Microbiology Focus

Volume 1.3, 2009

Fluka Analytical

Differentiation and Identification Media for *Salmonella*

Salmonella bacteria, responsible for food poisoning.

Differentiation and Identification Media for *Salmonella*

By Jvo Siegrist, Product Manager Microbiology…. *ivo.siegrist@sial.com*

Today's usage of classical culture methods to identify Salmonella, a potent food-borne pathogen

Salmonella contamination is the second leading cause of food-borne illness worldwide. Controlling outbreaks of *Salmonella* is an important task for food regulators, restaurants and the food industry in general.

The *Salmonella* family includes over 2,300 serotypes of bacteria, but two types, *Salmonella* enteritidis and *Salmonella typhimurium*, are responsible for about half of all human infections. Most outbreaks of *Salmonella* are traced back to dairy, poultry and meat products, but *Salmonella* can grow on nearly any food. Chicken, eggs and their derivative products are particularly high risk.

Kingdom: **Bacteria**
Phylum: **Proteobacteria**
Class: **Gamma Proteobacteria**
Order: **Enterobacteriales**
Family: **Enterobacteriaceae**
Genus: ***Salmonella***

Figure 1: ***Salmonella*** **Bacteria**

Microbiological control in the food industry plays a critical role in preventing *Salmonella* outbreaks. Tests and media used for identification of *Salmonella* take advantage of unique aspects of *Salmonella* physiology or biochemistry relative to other genera within the family Enterobacteriaceae. For example, bacteria from the genus *Salmonella* are mostly facultative anaerobes, oxidase-negative, catalase-positive and gram-negative rods. Most strains are motile and ferment glucose with production of both acid and gas.

The media used currently for the differentiation and identification of *Salmonella* are still based on the detection of carbohydrate fermentation indicated by a pH indicator (see also **Table 1** for carbohydrate fermentation abibility), the detection of proteolytic activity, hydrogen sulphide production and selectivity. Most modern media also combine some of this detection system to make the media more reliable. A listing of the most common selective and differential media appears in **Table 2** (page 3).

Did you know …

Salmonella can cause a chronic infection in some people?

These people are called **carriers** and can be a continued source of food contamination by excreting bacteria even though they are symptom-free.

Carbohydrate	Fermentation Acid	Fermentation Gas	Cat. No. of Fluka Cabohydtrate Discs
Adonitol	-	-	55876
Arabinose	+/-	+/-	80372
Cellobiose	-	-	56481
Dextrose	+	+/-	63367
Dulcitol	+/-	+/-	73044
Fructose	+/-	+/-	53901
Galactose	+	+/-	89608
Inositol	+/-	+/-	89614
Lactose	-	-	28816
Maltose	+	+/-	77653
Mannitol	+	+/-	94438
Mannose	+/-	+/-	94445
Melibiose	+	+	93196
Raffinose	-	-	94226
Rhamnose	+/-	+/-	93999
Salicin	-	-	92971
Sorbitol	+	+/-	93998
Sucrose	-	-	94309
Trehalose	+	+/-	92961
Xylose	+	+/-	07411

Table 1: **Typical carbohydrate fermentation ability of** ***Salmonella***

In addition, our current technology offers the chromogenic media, which makes identification even more reliable and faster as they detect a characteristic enzyme of the *Salmonella*. These reactions are based on the cleavage of a chromogenic substrate which results in a visible color change (see **Table 3**).

Brand	Cat #	Name
Fluka	00563	HiCrome™ MM Agar
Fluka	90918	HiCrome™ RajHans Medium, Modified
Fluka	78419	HiCrome™ Salmonella Agar
Fluka	05538	HiCrome™ Salmonella Agar, Improved
Fluka	84369	Salmonella Chromogen Agar
Fluka	01993	Salmonella Chromogen Agar Set

Table 3: **Chromogenic media for** ***Salmonella***

Brand	Cat #	Name
Fluka	A0715	Andrade Peptone Water
Fluka	28943	Andrade peptone water, Vegitone
Fluka	95388	Bismuth sulfite Agar
Fluka	15835	BPL Agar
Fluka	70134	Brilliant Green Agar, modified
Fluka	16026	Brilliant Green Phenol Red Lactose Sucrose Agar
Fluka	36408	Bromcresol Purple Broth
Fluka	22520	China Blue Lactose Agar
Fluka	55420	CLED Agar
Fluka	70135	DCLS Agar
Fluka	90035	DCLS Agar No. 2
Fluka	D2935	Decarboxylase Broth Base, Moeller
Fluka	D7809	Deoxycholate Citrate Agar
Fluka	E5399	Endo Agar
Fluka	70137	ENDO Agar (Base)
Fluka	16447	Glucose Bromcresol Purple Agar
Fluka	51490	Hektoen Enteric Agar
Fluka	60787	Kligler Agar
Fluka	61792	Leifson Agar
Fluka	66304	Lysine Decarboxylase Broth
Fluka	62915	Lysine Iron Agar
Fluka	70143	Mac Conkey Agar No 1
Fluka	19352	Mac Conkey Agar No 1, Vegitone
Fluka	M8302, 94216	MacConkey Agar with Crystal Violet, Sodium Chloride and 0.15% Bile Salts
Fluka	70144	MacConkey Broth
Fluka	75717, 16377	MacConkey broth purple
Fluka	63014	MacConkey MUG Agar
Fluka	51405	MacConkey-Agar (without salt)

