinations are synergistic in some bacteria and offer broad- spectrum coverage of both Gram- positive and Gram- negative pathogens."
- "What remain effective are synergistic congruous combinations. These combinations are proved to increase efficacy and suppress resistance"

**Cokol, M., Chua, H.N., Tasan, M., Mutlu, B., Weinstein, Z.B., Suzuki, Y., Nergiz, M.E., Costanzo, M., Baryshnikova, A., Giaever, G., *et al.* Systematic exploration of synergistic drug pairs. Mol Syst Biol. 2011. 7: 544-544.**

- "Drug synergy allows a therapeutic effect to be achieved with lower doses of component drugs."
- "Much higher than the previously reported background rate of antifungal synergy of 4-10%"

**Farha MA, Brown ED. Chemical probes of Escherichia coli uncovered through chemical-chemical interaction profiling with compounds of known biological activity. Chem Biol. 2010. 17(8):852-62.**

- "Our work revealed a high frequency of synergistic chemical-chemical interactions where the interaction profiles were unique to the various compound pairs."

**Borisy AA, Elliott PJ, Hurst NW, Lee MS, Lehar J, Price ER, Serbedzija G, Zimmermann GR, Foley MA, Stockwell BR, Keith CT. Systematic discovery of multicomponent therapeutics. Proc Natl Acad Sci . 2003. 100: 7977–7982.**

- "We observed unexpected synergistic interactions that may be attributable to the interconnected signaling networks existing within and between cells."

# Factors Impacting Lab Studies and Drug Efficacy

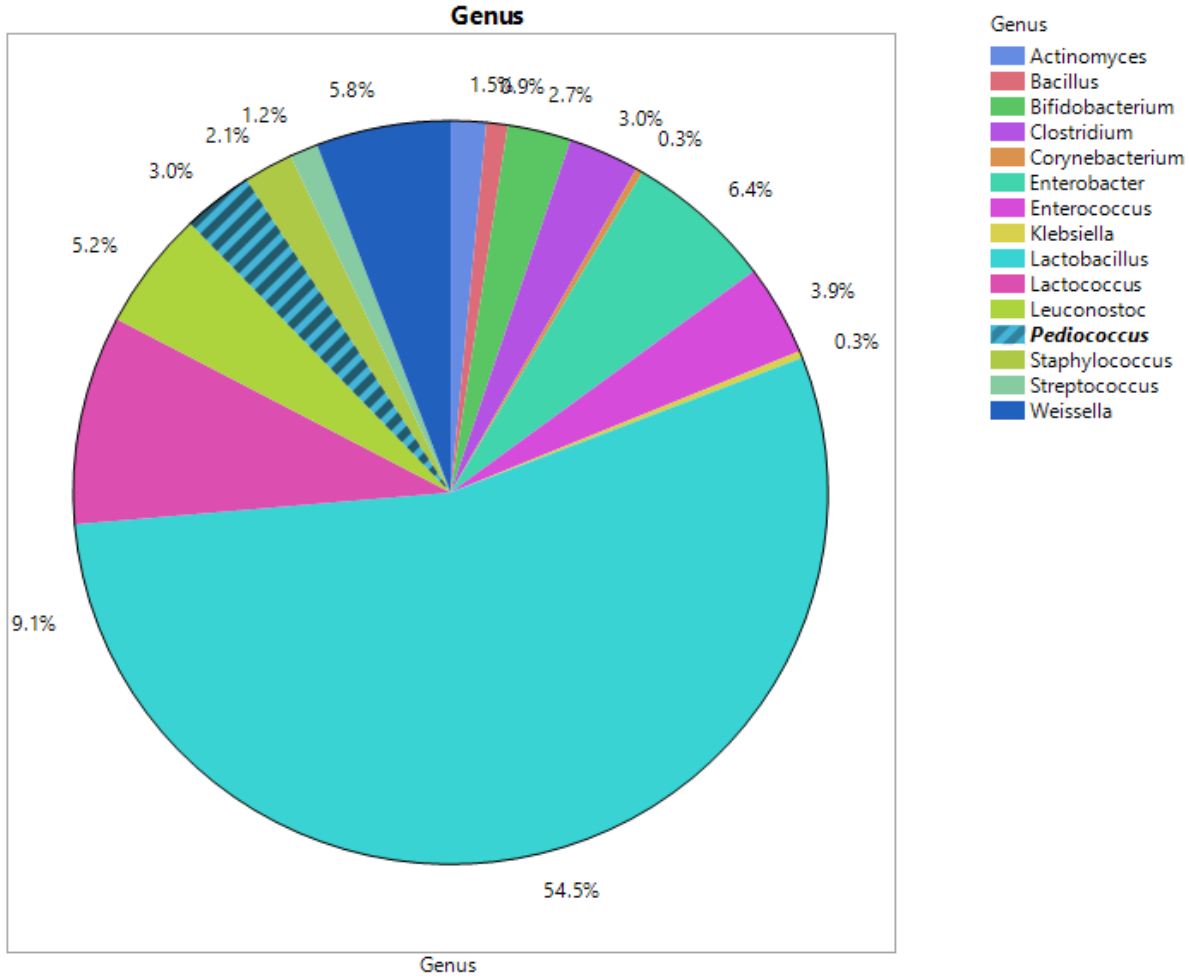
*Choosing an Organism for Laboratory Testing*

