

Nu-Tek Application Note: Successful Replacement of Animal Derived Components with Plant Derived Protein Hydrolysates in Media for the Growth of Probiotic Bacteria

A variety of bacterial species in the human gut, generally categorized as probiotic bacteria, promote general gastrointestinal health, and are positively correlated with broader physical and mental wellbeing¹⁻³. Many different lactic acid bacteria (LAB) species are probiotic and are typically grown in culture using traditional media formulations to meet their complex nutritional demands. Traditional media for propagation of LAB (e.g. deMan, Rogosa, and Sharpe (MRS) and M17) include complex undefined animal derived components such as beef extracts, casein hydrolysates and porcine hydrolysates. There is a growing demand and need for production of probiotics that are not reliant on animal derived components. This study shows the soy protein hydrolysate, HSP-A, can replace all animal derived components in MRS and M17 formulations to support the growth of *Lactobacillus acidophilus* ATCC 314 and *Lactococcus lactis* ATCC 11454. These results show HSP-A is effective ingredient for formulating animal derived component free (ADCF) media for production of probiotic bacteria.

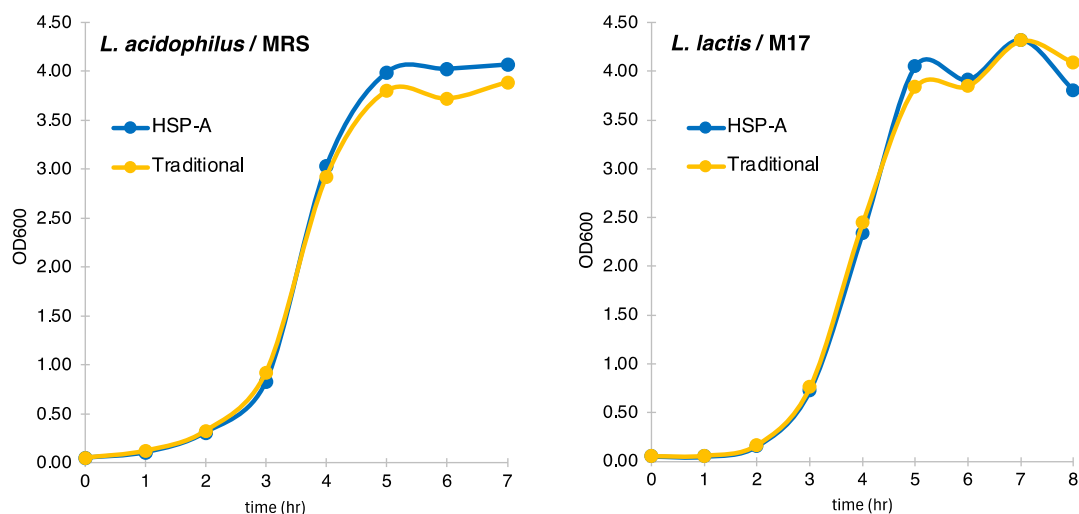


Figure 1. Growth curves of two different probiotic bacterial species in traditional and ADCF media formulations using soy peptone, HSP-A, compared to traditional animal derived media components (e.g. beef extract).

Sample	k (OD600)	r_i (OD600/hr)	$t_{0.5}$ (hr)	t_D (hr)	sigma
<i>L.a.</i> MRS/HSP-A	4.0	0.94	6.8	0.74	0.05
<i>L.a.</i> MRS/Traditional	3.8	0.92	6.6	0.76	0.11
<i>L.l.</i> M17/HSP-A	4.0	2.2	3.8	0.31	0.20
<i>L.l.</i> M17/Traditional	4.1	2.0	3.8	0.36	0.13

Table 1. Summary of growth curve data fit to logistic equation to compare carrying capacity (k), intrinsic growth rate (r_i) and doubling time (t_D). Sigma values are the residual sum of squares from the fit of the logistic curve to the data for each sample. The smaller the sigma value the better the fit of the logistic regression.

Methods

Static cultures of *Lactobacillus acidophilus* ATCC 314 and *Lactococcus lactis* ATCC 11454 were grown at 37°C with CO₂ Gas-pack anaerobe atmosphere sachets in a 1 L anaerobe chamber. Growth rates of LAB cultures were measured spectrophotometrically at a 600 nm wavelength with the appropriate dilution of samples that had OD₆₀₀ values exceeding 0.5. Data was recorded in Excel for analysis in RStudio to fit to a logistic function with logistic parameters generated using *growthcurver*⁴.

M17 Broth Formulations (per 1.0 L)

Component	Traditional	HSP-A ADCF
Pancreatic Digest of Casein	5.0 g	-
Soy Peptone	5.0 g	15 g
Beef Extract	5.0 g	-
Yeast Extract	2.5 g	2.5 g
Ascorbic Acid	0.5 g	0.5 g
Magnesium Sulfate	0.25 g	0.25 g
Disodium-β-glycerophosphate	19.0 g	19.0 g

MRS Broth Formulations (per 1.0 L)

Component	Traditional	HSP-A ADCF
Proteose Peptone No. 3	10.0 g	-
Beef Extract	10.0 g	-
Soy Peptone (HSP-A)	-	20 g
Yeast Extract	5.0 g	5.0 g
Dextrose	20.0 g	20.0 g
Polysorbate 80	1.0 g	1.0 g
Ammonium Citrate	2.0 g	2.0 g
Sodium Acetate	5.0 g	5.0 g
Magnesium Sulfate	0.1 g	0.1 g
Manganese Sulfate	0.05 g	0.05 g
Dipotassium Phosphate	2.0 g	2.0 g

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3. Mörkl, S., Butler, M.I., Holl, A. *et al.* Probiotics and the Microbiota-Gut-Brain Axis: Focus on Psychiatry. *Curr Nutr Rep* **9**, 171–182 (2020). <https://doi-org.ezproxy.stthomas.edu/10.1007/s13668-020-00313-5>
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