Brand	Cat #	Name
Fluka	69965	Mossel Broth
Fluka	43052	Muller-Kauffmann Tetrathionate Broth, Base (ISO)
Fluka	75315	OF Test Nutrient Agar
Fluka	81648	Pril® Mannitol Agar
Fluka	04584	Rappaport Vassiliadis Broth acc. to DIN EN ISO 6579:2002
Fluka	17173	Rappaport Vassiliadis Broth, modified
Fluka	R0773	Rappaport Vassiliadis Medium
Fluka	92322	Rappaport Vassiliadis medium (base), modified, semi-solid
Fluka	84368	Salmonella Agar according to Önöz
Fluka	84370	Salmonella Enrichment Broth
Fluka	70153	Selenite Broth (Base)
Fluka	84922	Selenite Cystine Broth
Fluka	85438	SIM Medium
Fluka	85463	Simmons Citrate Agar
Fluka	85640	SS-Agar
Fluka	86352	TBG Broth
Fluka	88151	Tetrathionate Broth
Fluka	88148	Tetrathionate Enrichment Broth according to Muller-Kauffmann
Fluka	44940	Triple Sugar Iron Agar
Fluka	51463	Urea Broth
Fluka	42376	Violet Red Bile Agar, Vegitone
Fluka	70189, 79873	Violet Red Bile Glucose Agar
Fluka	17213	Violet Red Bile Glucose Agar without Lactose
Fluka	53605	Violet Red Bile Glucose Agar without Lactose, Vegitone
Fluka	41270	Violet Red Bile Lactose Dextrose Agar
Fluka	95273	VRB MUG Agar
Fluka	95586	XLD Agar
Fluka	76721	XLT4 Agar (Base)

Table 2: ***Salmonella*** **selective and differential media (list not complete. For more see *sigma-aldrich.com/salmonella*)**

Figure 2: HiCrome™ Salmonella Agar, Improved

Test Organisms (ATCC)	H_2S production
Escherichia coli (25922)	-
Salmonella serotype Enteritidis (13076)	+
Salmonella serotype Typhimurium (14028)	+

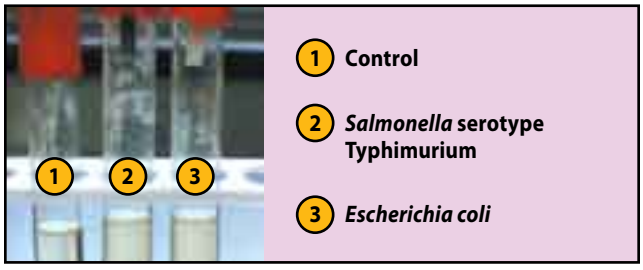


Figure 3: Hydrogen Sulfide Test Strips

Detection of hydrogen sulfide Production of microorganisms such as *Salmonella*

A large number of bacteria can produce H_2S in small amounts from sulfur containing amino acids in carbohydrate media. When combined with lead acetate, the H_2S will produce a black precipitate, giving rise to a visible black coloured reaction on the paper strip. The lead acetate method is very sensitive, allowing the detection of trace levels of hydrogen sulphide.

Test with strips: Inoculate peptone water (Fluka Cat. No. 70179) with the suspect organism. Insert a lead acetate paper strip between the plug and inner wall of tube, above the inoculated medium and incubate at 35 °C for 18-24 hours. A positive reaction appears as a blackening of the lower part of the strip. In the case of negative response, no blackening should appear (see **Figure 3**).

NEW!

A rapid test based on the rRNA detection. The test takes less than 2.5 hours, is on microtiter plate format and needs no expensive equipment.

Figure 4: HybriScan Kit

Test	Number	Brand	Cat. No.
HybriScan®D *Salmonella*	96 tests	Fluka	55662
HybriScan®I *Salmonella*	48 tests	Fluka	49415

Vibrio Detection

By Jvo Siegrist, Product Manager Microbiology…. *ivo.siegrist@sial.com*

Vibrio cholerae causes cholera in humans, Vibrio parahaemolyticus and Vibrio vulnificus are the leading cause of seafood-associated gastroenteritis.