**Consortium vs. Isolates**

**Which isolates to utilize?**

# Choosing an Organism for Laboratory Testing

*Phibro Data Compilation*

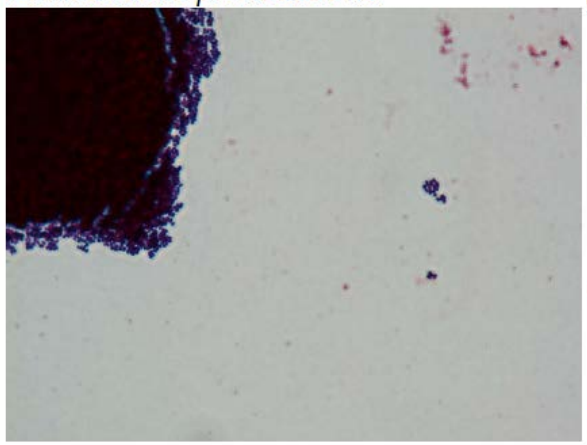



- **Predominant Genera:**
  - Lactobacillus = 54.5%
  - Lactococcus = 9.1%
  - Enterobacter = 6.4%
  - Weissella = 5.8%
  - Staphylococcus = 5.2%

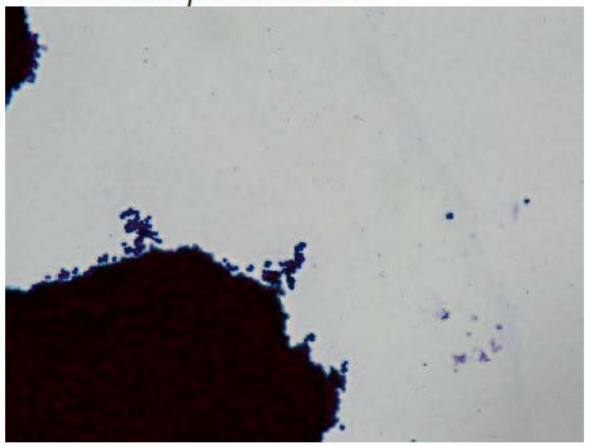
# Choosing an Organism for Laboratory Testing

