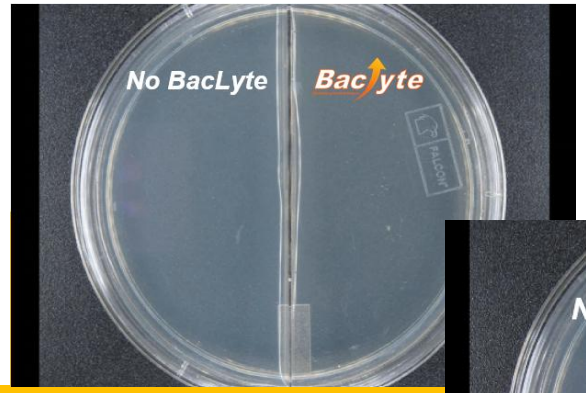
Non-Confidential Proof of Concept Data

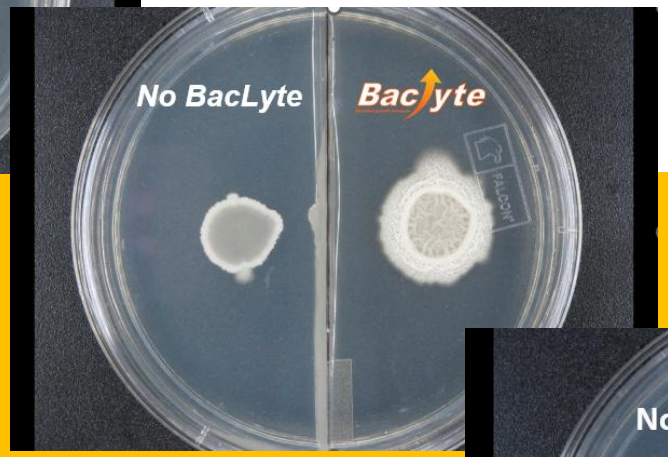
Lyte Gro

STEROIDS FOR MICROBES

12-hour culture of bacillus spores on agar plate



0 hrs



6 hrs

12 hrs

Grow quicker

Grow more

Grow better

More microbes

More productivity

Increased yield

Shorter process times

Effects of BacLyte

There is nothing currently in the market which does what BacLyte does

BacLyte's main effects are:

1) Reduces lag phase – enables fermentations to start earlier so enabling a longer production cycle

2) Accelerates the rate of microbial replication - meaning that you get more microbes quicker

3) Dampens Quorum Sensing - enables more densely packed cultures

4) Allows growth of microbes in much simpler culture media – reduces media and downstream processing costs

Combine these effects and BacLyte enables industry to grow things faster and cheaper and improves product yields across a broad range of species and applications.

Through our own research and in collaboration with leading companies who evaluated BacLyte we have been able to demonstrate BacLyte's profound effect upon the growth and metabolism of a range of bacterial species and saccharomyces yeast. This document highlights a small fraction of the data that we have generated to date.

Some of the figures use the "Mediaboost" rather than BacLyte – this is the product name under which Merck KGaA sells our product in the R&D sector

Compatibility of BacLyte with Relevant Bacterial Species and Culture Media

Microbe/ Media Type	LB	1/3 LB	BM2	M9	IMDM	DMEM	YPD	1/3 YPD	MRS	1/3 MRS
Bacillus subtilis	✓				✓	✓				
Lactobacillus rhamnosus	✓	✓	x	x		✓			✓	✓
Lactobacillus reuteri					✓				✓	✓
Lactobacillus salivarius									✓	✓
Bacillus coagulans					✓					
Lactococcus lactis					✓					
Escherichia coli	x				✓					
Saccharomyces cerevisiae	x		✓	✓	✓		✓	✓		
Pseudomonas aeruginosa	x			✓						

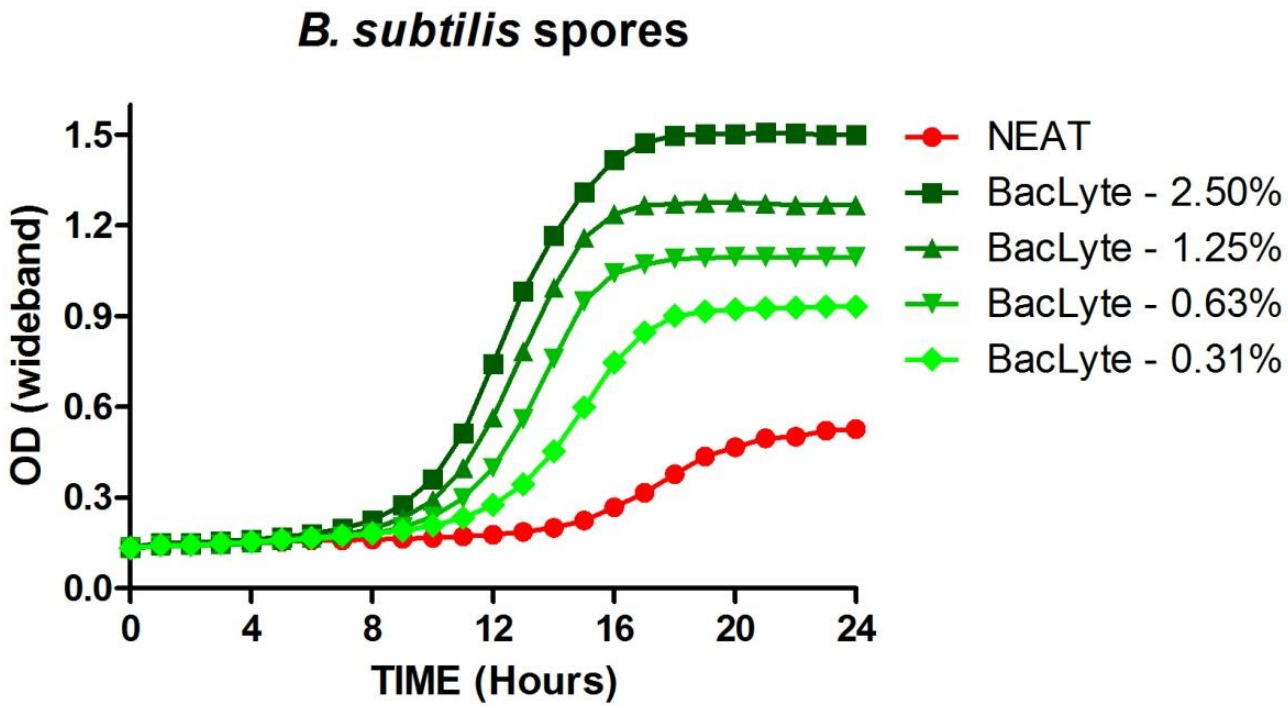
(✓) Indicates that a relative increase in growth was observed when compared to media without BacLyte,

(X) Indicates that no growth improvement was observed with BacLyte.

Colored cells indicate media and species not tested together with BacLyte.

