

Comparing the Nutritional Requirements of the Bile Acid-Metabolizing Gut Bacteria *Clostridium hylemonae* and *Clostridium hiranonis*

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Introduction

Clostridium hiranonis is an obligate anaerobe that was found to be gram positive, spore-forming, and non-motile. The straight or slightly curved rod-shaped cells occur singly or in pairs (2). Also, *Clostridium hylemonae* has shown similar characteristics (3). Both of these *Clostridium* species can convert the primary bile acid cholic acid to a potentially toxic compound, deoxycholic acid (4). These bile acids aid in the process of digestion by absorbing cholesterol, fats, and fat-soluble vitamins. Most of the bile acids are then absorbed and returned to the liver. They are absorbed from the intestines through the ileum, and then travel back to the liver through the portal blood circulation (1). This whole cycle is called the enterohepatic circulation. Furthermore, cholic acid is one of the most common bile acids in the intestine, and it is produced mainly by the deconjugation of bile salts, such as taurocholic acid and glycocholic acid. Increased levels of deoxycholic acid in the bile acid pool have been associated with an increased risk of cholesterol gallstone disease (4). Gallstones form when substances, like cholesterol, in the bile crystallize and become solid.

Purpose

To determine the vitamin and amino acid requirements of *C. hylemonae* and *C. hiranonis*

Methods

C. hylemonae TN-271 and *C. hiranonis* TO-931 were grown anaerobically under 100% CO₂ conditions in Bellco tubes containing 10 ml of medium (Table 1). All the cultures were incubated at 37°C, and the growth was measured with a spectrophotometer at 600 nm.

Vitamin and amino acid stock solutions were prepared anaerobically with filter sterilization then degassing with argon into crimp-sealed serum bottles:

- Vitamin mix contained *p*-aminobenzoate, biotin, cyanocobalamin, folate, lipoate, nicotinate, pantothenate, pyridoxal, riboflavin, thiamine.
- Amino acid mix contained alanine, arginine, asparagine, aspartate, cysteine, glutamate, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine.

Medium	Components
BHI	Glucose
	Brain Heart Infusion
	Yeast Extract
	Resazurin
	Sodium Bicarbonate
	Cysteine (reducing agent)
UM	Yeast Extract
	Glucose
	Mineral Solution
	Trace Metal Solution
	Resazurin
	Sodium Bicarbonate
	Cysteine (reducing agent)
DM	Glucose
	Mineral Solution
	Trace Metal Solution
	Resazurin
	Sodium Bicarbonate
	Cysteine (reducing agent)
DM + VM	Glucose
	Mineral Solution
	Trace Metal Solution
	Resazurin
	Sodium Bicarbonate
	Cysteine (reducing agent)
Vitamin Mix	
DM + AM	Glucose
	Mineral Solution
	Trace Metal Solution
	Resazurin
	Sodium Bicarbonate
	Cysteine (reducing agent)
DM + VM + AM	Glucose
	Mineral Solution
	Trace Metal Solution
	Resazurin
	Sodium Bicarbonate
	Cysteine (reducing agent)
Vitamin Mix	
Amino Acid Mix	

