# Resolving the Nutritional Requirements of *Clostridium scindens*, a Bile Acid-Metabolizing Gut Bacterium

**Ashley M. Inboden and Steven L. Daniel**
**Eastern Illinois University, Department of Biological Sciences, Charleston, IL**

## Abstract

Biotransformation of cholate (a primary bile acid) in the gut results in the production of deoxycholate. Increased deoxycholate levels have been correlated with an increased risk for high cholesterol, gallstone disease, and colon cancer. In the gut, the obligate anaerobe *Clostridium scindens* is actively involved in the conversion of cholate to deoxycholate. Thus, *C. scindens* is important to human health, and our goals were to define the nutritional requirements of this key gut bacterium. *C. scindens* VPI 12708 was grown anaerobically in an undefined broth medium (UBM; minerals, metals, bicarbonate, 100% $CO_2$ gas phase, cysteine [reducing agent], 0.1% yeast extract, and 20 mM glucose) at 37°C. When transferred from UBM to a defined broth medium (DBM; UBM lacking yeast extract), *C. scindens* did not grow. However, growth was supported when DBM was supplemented with both a vitamin mix (*p*-aminobenzoate, biotin, cyanocobalamin, folate, lipoate, nicotinate, pantothenate, pyridoxal, riboflavin, thiamine) and an amino acid mix (alanine, arginine, asparagine, aspartate, cystine, glutamate, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine). Deletion of individual vitamins from the mix indicated that biotin, lipoate, riboflavin, and pantothenate were required by *C. scindens* for growth. This is the first report of the vitamin requirements of *C. scindens*. Studies are currently underway to determine the amino acid requirements of *C. scindens*.

## Introduction

The gut bacterium *Clostridium scindens* is a gram-negative rod, and is shown to be an obligate anaerobe, meaning lives only in the absence of free oxygen. This bacterium is commonly found in the gut, particularly areas associated with bile acid production **(1)**. Bile acids are produced in the liver from cholesterol and are used primarily for absorbption of fats. In humans, cholic acid and chenodeoxycholic acids are the primary bile acids. Bile acids are stored in the gall bladder and released into the small intestine to aid in digestion and absorption of lipids and lipid soluble nutrients. Most bile salts are actively reabsorbed from small intestines and returned to the liver. However, a small fraction of bile salts escape the reabsorbing process and enter the colon where they are biotransformed by bacteria to secondary bile acids. One gut bacterium involved in the biotransformation of primary bile acids is *Clostridium scindens* **(2)**. This bacterium converts cholic acid via 7$\alpha$-dehydroxylation to deoxycholic acid, a secondary bile acid. The product of biotransformation of the bile salts is called secondary bile acids. One such secondary bile acid is deoxycholic acid, the result of 7$\alpha$-dehydroxylation of cholic acid. The liver of humans cannot synthesize deoxycholic acid; therefore the amounts of this secondary bile acid vary greatly (from 1%-40%) in human bile. Increased deoxycholic acid levels in blood, bile, and feces have been correlated with an increased risk for high cholesterol, gallstone disease, and colon cancer **(3)**.

## Objectives

(1)Determine the nutrient requirements of *C. scindens*

(2)Determine the types and amounts of end products produced during glucose fermentation.

(3)Determine *C. scindens'* role in secondary bile acid production.

## Acknowledgments

Thanks is given to Eastern Illinois University Department of Biological Sciences for their support through a Biological Sciences Undergraduate Research Grant.

Thanks is also given to Dr. Kinchel Doerner from Western Kentucky University for providing the culture of *C. scindens*.

## Literature Cited

(1)Kitahara, M., F. Takamine, et al. (2000). "Assignment of Eubacterium sp. VPI 12708 and related strains with high bile acid 7α-dehydroxylating activity to *Clostridium hylemonae* sp. nov. isolated from human faeces." International Journal of Systematic and Evolutionary Microbiology **50**: 971-978.

(2)Hylemon, P. B. and J. Harder (1999). "Biotransformation of monoterpenes, bile acids, and other isoprenoids in anaerobic ecosystems." FEMS Microbiology Reviews 22: 475-488.

(3) Baron, S. F. and P. B. Hylemon (1997). Biotransformation of bile acids, cholesterol, and steroid hormones. Gastrointestinal Microbiology. 1: 470-510.

## Methods

*C. scindens* VPI 12708 was grown anaerobically under 100% $CO_2$ in 18x150mm Bellco tubes containing 10mL of culture medium **[Table 1]**. All cultures were incubated at 37˚C.