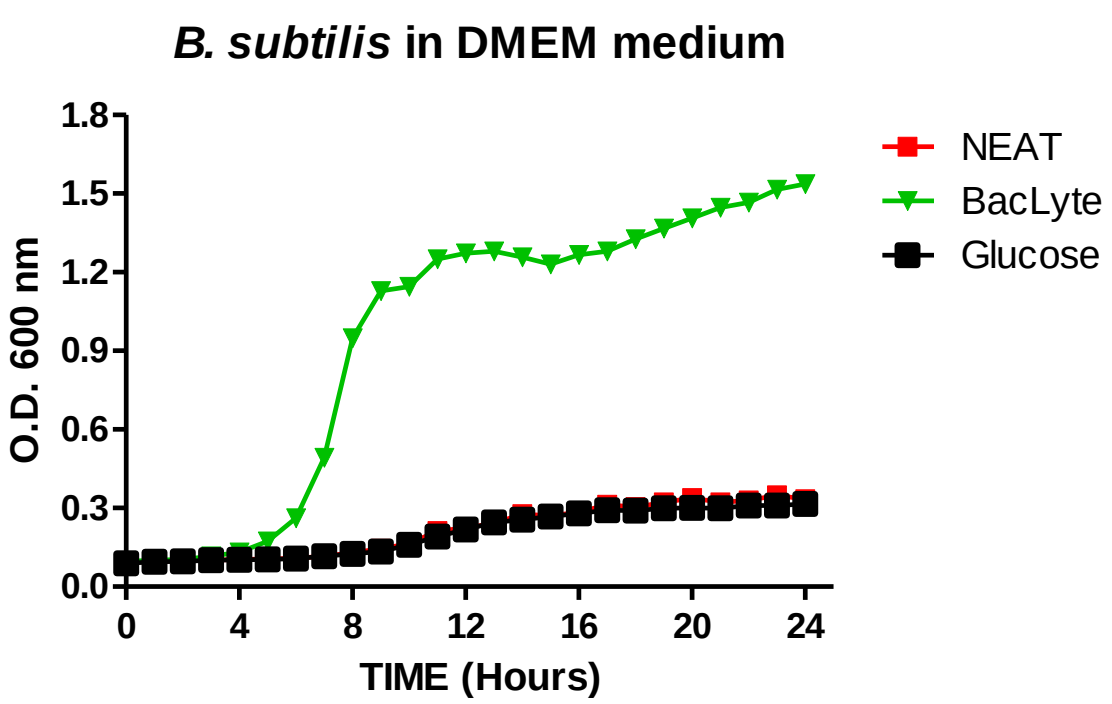
Bacillus

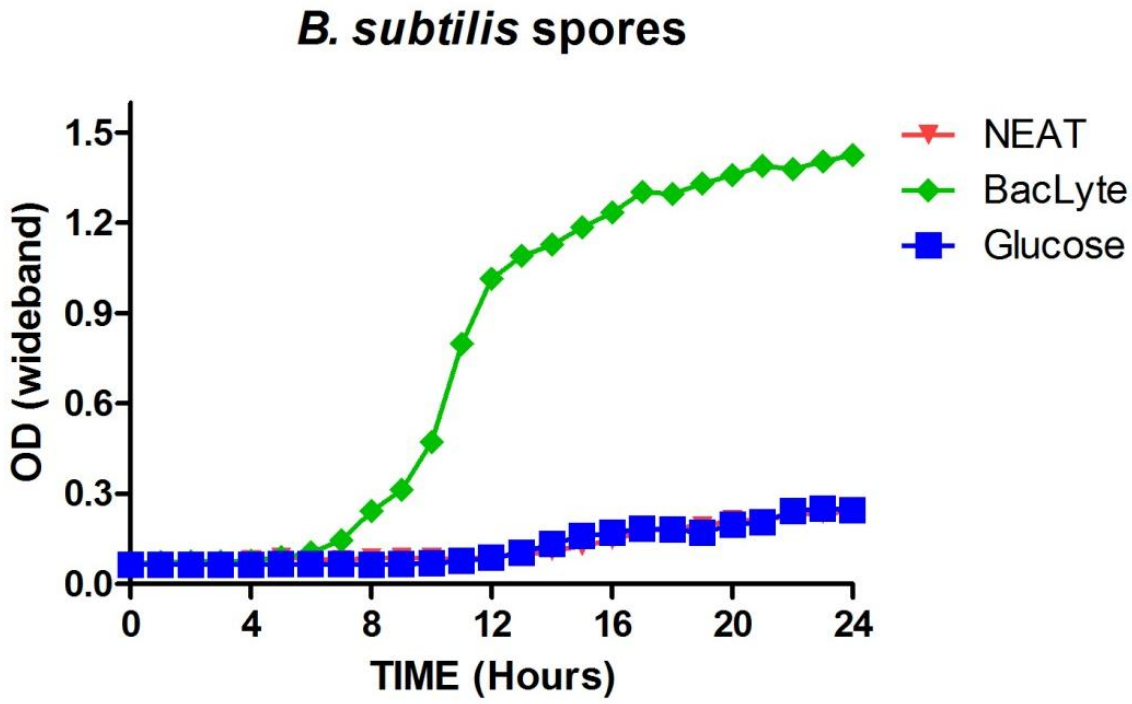
Germination of Bacillus spores in LB media clearly demonstrates a dose-dependent effect – which is also seen with other microorganisms we have tested



Approximately 10 *B. subtilis* spores were incubated in LB with or without increasing concentrations of added BacLyte. Data points represent means of triplicate cultures and S.D. did not exceed 10% of mean. There is a significant titration effect observed with added concentration of BacLyte corresponding to reductions in lag phase and final stationary phase OD.

Growth in minimal media



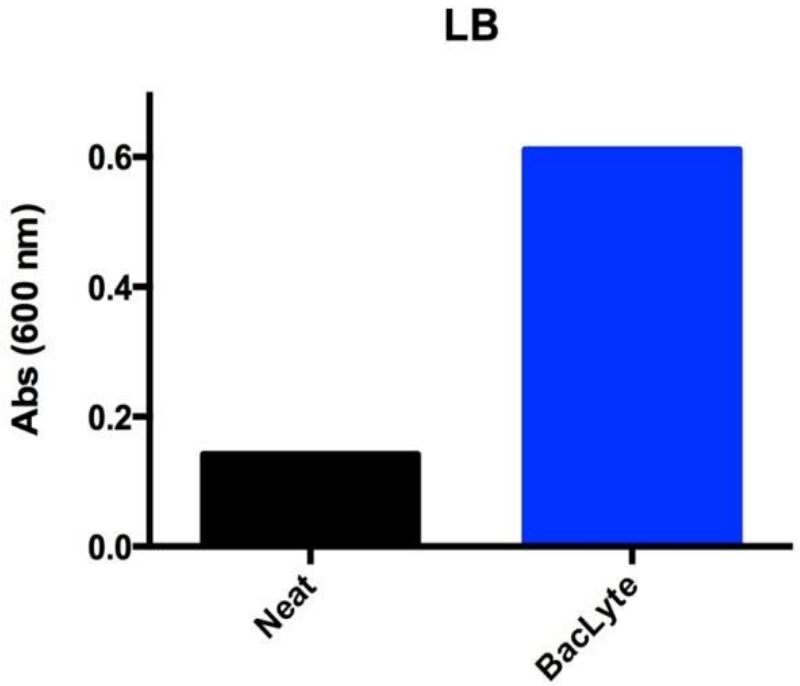


Same conditions as in slide #2 with the exception that DMEM or IMDM was used instead of LB. The concentration of BacLyte was 2% v/v. An equivalent amount of a 50% glucose solution to that used for BacLyte was also employed to demonstrate that BacLyte growth promoting effect was not due to additional carbon source. Data points represent means of triplicate cultures and S.D. did not exceed 10% of mean.

Enabling of growth in simpler media could lead to simpler downstream processing steps in isolating proteins since medium only contains known simple salts and BacLyte which is protein-free.

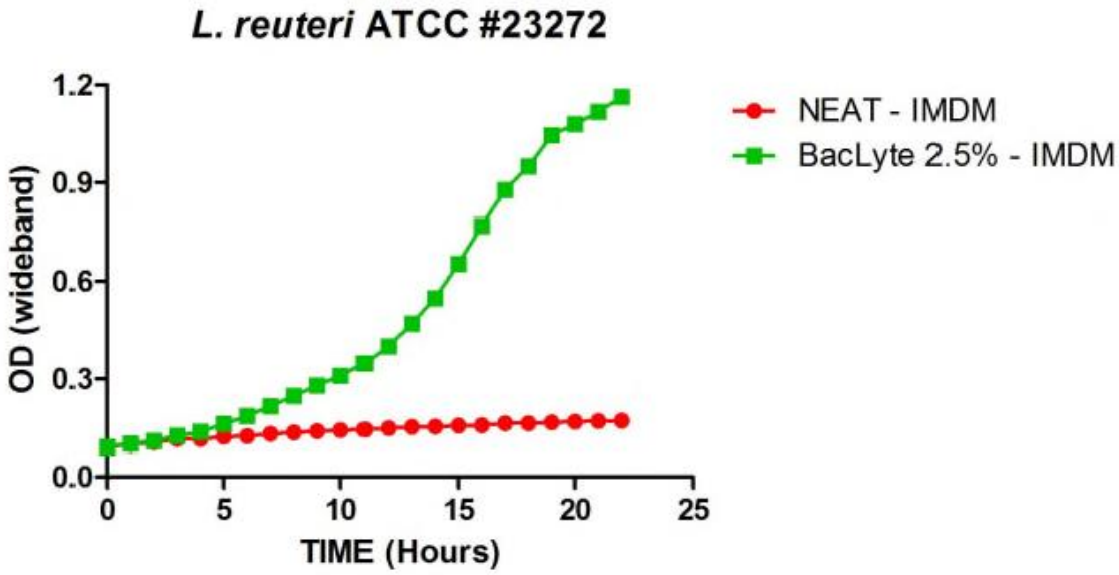
Lactic acid bacteria

This graph shows how 0.5% w/v Baclyte induces the ability of Lactobacillus rhamnosus to grow in rich Luria Bertoni media.