Figure 1. Growth of *Clostridium hylemonae*

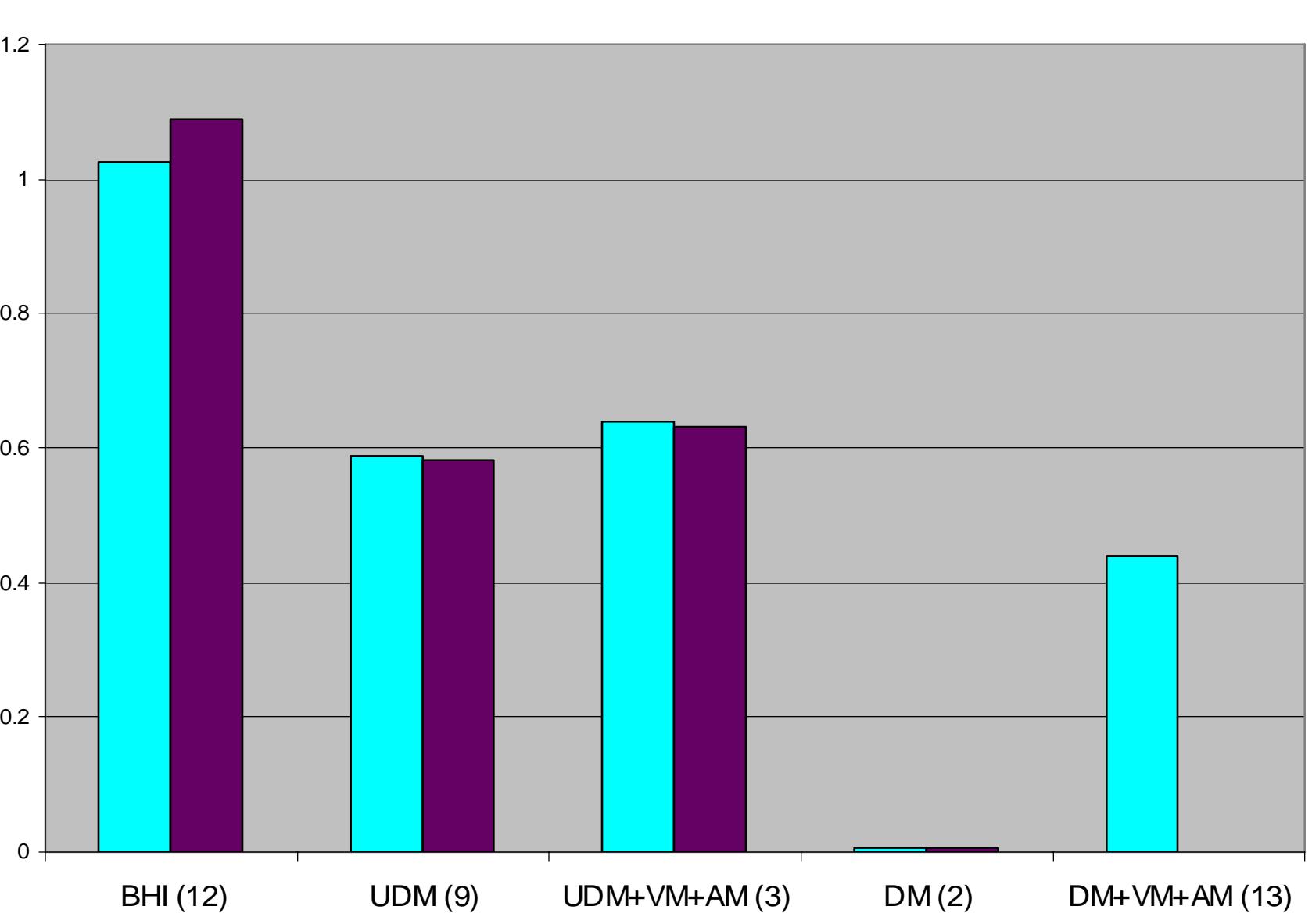
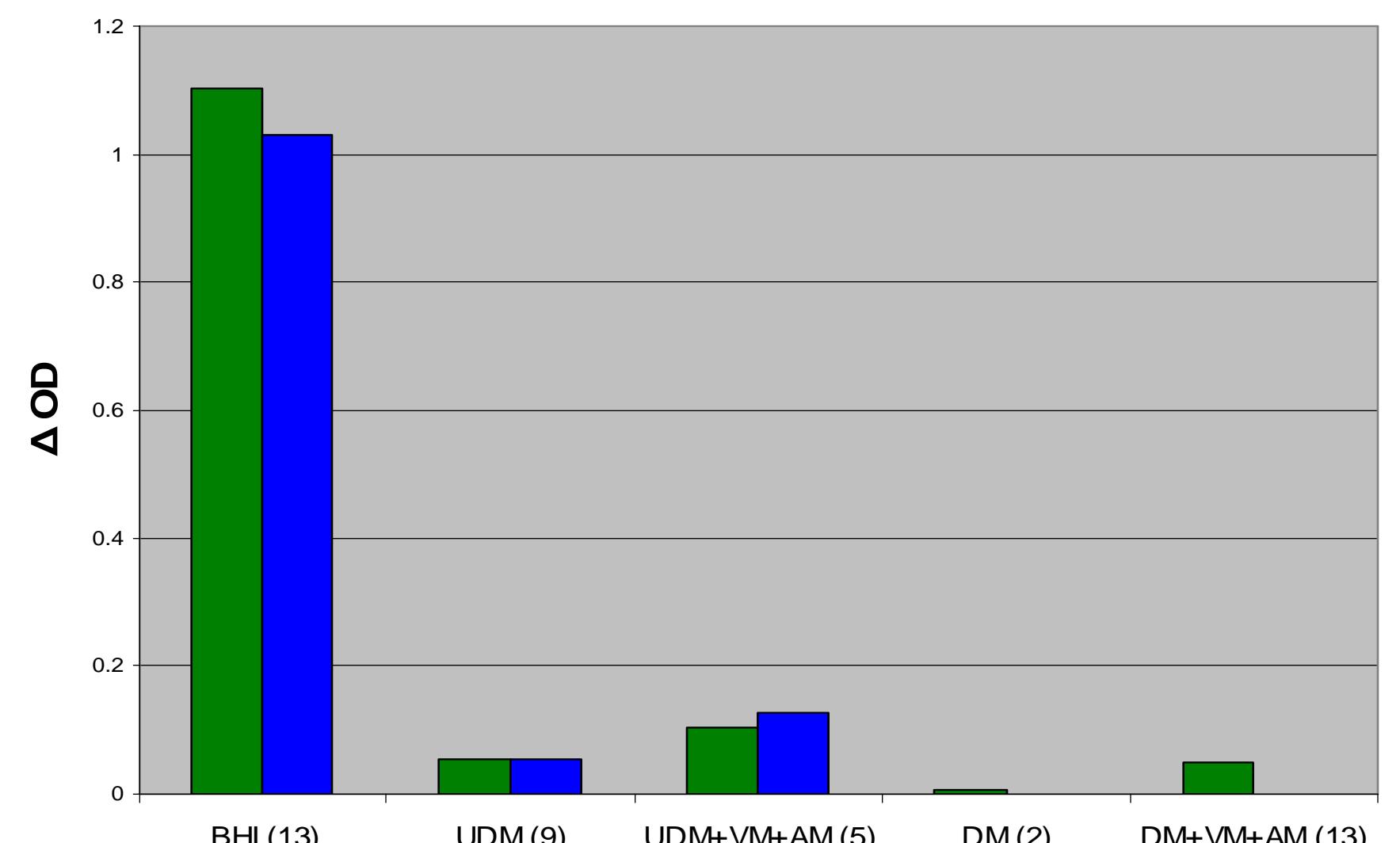