*Images from Gram stains in Phibro laboratory*

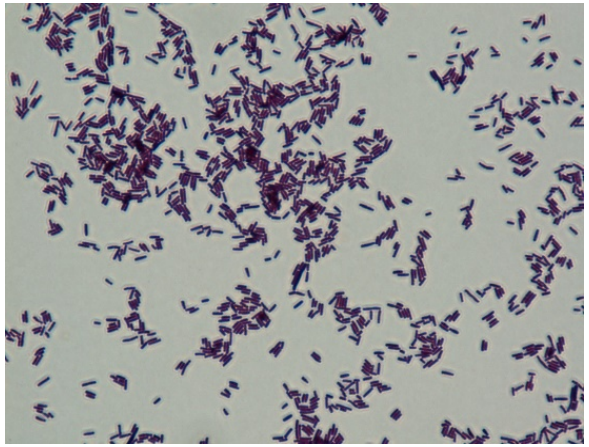
*Pediococcus pentosaceus*

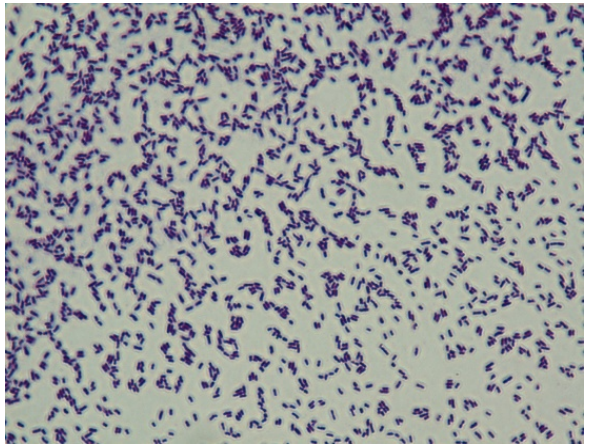


*Pediococcus pentosaceus*



- Due to biofilm formation individual cells are not visible
- *P. pentosaceus* is not an ideal organism to work with for scientific studies unless there is a very specific goal related to biofilm formation and treatment





- Typical rod and cocci bacterial images (non-biofilm producers)
- Phibro isolates hundreds-thousands of bacterial strains per year to keep a library of representative bacterial strains

# Factors Impacting Lab Studies and Drug Efficacy

*Lactrol® vs. Alternative VM Products*

## Lactrol

### *Designed for Ethanol Production*

- **100% activity**
  - ~50% by weight
- **Formula purpose**
  - Corrects for raw VM activity variation
  - Creates hydrophilicity
  - Improves flow characteristics