Vibrios are motile, curved or comma-shaped bacilli and have a single polar flagella with sheet proteins. They are often found in open water, freshwater and saltwater. Vibrios are facultative aerobe and Gram-negative bacterium and do not form spores. The metabolism can be oxidative and fermentative. Most species are oxidase-positive expect *V. metschnikovii*. Some vibrios such as *Vibrio fischeri* exhibit bioluminescence (Quorum sensing) under certain conditions. In most ways vibrios are close to Enterobacteriaceae, but share also some properties with pseudomonads. They can be differentiated from enteric bacteria by oxidase-positive reaction and motility. Differentiation from *Pseudomonas* can be made based on the ability of vibrios to undergo oxidative and fermentative metabolism.

Most vibrios are not fastidious and a simple C-source like glucose serves as an energy source. As it is a typically marine organism, most species require 2-4% NaCl or other salts and trace elements present in sea water for optimal growth. Some species are like *Pseudomonas* and can use diverse energy sources and show great versatility in their metabolism.

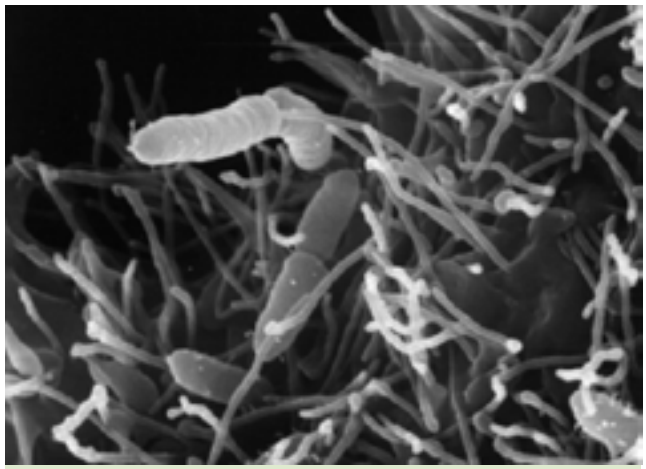
The widely used media for *Vibrio* isolation are TCBS Agar and Alkaline Peptone Water. However, accompanying sucrose-fermenting bacteria may pose a problem in the identification of *Vibrio* species on TCBS Agar[3]. The TCBS Agar contains a mixed indicator of bromothymol blue and thymol. This system reacts upon acid production from sucrose fermentation. On a chromogenic medium like HiCrome Vibrio Agar (see **Table 2**), the color development by *Vibrio* species is not affected by the presence of colonies of other bacteria. This is because, the amount of color developed depends on the reaction of the bacterial β-galactosidase with the substrate contained in the media. The TCBS Agar also contains a sodium thiosulfate and ferric citrate indicator system which detects the production of hydrogen sulphide.

Did you know …

…the origin of the word Vibrio?

The bacterium was given the Latin name *Vibrio* because it possesses a flagellum and appears to **vibrate**.

Pepton from animal origin provides carbonaceous, nitrogeneous and essential nutrients to the *Vibrio* species to promote growth. High concentrations of sodium chloride in the medium are used to get an inhibitory effect on the accompanying microflora. Sodium thiosulphate, sodium citrate and sodium cholate are used as well to inhibit the growth of gram-positive and some gram-negative bacteria, but not members of Enterobacteriaceae. The strongly alkaline pH of the medium is also an important tool to get selectivity for *Vibrio* species.



Kingdom:	**Bacteria**	Order:	**Vibrionales**
Phylum:	**Proteobacteria**	Family:	**Vibrionaceae**
Class:	**Gamma Proteobacteria**	Genus:	***Vibrio***

Figure 1: ***Vibrio vulnificus*** **is the cause of seafood-related mortality. Scientists from Northwest Fisheries Science Center have isolated and characterized a key surface protein involved in the ability of** ***Vibrio vulnificus*** **to attach to shellfish, such as oysters (microscopic image from Northwest Fisheries Science Center, Seattle, USA)**

V. cholerae is a non-invasive bacteria, affecting the small intestine by producing the cholera enterotoxins. The result is a life-threatening watery diarrhea because of activation of the adenylate cyclase in the intestinal cells. This reaction causes water and electrolytes from blood and tissues to be pumped into the intestinal tract. The rapid loss of fluids leads to dehydration, anuria, acidosis and shock. An additional loss of potassium ions may result in cardiac complications and circulatory failure. The mortality rate is very high (50-60%) if the disease is not treated. Infection source is the water or food contaminated with human feces.