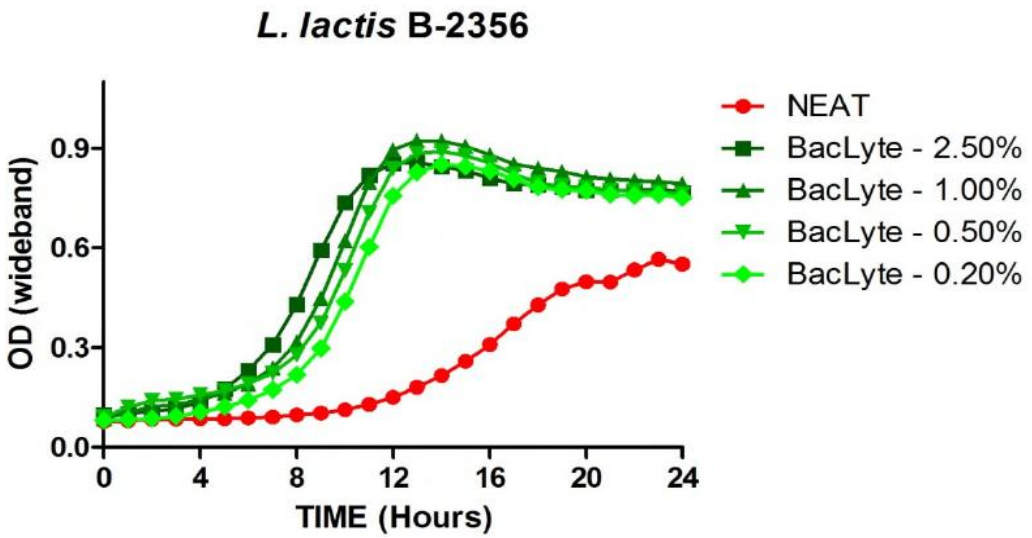


This graph shows how 0.5% w/v Baclyte induces the ability of Lactobacillus rhamnosus to grow in normal unsupportive minimal DMEM media.

Similarly, supplementation of the media with Baclyte (2.5% w/v) enables the otherwise fastidious Lactobacillus reuteri to grow in normally unsupportive minimal IMDM media.

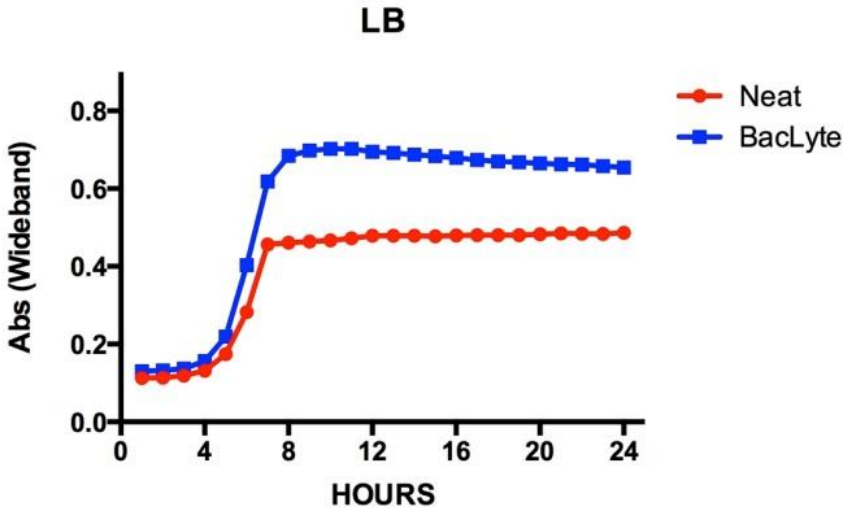


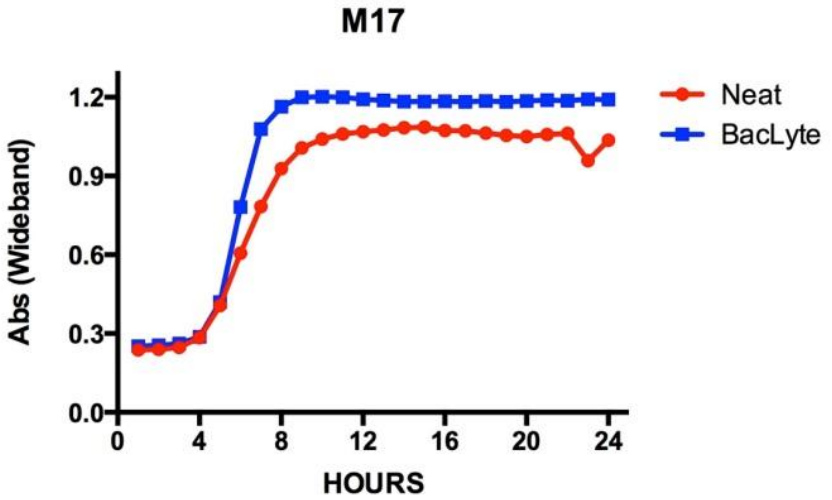
This is a mammalian cell culture medium that is not even designed for bacteria.
<u>The culture was also grown in aerobic conditions</u>



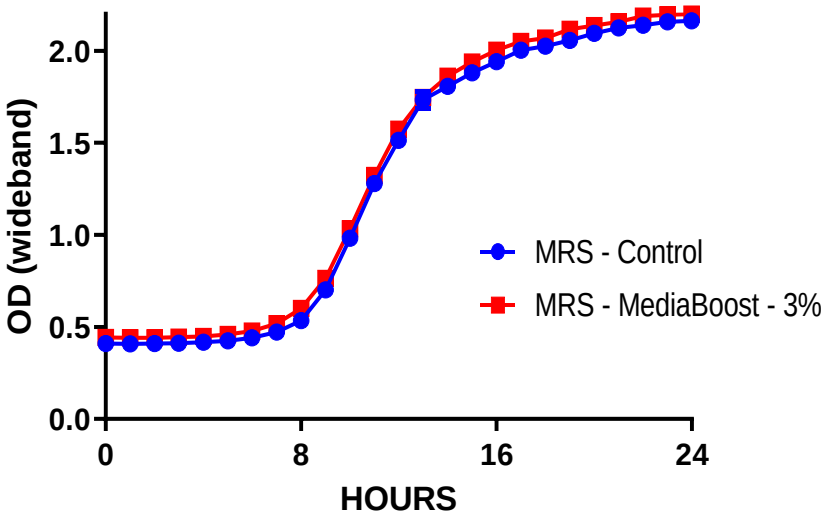
This graph demonstrates the <u>improvements in growth rate and final OD</u> of a Lactobacillus lactis culture achievable with the addition of Baclyte at a range of concentrations in rich MRS (de Man Rogosa Sharpe) media

Similarly addition of 0.5% Baclyte into rich M17 and Luria Bertoni medias increased growth rate and final OD of *L. lactis*.