Vitamin and amino acid stock solutions were prepared anaerobically with filter sterilization then degassing with argon into crimp-sealed serum bottles. Vitamin stock solution contained *p*-aminobenzoate, biotin, cyanocobalamin, folate, lipoate, nicotinate, pantothenate, pyridoxal, riboflavin, thiamine. Amino acid stock solution contained alanine, arginine, asparagine, aspartate, cystine, glutamate, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine. Individual vitamin mixes were prepared anaerobically with filter sterilization then degassing with argon into crimp-sealed serum bottles. Cultures were maintained using DBM supplemented with both vitamin and amino acid stock solutions.

Growth in culture media was measured with a spectrophotometer at 600 nm.

### Table 1. Media Comparisons

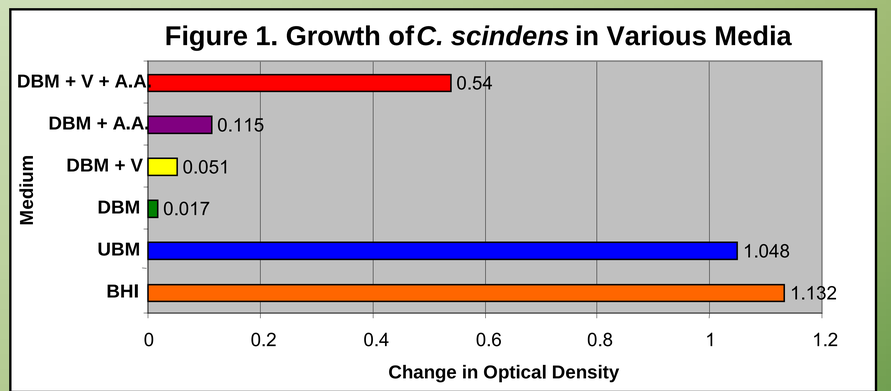
| Medium | Components |
|---|---|
| BHI | Glucose |
| | Yeast Extract |
| | Brain Heart Infusion Broth |
| | Resazurin |
| | Sodium Bicarbonate |
| | Cysteine |
| UBM | Glucose |
| | Yeast Extract |
| | Mineral Solution |
| | Trace Metal Solution |
| | Resazurin |
| | Sodium Bicarbonate |
| | Cysteine |
| DBM | Glucose |
| | Mineral Solution |
| | Trace Metal Solution |
| | Resazurin |
| | Sodium Bicarbonate |
| | Cysteine |
| DBM + V | Glucose |
| | Mineral Solution |
| | Trace Metal Solution |
| | Resazurin |
| | Sodium Bicarbonate |
| | Cysteine |
| | Vitamin Stock Solution |
| DBM + A.A. | Glucose |
| | Mineral Solution |
| | Trace Metal Solution |
| | Resazurin |
| | Sodium Bicarbonate |
| | Cysteine |
| | Amino Acid Stock Solution |
| DBM + V + A.A. | Glucose |
| | Mineral Solution |
| | Trace Metal Solution |
| | Resazurin |
| | Sodium Bicarbonate |
| | Cysteine |
| | Vitamin Stock Solution |
| | Amino Acid Stock Solution |

## Results and Discussion

- Brain Heart Infusion (BHI) broth and UBM both supported good growth of *C. scindens* **[Fig.1]**.
- When the yeast extract was removed from UBM (see DBM), growth of *C. scindens* was not supported **[Fig. 3]**, indicating that this bacterium required one or more growth factors in order for growth to occur.
- Growth of *C. scindens* was not supported in DBM supplemented with either a vitamin solution (DBM + V) or an amino acid solution (DBM + A.A.) **[Fig. 1]**
- Supplementing DBM with both vitamin and amino acid solutions (see DBM + V + A.A.) served as an effective source of growth factors and supported the growth of *C. scindens* **[Fig. 1]**.
- Deletion of individual vitamins from the mix indicated that biotin, lipoate, riboflavin, and pantothenate were required by *C. scindens* for growth **[Fig. 2]**.
- Studies are currently underway to determine the amino acid requirements of *C. scindens.*

➢ **Resolving the nutritional requirements of *C. scindens* will provide us the tools for understanding how carbon and energy flow impact bile acid metabolism by this important gut bacterium.**

### Figure 1. Growth of *C. scindens* in Various Media




### Figure 2. Growth of *C. scindens* in DBM + A.A. + (V-Vitamin)