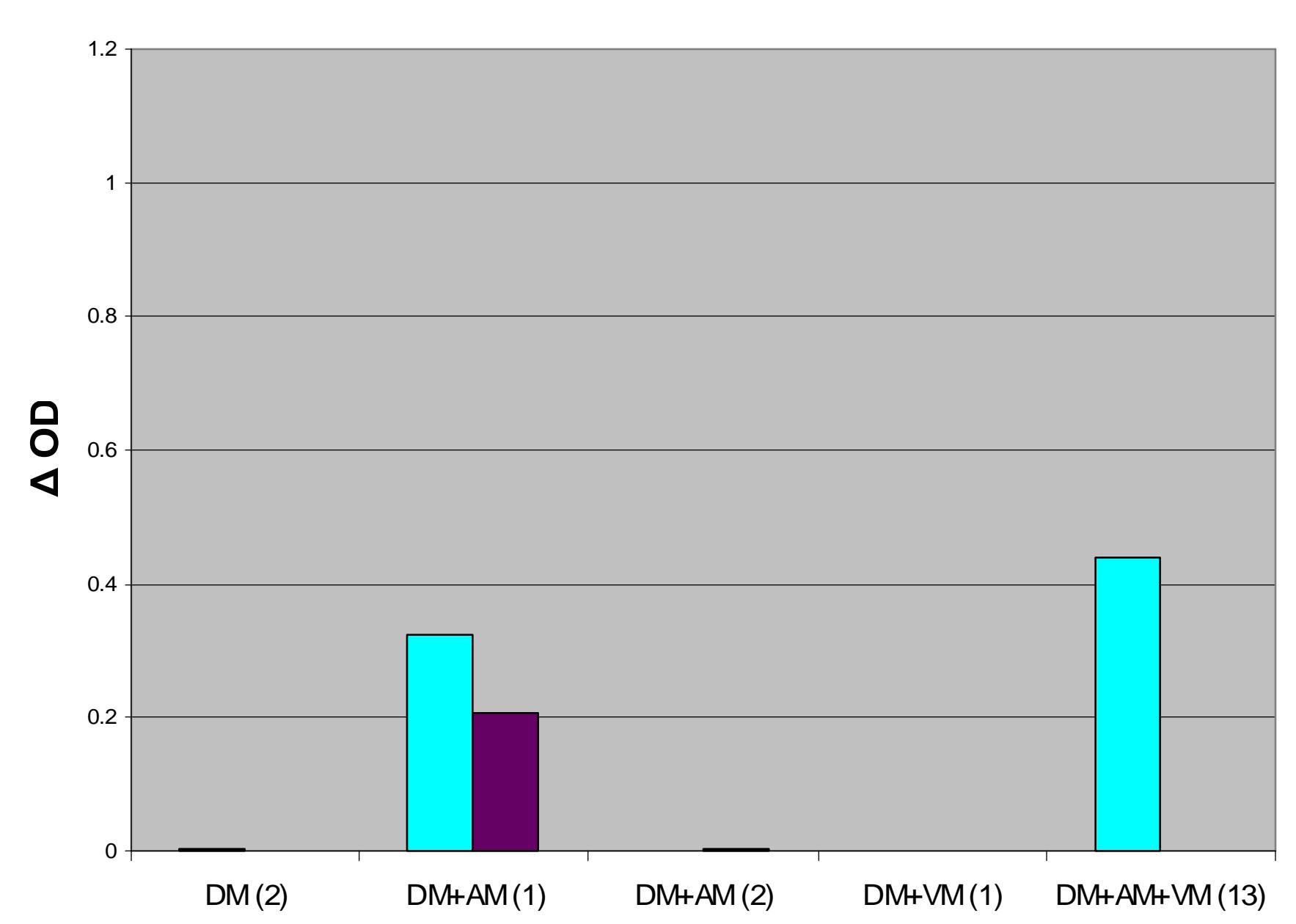