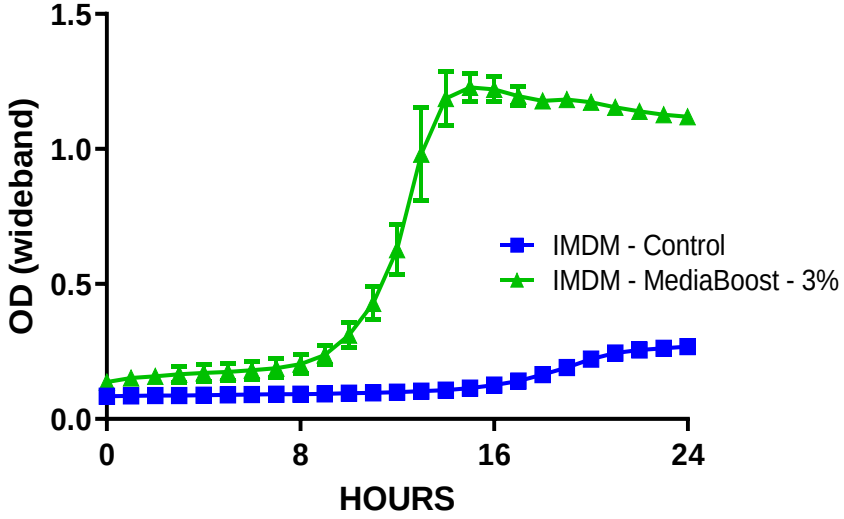




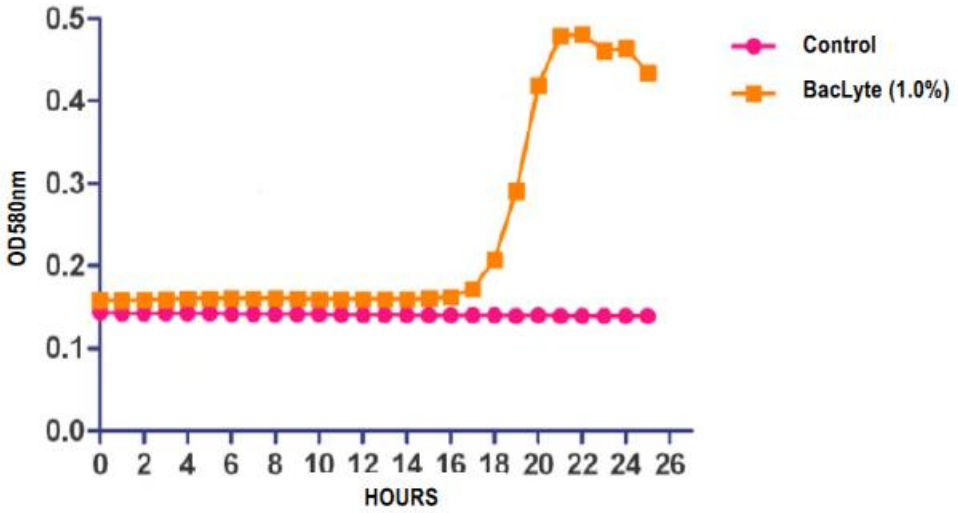
Growth of *Bifidobacterium animalis* in MRS



Growth of *Bifidobacterium animalis* in IMDM



These slides shows how 3% w/v Baclyte enables Bifidobacterium animalis can be grown in both rich MRS and normally unsuitable basic IMDM media

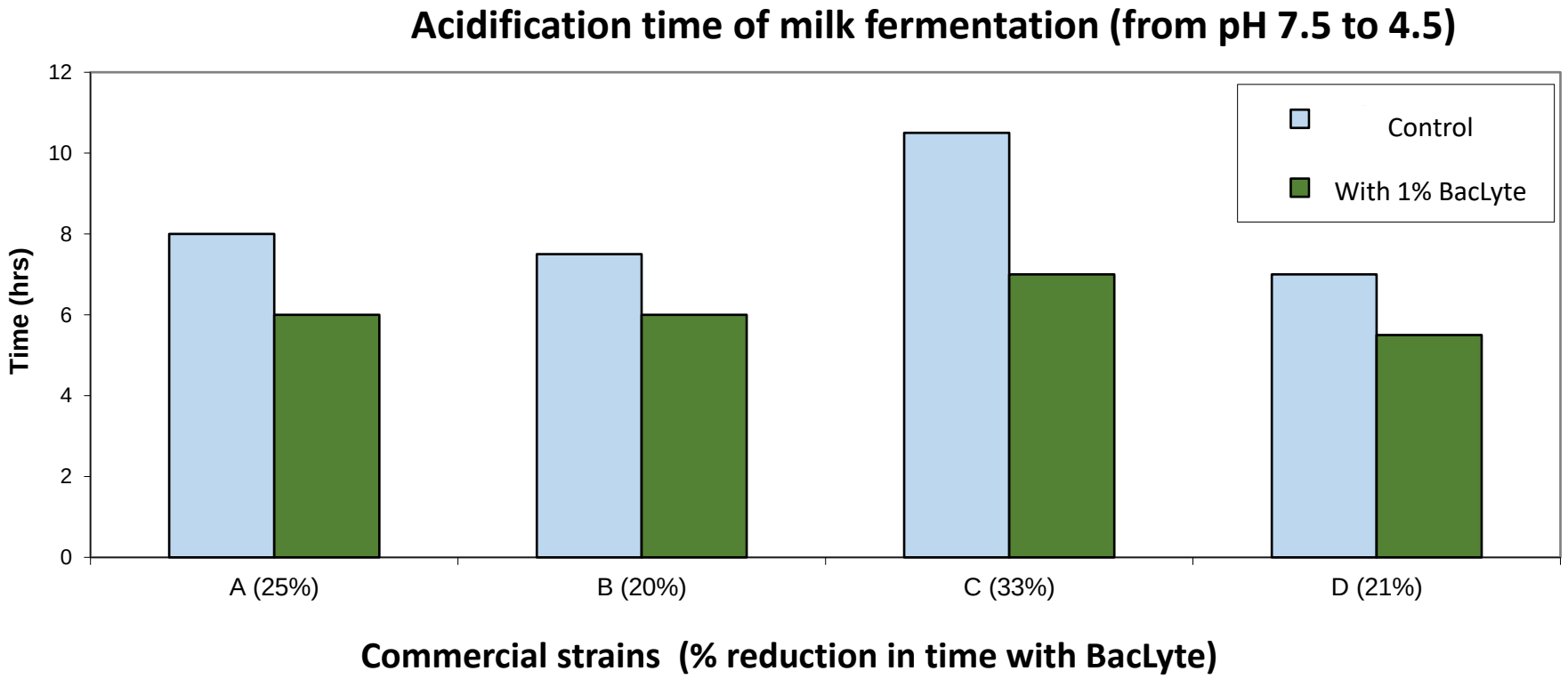


This graph shows how supplementation with 1% w/v Baclyte in rich MRS media is able to “resurrect” 3-year old unviable bifidobacteria from a lyophilised probiotic tablet

Yoghurt production

A number of evaluations were carried out by leading European probiotic manufacturers seeking to evaluate an early version of BacLyte's use in bulk milk fermentations. We have since tripled the activity of BacLyte.

An example of results when testing LAB E with *Streptomyces thermophilus* strains (4 strains tested)

