Technical Brief:

Animal-Product-Free LB Broth (Miller, Lennox, Luria)

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LB both has been the venerable culture medium formulation used by researchers for over 50 years. First introduced in 1955 by two groups[1,2,3,] LB broth has three different variations, designated as LB Miller[4], LB Lennox and LB Luria after the researchers who developed the formulations. LB is a simple medium composed of three ingredients: yeast extract, sodium chloride and casein hydrolysate. Table 1 gives the formulation for each of the three different variations.

Compositions of LB Broths (g/L).			
Ingredient	**Miller**	**Lennox**	**Luria**
Yeast Extract	5	5	5
NaCl	10	5	0.5
Casein Hydrolysate	10	10	10

Table 1.

In recent years, the use of animal-derived raw materials has become a concern as a source of harmful contaminants to manufacturers of biopharmaceutical products.[5] Initially, this concern was confined to the manufacturing end of the industry. As the regulatory environment has evolved in regard to the use of animal-derived materials, greater restrictions are being placed at earlier stages of product development. The consequence is a pressing need for media formulations which are not made from animal sources.

To address this, we have developed a non-animal protein hydrolysate to replace the casein hydrolysate. An obvious choice for the casein replacement would be plant-derived hydrolysates, however, no one plant protein source has the same amino acid composition as casein. Therefore, the direct replacement of casein hydrolysate with a plant protein hydrolysate would not yield a medium with the same nutritional composition. To overcome this limitation, we have designed a blended plant-based protein hydrolysate that matches casein hydrolysate in composition and in performance.

Results and Discussion

Using an in silico model, several candidate blends were devised. The algorithm applied first searched among 30 different plant protein hydrolysates for those with amino acid compositions that most closely matched casein's composition for each individual amino acid. The model then used an iterative process of forming hypothetical blends that resulted in eight possible variations with compositions closely matching casein. These base formulations were then used to derive a mathematical model for optimizing the blending.

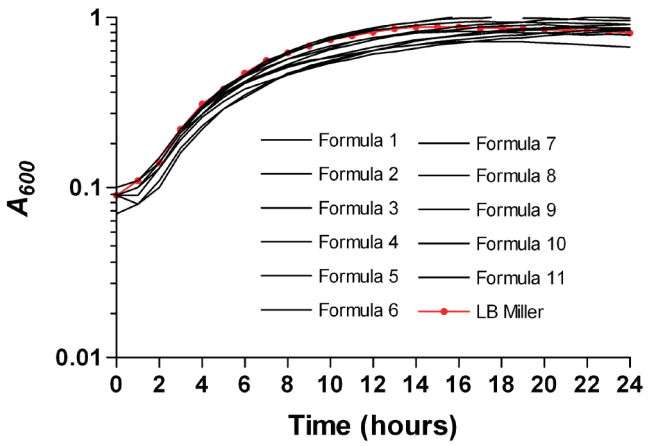

Figure 1. Growth curves for strain HMS174 cultured in various APF LB formulations. To 1 ml of each medium in a 24-well culture dish, a 0.1 ml aliquot of an overnight culture grown in LB (Miller) was added. The cultures were incubated in a Tecan Genios microplate reader with shaking at 37°C. The absorbance at 600 nm was measured at 1 hour intervals. Duplicate wells of non-inoculated medium served as blanks. The net absorbance values were plotted to generate the growth curves.

To determine the best formulation, the candidate hydrolysates were blended according to a simplex lattice mixture design.[6] Eleven prospective formulations were tested empirically by culturing *E. coli* strain HMS174 in medium composed of 5 g/L yeast extract, 10 g/L NaCl and 15 g/L blended hydrolysate. Growth was monitored spectrophotometrically over a 24 hour period and the growth rate and yield calculated. Figure 1 shows the results of this initial screen. The growth parameter values were used to construct a mathematical model which to predicted blends that would produce a growth rate and yield that matched the LB reference medium. This model predicted two possible formulations, designated 12 and 13. Two additional formulations, designated 14 and 16, were predicted by examining the compositions that gave the same growth characteristics as the reference medium and selecting compositions intuitively based on this comparison. Figure 2 (top panel) shows the growth curves of strain HMS174 cultured in these formulations. The growth rates of strain HMS174 in all four formulations were statistically

identical (p = 0.41) to the reference LB medium. Formulations numbered 14 and 16 gave the closest values and were selected for a second analysis (Fig. 2, bottom panel). In this test, the growth rates were again identical between the three media. The biomass yields were the same up to about 12 hours of growth after which time the absorbance values for the reference LB cultures appeared to decrease as compared to the new formulations. The basis for this is not known.

The growth of other commonly used *E. coli* strains were tested using Formula 16. Figure 3 shows that a wild-type strain, W3110, along with four commonly used strains exhibited nearly identical growth characteristics in the animal-product-free Formula 16 as in the standard LB broth. There was a slightly higher yield in some cases for the APF Formula 16. Strain JM109 grew less well in the standard LB formula than in the APF formula, however, its growth rate and yield in this experiment was typical for strain JM109 in our laboratory (data not shown).