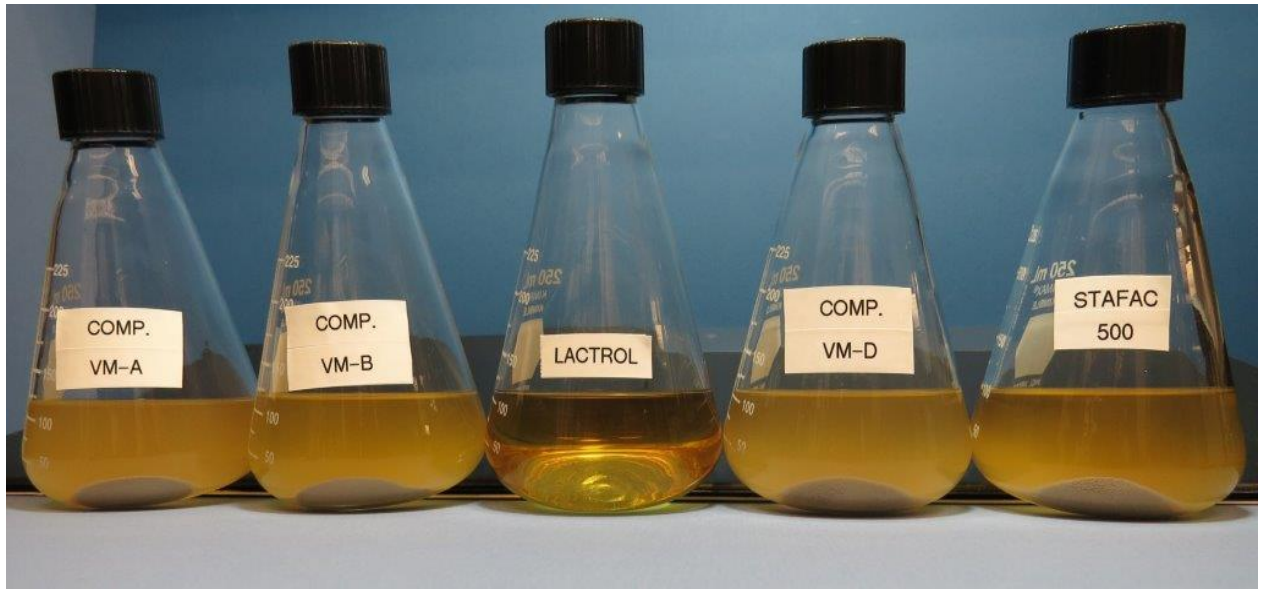
## Stafac 500

### *Designed for Animal Feeds*

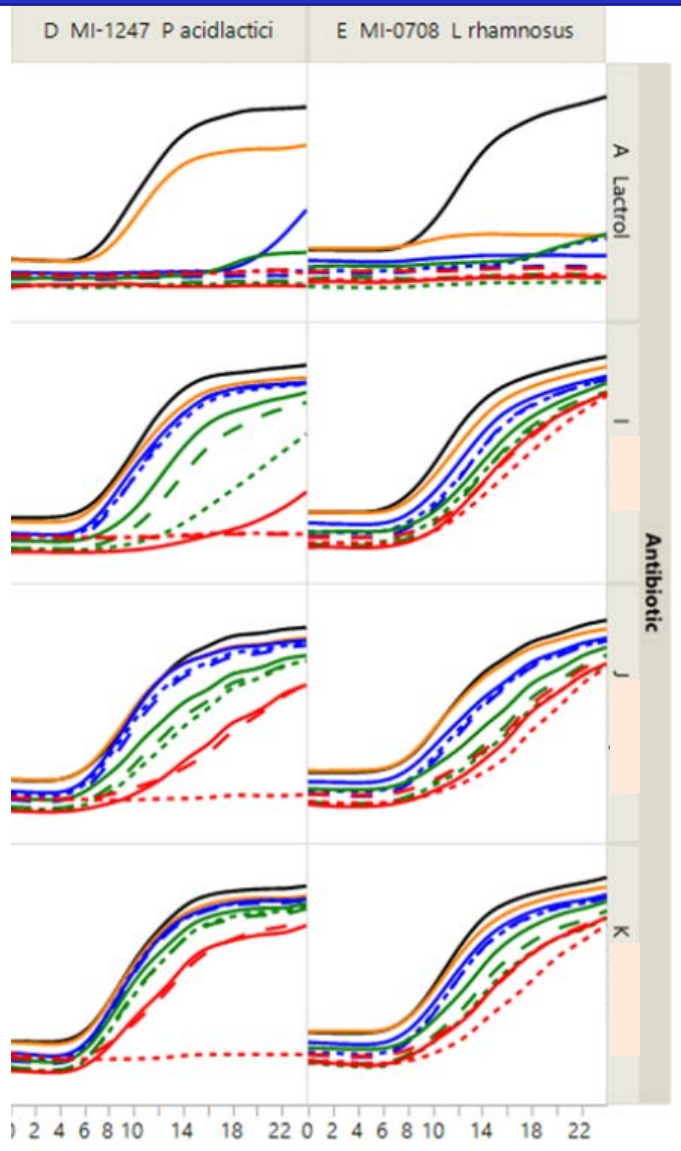
- **50% activity**
  - ~25% by weight
- **Formula purpose**
  - Corrects for raw VM activity variation
  - Creates hydrophobicity
  - Improves flow characteristics
- **All competitive products in ethanol market are derived from Stafac line**