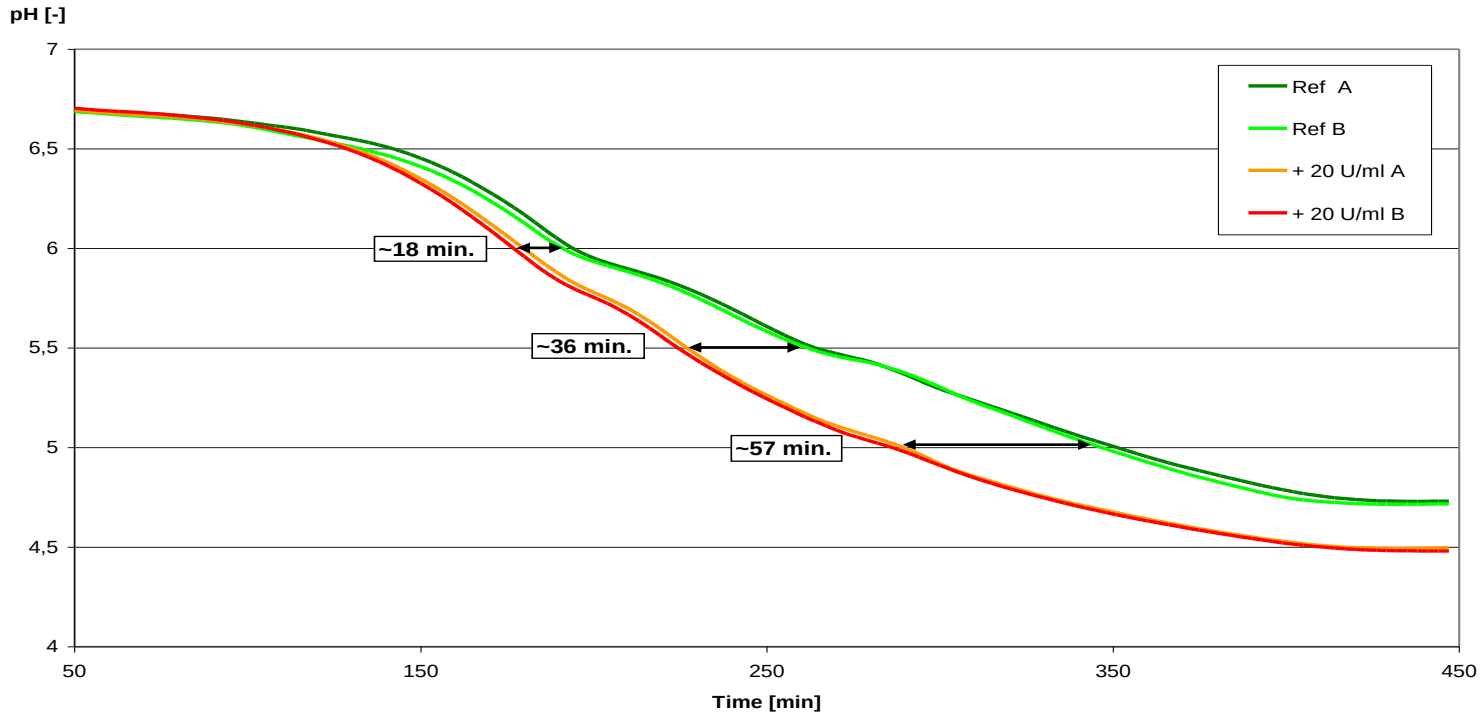


Acidification time was reduced by an average of 24.6% across all four strains tested

Similar results were also obtained Lb. acidophilous

In a second third-party evaluation of (the earlier version of) BacLyte's ability to accelerate milk acidification the results were conclusive. From the Final Evaluation Report:

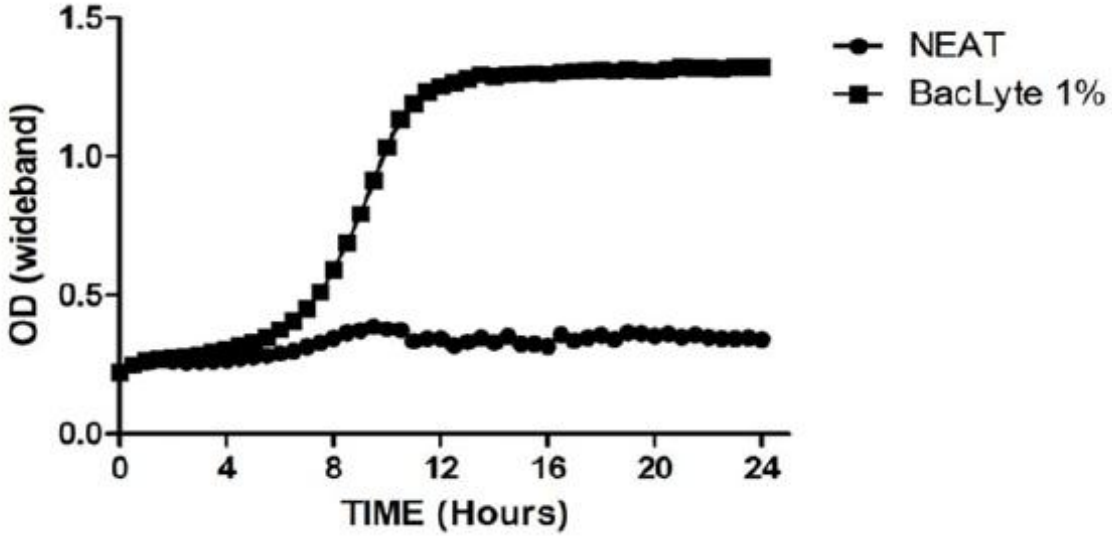
"Overall nine different strains were tested with LAB Enhancer generating an enhancement of the activity in 7 of those 9 strains." "The extract showed promising results for Streptococcus (see graph above), Lactobacillus & complex cultures."



"Only two Lactococci strains showed no response – the other seven experiments provide benefits between 10 and 120 minutes depending on the strain and the chosen pH-feature point. There was a clear correlation between the amount of extract used and the measured benefit."

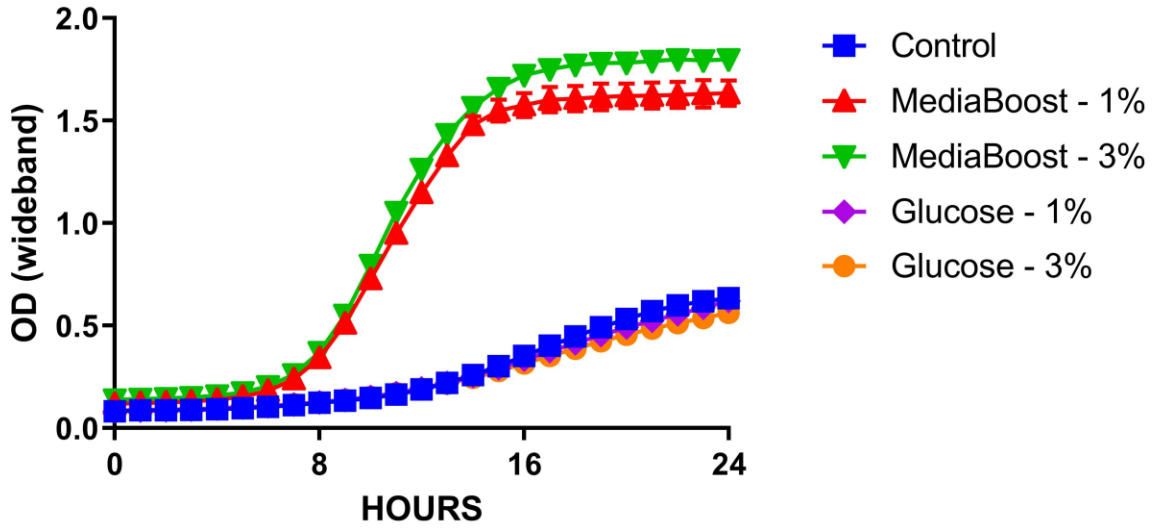
Yeast

Growth of *Saccharomyces cervisiae* in SAPI medium

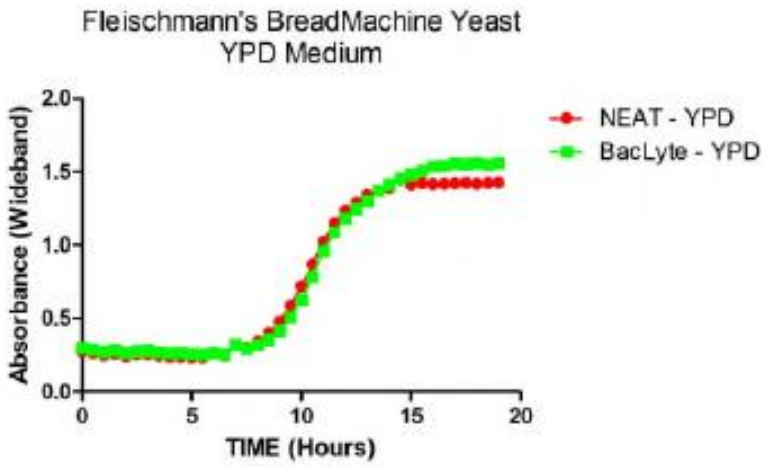


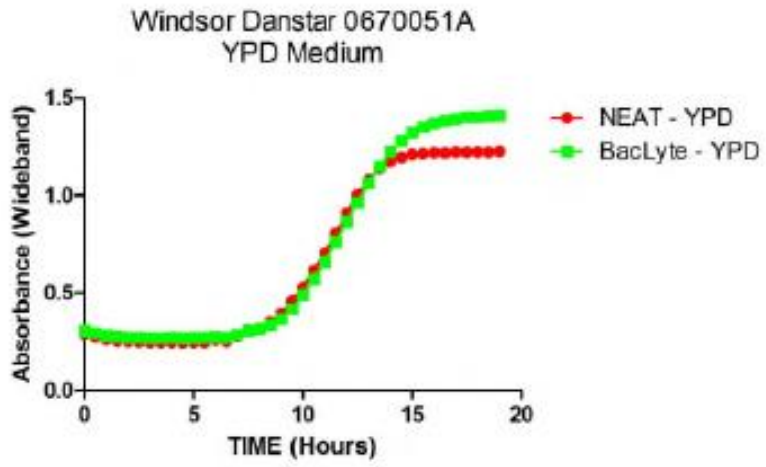
This graph demonstrates how 1.0% BacLyte supplementation supports growth of S.cerevisae yeast in a minimal SAPI media which is normally unsupportive of vegetative growth.