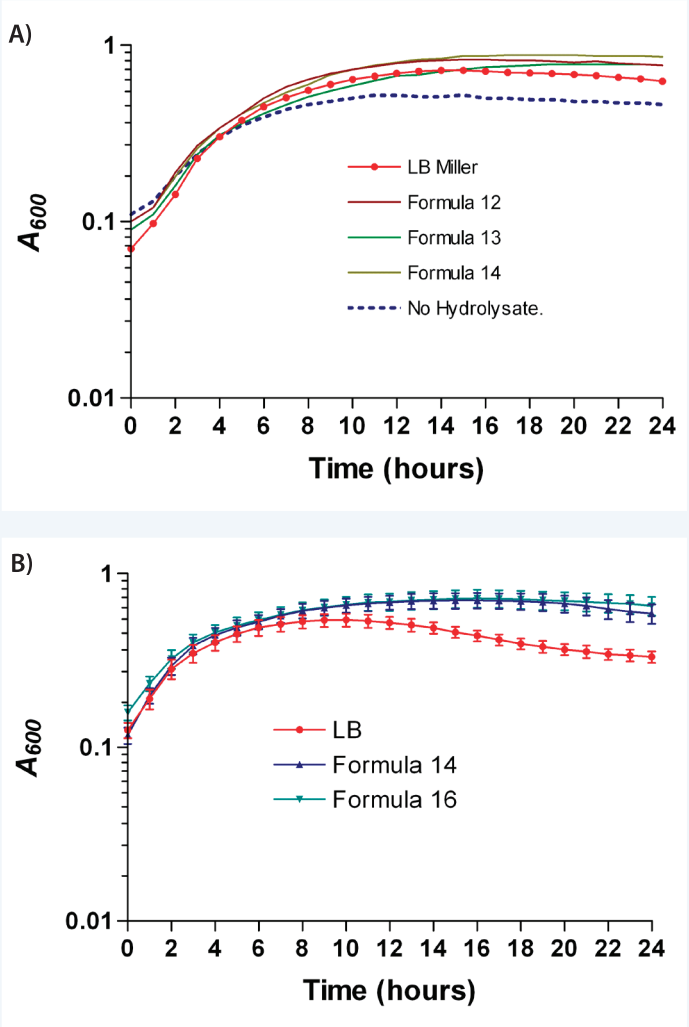

Figure 2. Growth curves of strain HMS174 cultured in APF LB formulas predicted by the initial screen. The cultures were set up and incubated as described in Figure 1. The top panel shows growth in Formulas 12, 13 and 14 along with medium containing only yeast extract and NaCl ("No hydrolysate"). The bottom panel shows growth in Formulas 14 and 16 with seven replicate cultures.

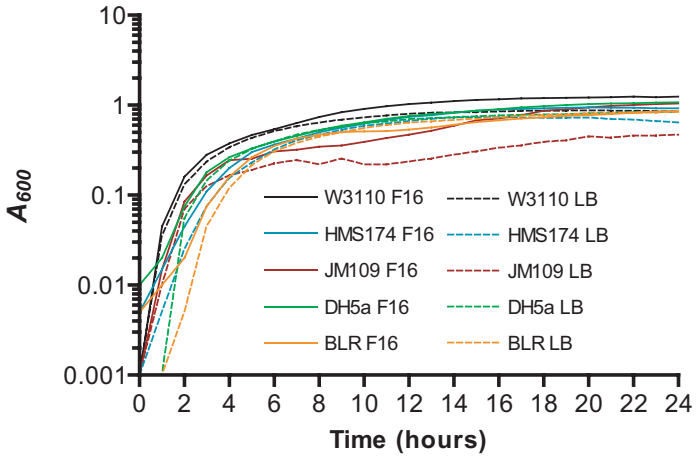

Figure 3. Growth curves for wild-type and four commonly used *E. coli* strain. Duplicate cultures of each strain were grown as described in Figure 1 in LB (Miller) or APF LB Formula 16.

From the above experiments, APF Formula 16 was deemed to be the one which most closely matched the standard LB formulations. While there were formulations that showed higher growth rates and yields, the intent was to match the growth of various strains of *E. coli* to that observed in standard LB broth. In cases where higher growth rates and yields may be important, such as in the production of a recombinant protein, formulations giving higher biomass could be advantageous. Table 2 gives the composition of APF LB in the three different versions using the newly designed casein hydrolysate replacement and Table 3 shows the final amount of each amino acid per liter of culture.

Compositions of *Animal-Product-Free* LB Broths (g/L).			
Ingredient	**Miller**	**Lennox**	**Luria**
Yeast Extract	5	5	5
NaCl	10	5	0.5
Atholate™	20	20	20

Table 2.

Amino acid composition in LB media (mg per liter).						
mg/L	**ala**	**arg**	**asp**	**cys**	**glu**	**gly**
Casein Hydrolysate	301	313	674	30	1,858	189
Athena's APF Blend 16	367	660	868	125	2,438	369
	met	**phe**	**pro**	**ser**	**thr**	**trp**
Casein Hydrolysate	272	398	884	514	418	98
Athena's APF Blend 16	164	469	698	479	341	54
	his	**ile**	**leu**	**lys**	**tyr**	**val**
Casein Hydrolysate	222	436	746	677	282	590
Athena's APF Blend 16	206	386	672	414	302	428
Totals:	Casein Hydrolysate:			8,902		
	Athena's *APF* Blend 16			9,437		

Table 3.

See Next Page for References

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