Figure 2. Growth of *Clostridium hiranonis*



Numbers inside of the () in the figures above indicate the number of transfers. Also, on Figures 1, 2, and 4 the DM+VM+AM are just a single tube and all others are in duplicate tubes.

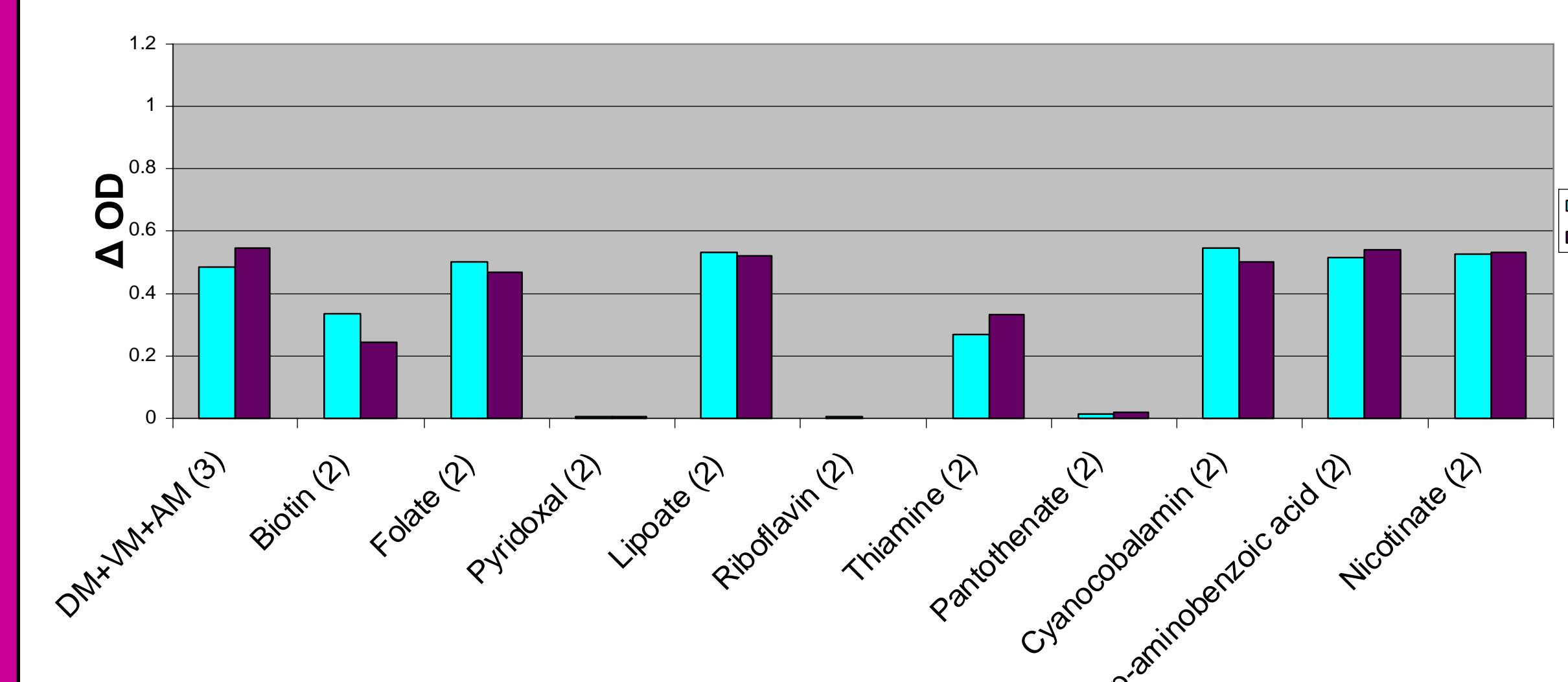
Figure 3. Growth of *Clostridium hylemonae*



Results and Discussion

- BHI broth supported good growth of *C. hylemonae* and *C. hiranonis* [Fig. 1 & 2].
- When transferred to an UM growth by *C. hylemonae* was suppressed some, but growth by *C. hiranonis* was minimal
- When yeast extract was removed from UM (see DM), growth of both bacteria was not supported [Fig. 1 & 2], indicating that these bacterium required one or more growth factors in order for growth to occur.
- Supplementing DM with both vitamin and amino acid solutions (see DM + VM + AM) served as an effective source of growth factors and supported the growth of both gut bacteria [Fig. 1 & 2].
- Supplementing DM with either the amino acid mix or the vitamin mix failed to support the growth of *C. hylemonae* [Fig. 3]. This shows that it requires both for optimal growth.
- Relative to *C. hylemonae*, deletion of individual vitamins from the mix indicated that pyridoxal, riboflavin, and pantothenate were required by *C. hylemonae* for growth [Fig. 4].
- Data for 3rd transfer of *C. hylemonae* and the individual vitamins is currently in progress.
- Studies are currently underway to determine the individual vitamin requirements of *C. hiranonis* and the amino acid requirements of both.
- Resolving the nutritional requirements of these bile acid-metabolizing gut bacteria will provide us the tools for understanding the physiology and metabolism of these bile acid-metabolizing gut bacteria.

Figure 4: Growth of *Clostridium hylemonae* in DM + AM + (VM-Vitamin)



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