Growth of *Saccharoyces cervisiae* in BM2 medium



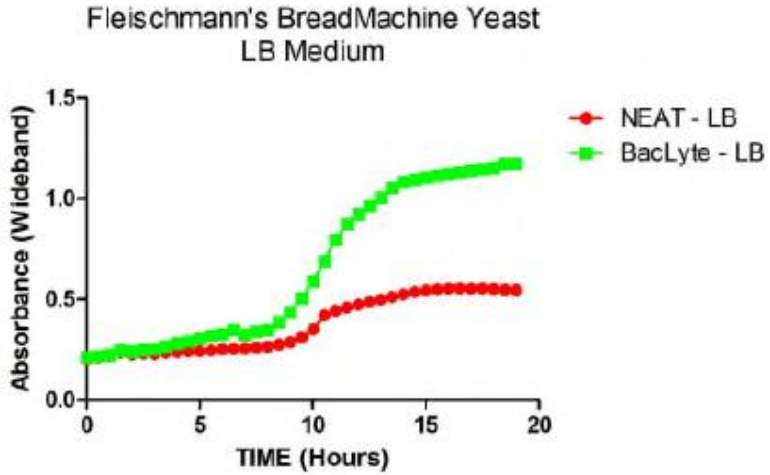
The above graph demonstrates how different levels of BacLyte supplementation supports growth of S.cerevisae yeast in a minimal BM2 media which is normally unsupportive of vegetative growth. Glucose controls have been added to show that growth effects were not sugar-dependant

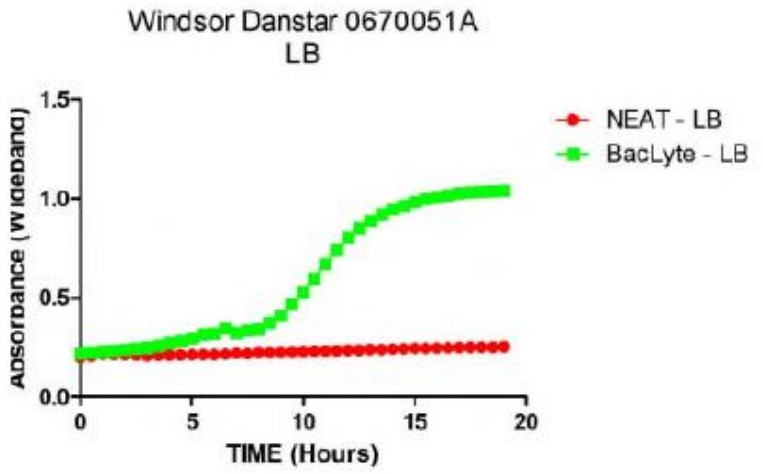




This pair of graphs highlight how a 0.5% w/v Baclyte supplementation of two Saccharomyces bakers yeasts results in improvements of final fermentation ODs in rich YPD media which is by nature optimised for yeast culture.

More profound is the effect 0.5% Baclyte supplementation has on these yeasts when grown Luria Bertani media which (whilst being a rich media) does not normally contain the right mix of nutrients to effectively support yeast growth.



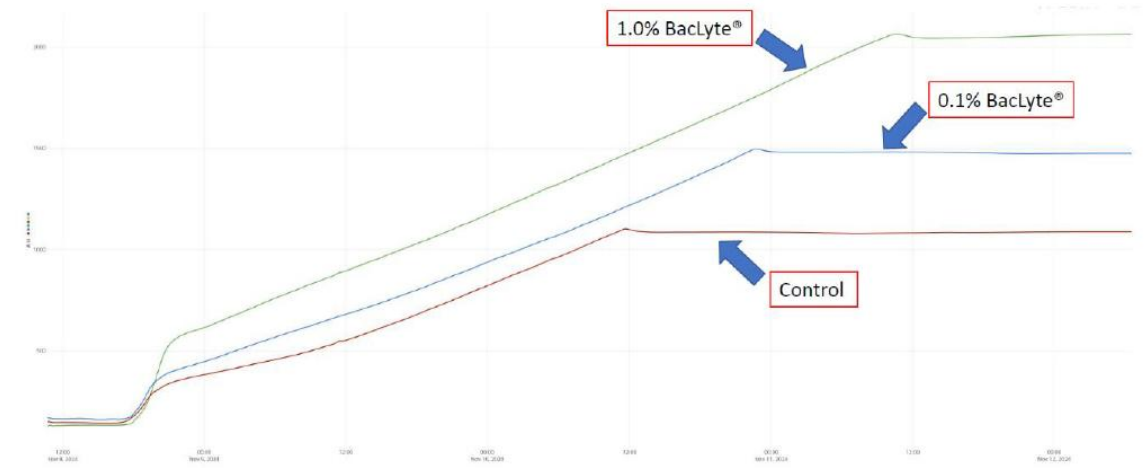


Enhancement of biomass by use of BacLyte® or Banana Juice 3X

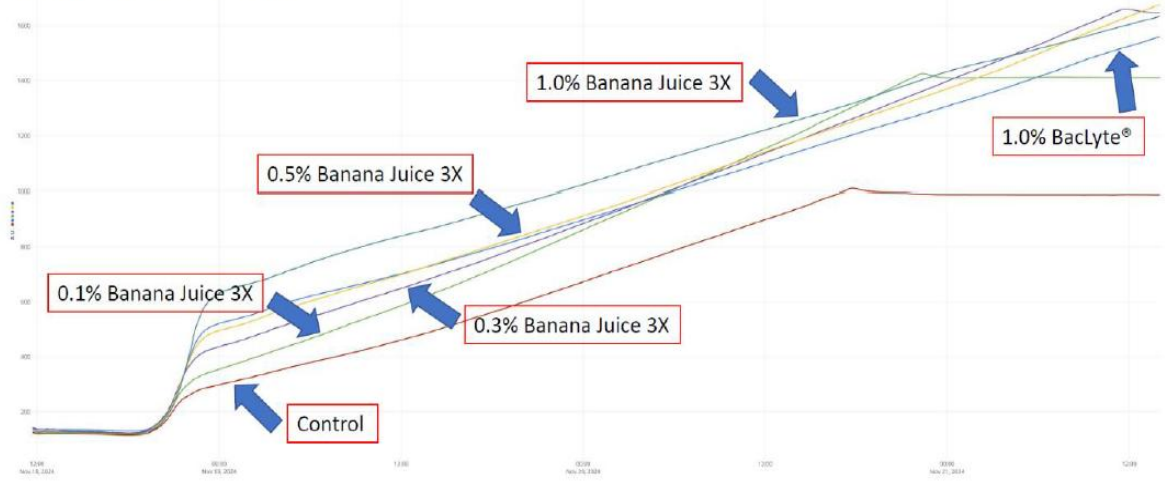
We evaluated the ability of BacLyte® products, “BacLyte Gold” and “Banana Juice 3X”, to increase yeast fermentative growth and biomass. BacLyte Gold is the highest purified product produced by LyteGro while Banana Juice 3X is a less processeculture supplement.

These two BacLyte products were evaluated as an additive to seeder cultures of yeast **only** with the goal of increasing growth during the main bulk fermentation ultimately resulting in greater biomass yields.