# Lactrol vs. Alternative VM Products

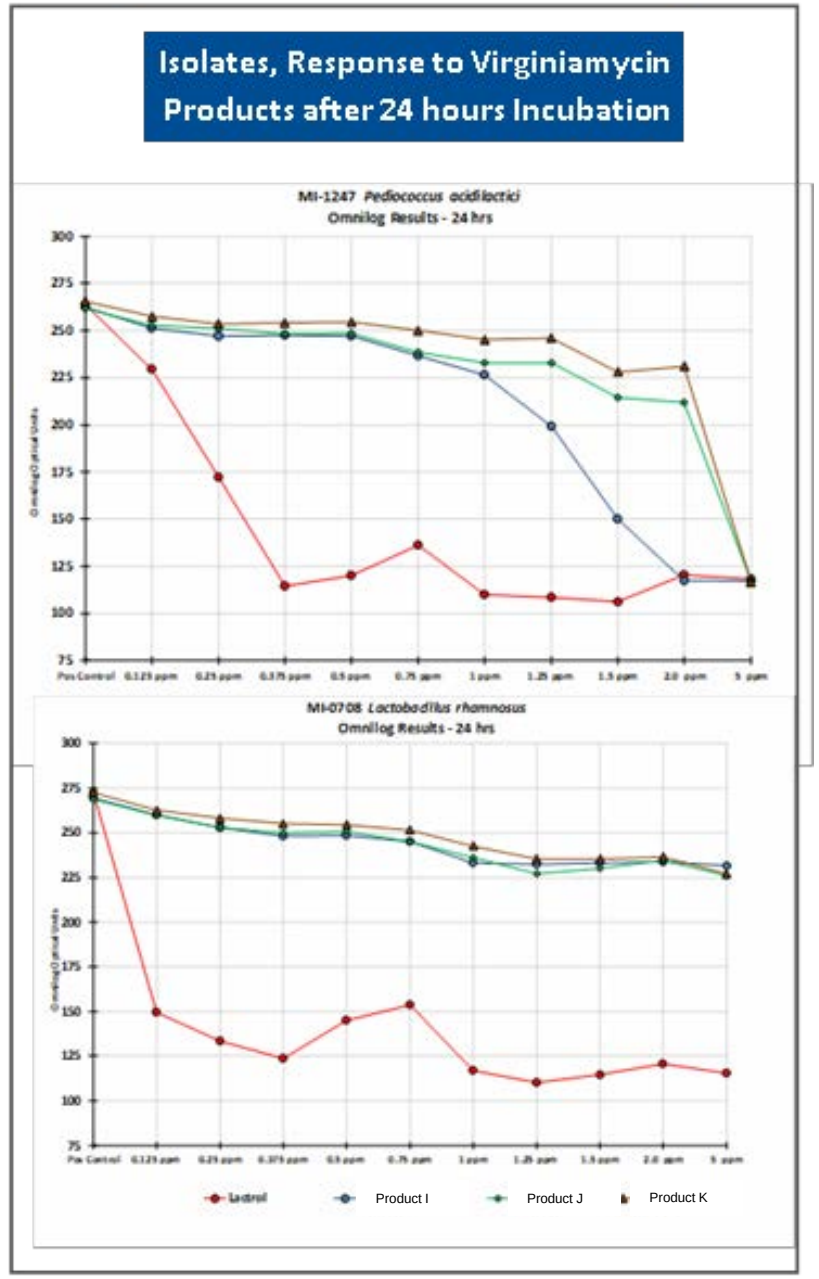
*Presence of Additives in Alternative Products Can Create Solubility Issues*



# Isolates, Response to Virginiamycin Products over 24 hours




Phibro Ethanol's laboratory selected bacterial isolates at random for subjective testing to various antimicrobials at varying concentrations. As demonstrated, all of the treatment doses of Lactrol were effective in treating the bacterial strains. Competitive VM products did not perform as expected indicating likely solubility issues due to the additives included in Stafac derived VM products.



# SUMMARY

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|---|---|

# QUESTIONS?