Saccharomyces cerevisiae



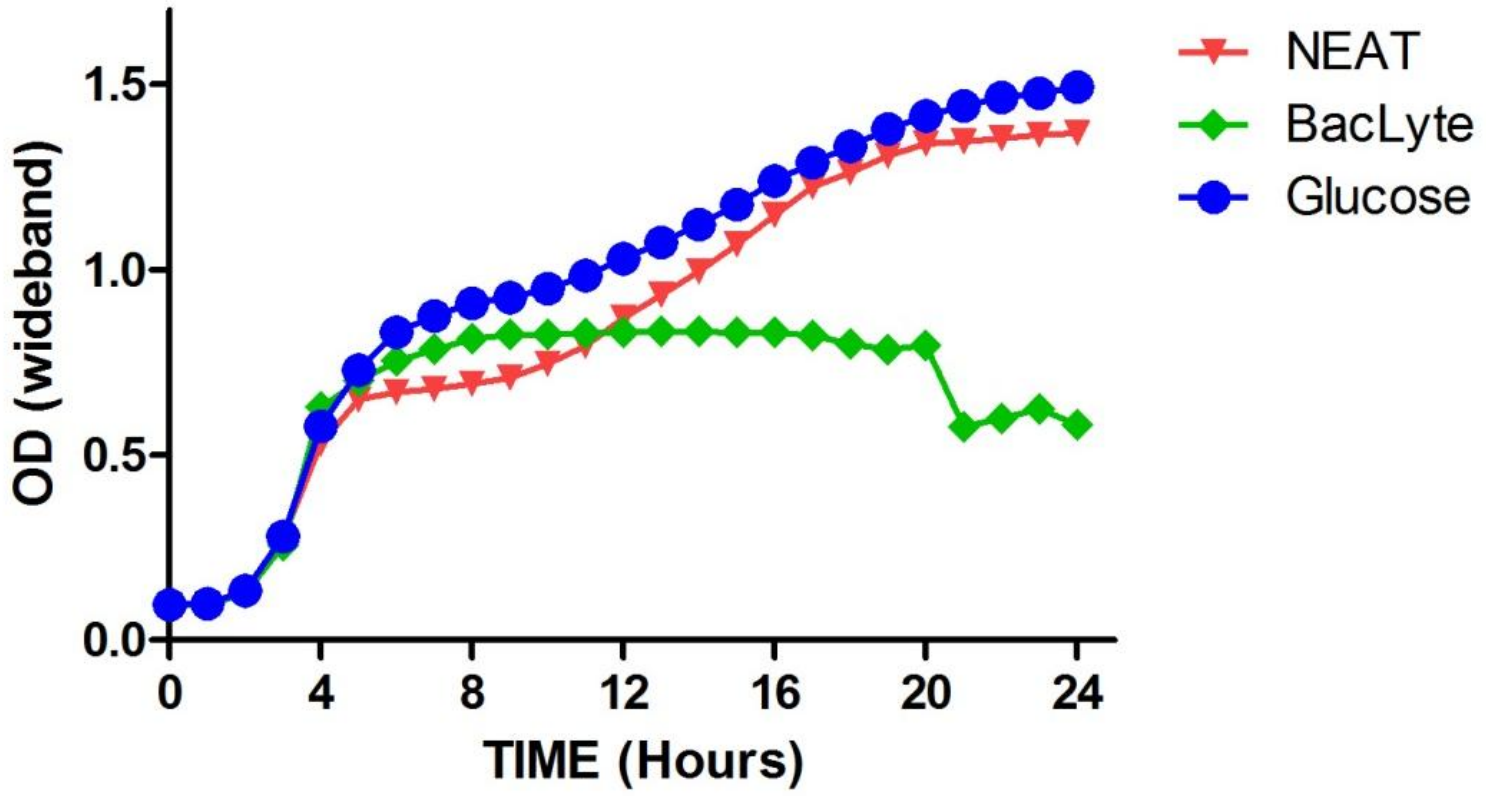
Saccharomyces cerevisiae



The results unequivocally demonstrate the ability of the two BacLyte products to greatly increase biomass when introduced as part of the seeder culture. No further supplementation of the main culture was needed. Additionally, the onset of lag phase was also significantly increased over controls.

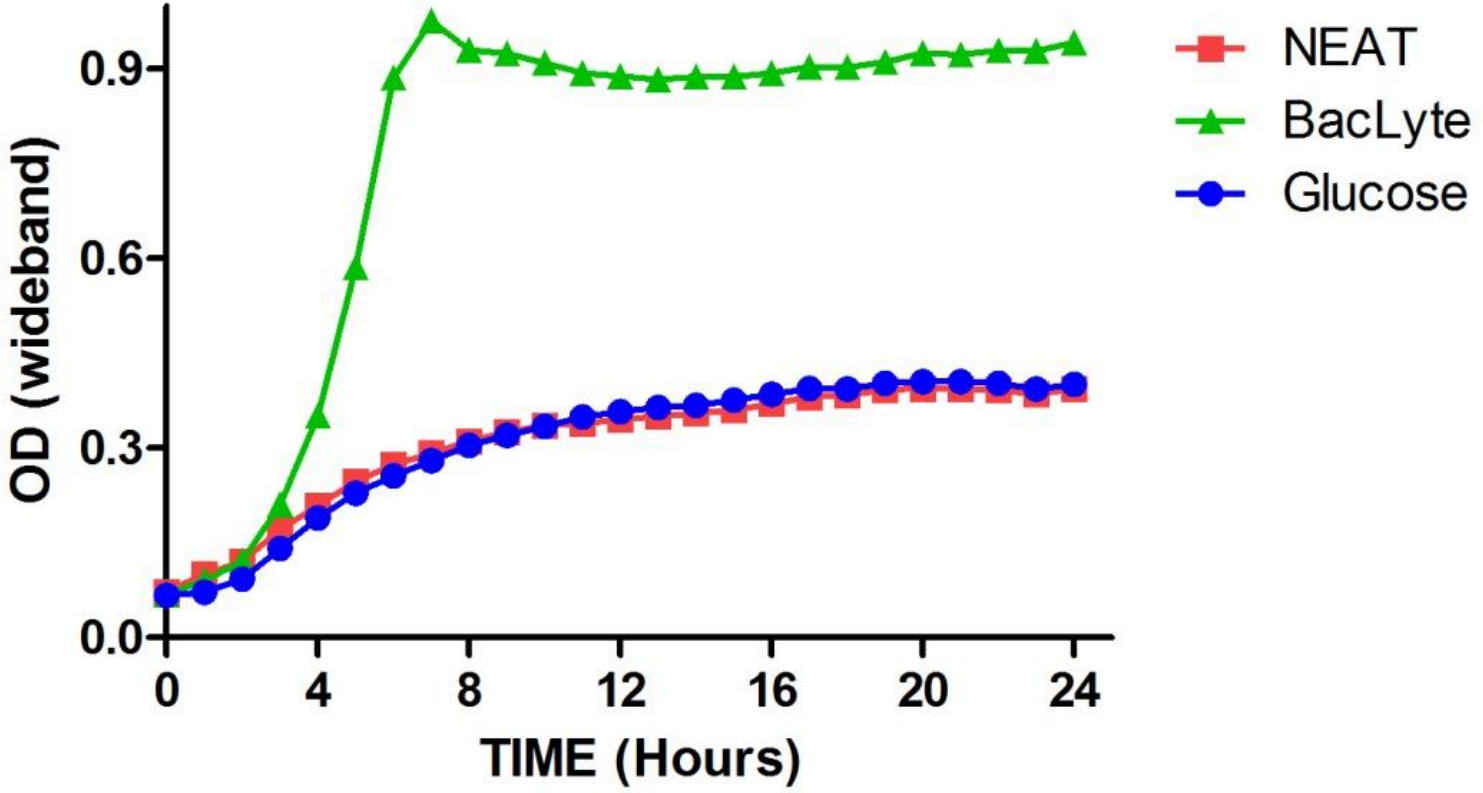
Similar results were also seen with Pichia and Brettanomyces species

Escherichia coli *Rich media*



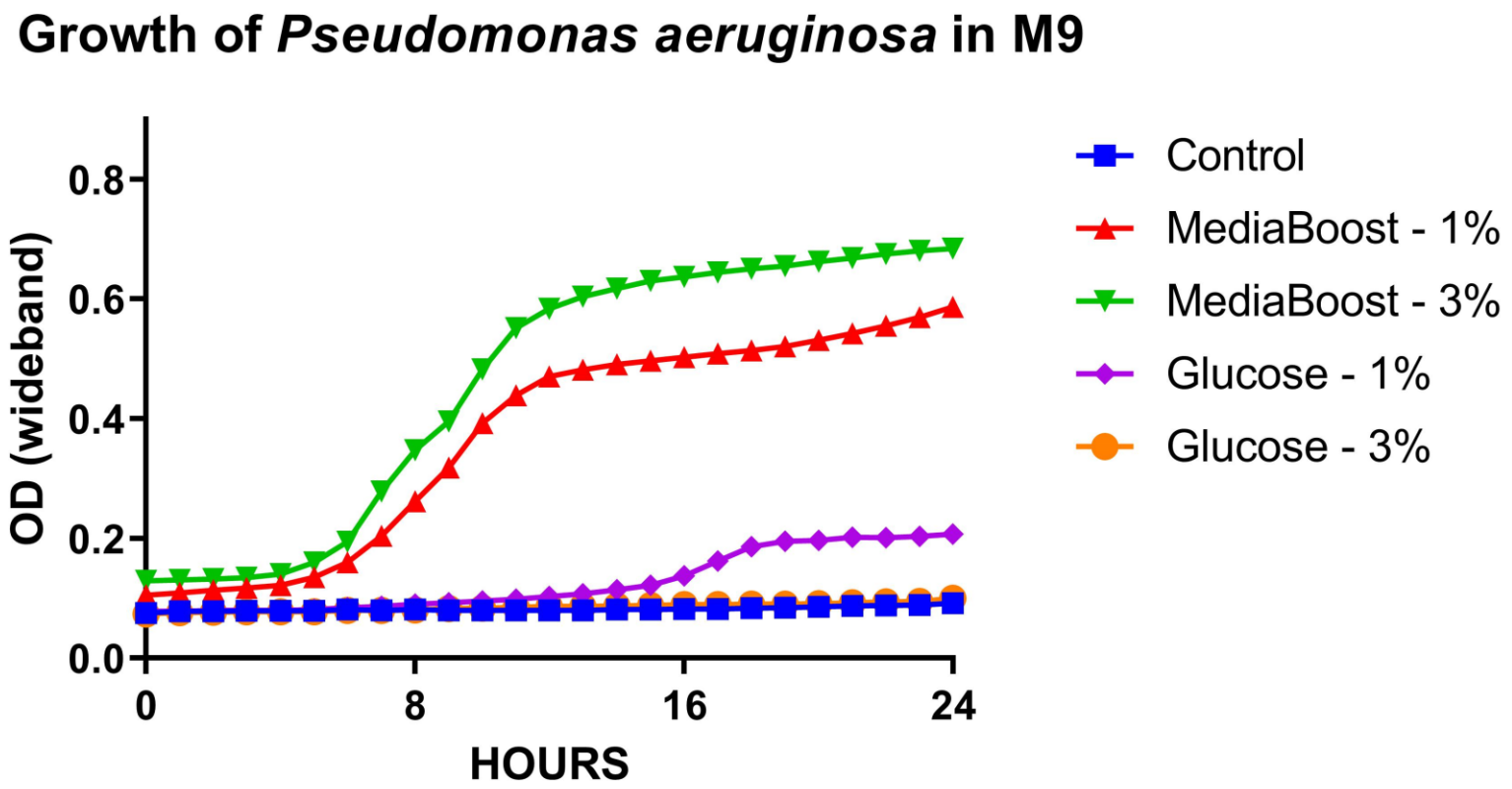
Approximately 100 CFUs of *E. coli* were incubated in LB with or without added 2% v/v BacLyte. An equivalent amount of a 50% glucose solution to that used for MediaBoost was also employed to demonstrate that inability of BacLyte. to promote growth was not due to additional carbon source. Data points represent means of triplicate cultures and S.D. did not exceed 10% of mean.

Minimal media



Same conditions as previous slide with the exception that IMDM was used instead of LB. The concentration of BacLyte was 2% v/v. An equivalent amount of a 50% glucose solution to that used for BacLyte was also employed to demonstrate that BacLyte growth promoting effect was not due to additional carbon source. Data points represent means of triplicate cultures and S.D. did not exceed 10% of mean.

Other species



The above graph demonstrates how different levels of BacLyte supplementation supports growth of P. aeruginosa in a minimal M9 media which is normally unsupportive of vegetative growth. Glucose controls have been added to show that growth effects were not sugar-dependant

Hospital Pathogens

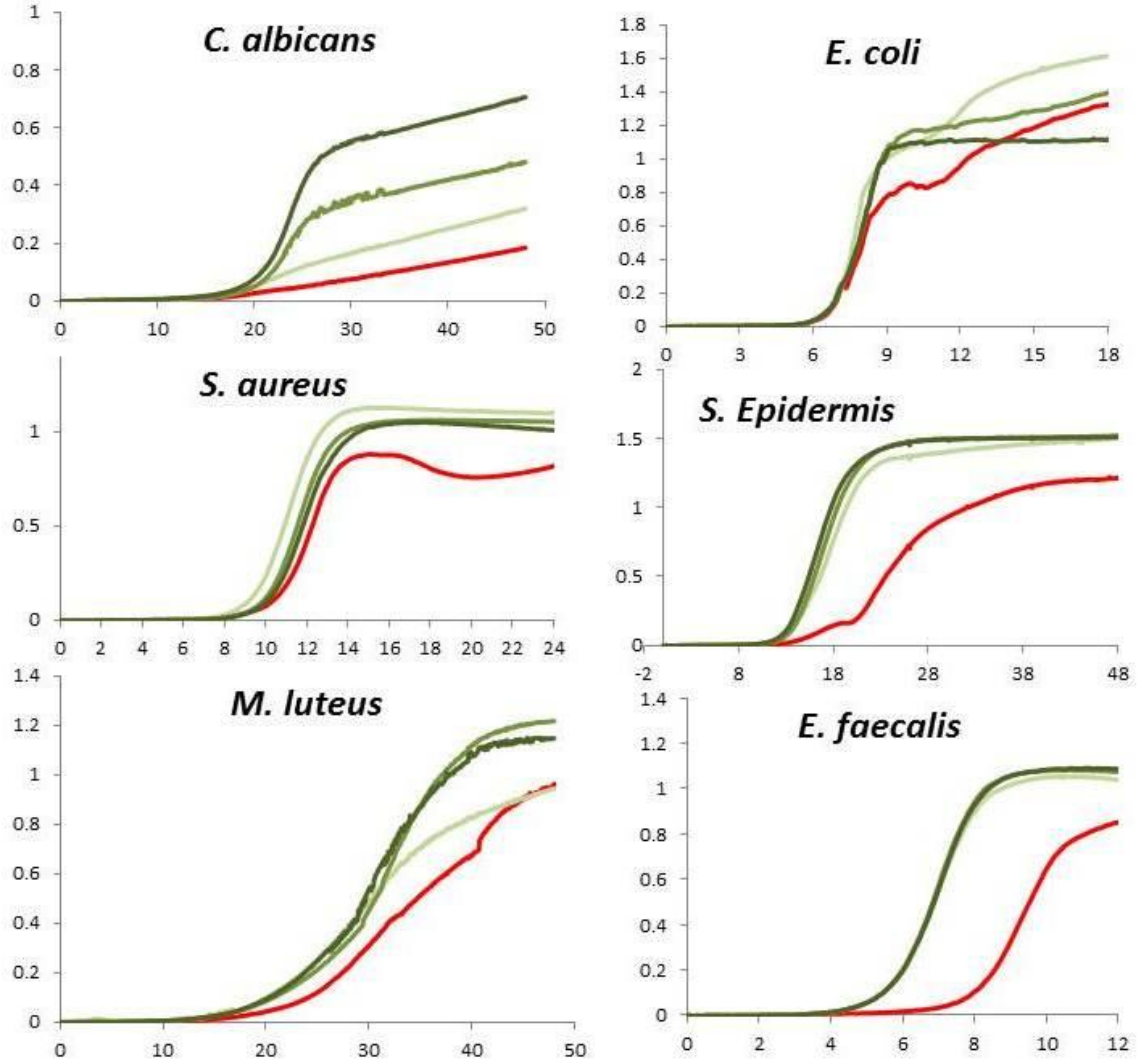
The effects of three levels of BacLyte supplementation on growth of a range of common hospital pathogens was tested by a leading international diagnostics company.

Three concentrations of BacLyte used were:

- 0.5% v/v (pale green)
- 1% v/v (mid green)
- 2% v/v (dark green)

Controls without any BacLyte supplementation were performed (red).

Growth was significantly improved across all samples with some species exhibiting shorter lag phase too.



Get in touch if you would like to explore how BacLyte can improve your microbial production

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