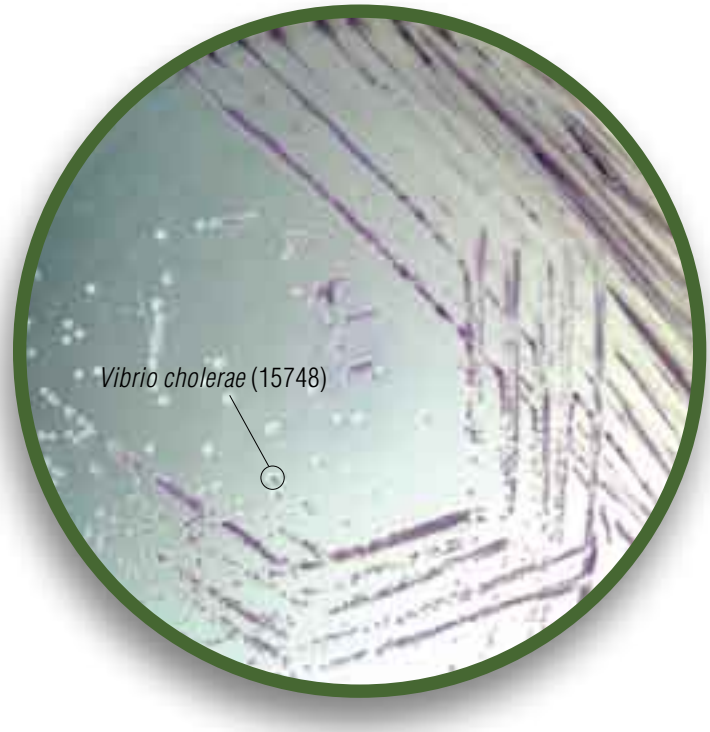


Figure 2: HiCrome Vibrio Agar, a selective Agar with chromogenic system as differential system.

V. parahaemolyticus causes gastroenteritis. It is an invasive organism affecting primarily the colon tissue and excretes a presently unidentified toxin. The origin of an infection leads in most cases back to contaminated raw and improper refrigerated seafood or a fecal contamination of water and food.

V. vulnificus lives in warm seawater and is halophile, meaning they require salt for growth. Contaminated seafood which is eaten raw or is undercooked is in most case the source of infections and cause gastroenteritis, or a syndrome known as "primary septicemia." Also open wounds that are exposed to seawater can lead to a wound infection.

	V. cholerae	V. parahaemolyticus	V. vulnificus
Growth in nutrient broth			
without NaCl	+	-	-
with 1% NaCl	+	+	+
Oxidase	+	+	+
Nitrate reduction	+	+	+
myo-Inositol fermentation	-	-	-
Arginine dihydrolase	-	-	-
Lysine decarboxylase	+	+	+
Ornithine decarboxylase	+	+	V
ONPG	+	+	+

Table 1: Typical biochemical reactions

	Brand	Cat. No.	Name	ISO
Nonselective media	Fluka	43856	Alkaline Peptone Water (ISO)	8914
	Fluka	77185	Peptone Water	
	Fluka	T2117	Thiol broth	
Nonselective media w/differential system	Fluka	62895	Lysine Decarboxylase Salt Broth	
	Fluka	75315	OF Test Nutrient Agar	
	Fluka	22091	Tryptic Soy Agar with supplement: TTC solution	8914
Selective media	Fluka	49281	Glucose Salt Teepol Broth	
Selective media w/differential system	Fluka	17134	CPC-Agar (Base)	
	Fluka	70135	DCLS Agar	
	Fluka	90035	DCLS Agar No. 2	
	Fluka	92323	HiCrome™ Vibrio Agar	
	Fluka	86348	TCBS Agar	8914

Table 2: Media for enrichment, detection and differentiation of Vibrio species

Brand	Cat. No.	Name
Fluka	P9602	Polymyxin B Selective Supplement
Fluka	17779	TTC Solution

Table 3: Supplements for *Vibrio* media

Brand	Cat. No.	Name
Fluka	18502	Oxidase Reagent acc. Gordon-McLeod
Fluka	40560	Oxidase Strips
Fluka	70439	Oxidase Test
Fluka	07345	Oxidase Reagent acc. Gaby-Hadley A
Fluka	07817	Oxidase Reagent acc. Gaby-Hadley B
Fluka	49940	ONPG Disks
Fluka	51138	Nitrate Reagent Disks Kit
Fluka	38497	Nitrate Reagent A
Fluka	39441	Nitrate Reagent B
Fluka	77730	Gram Staining Kit

Table 4: Biochemical products and kits for *Vibrio* identification and differentiation

References:

1. Thompson et. al (ed.), The Biology of Vibrios, ASM Press, chapter1, pg 3 (2006)
2. E.I. Alcamo, Fundamentals of Microbiology, 6th ed, Jones and Bartlett Publishers, Inc. pg 254, 244 (2001)
3. Clesceri, Greenberg and Eaton (ed.), Standard Method for the Examination of Water and Waste Water, 20th ed. American Public Health Association, Washington, D. C. (1998)
4. H.Y. Kudo et. al, Improved Method for Detection of Vibrio parahaemolyticus in Seafood. ASM. Vol 67, No. 12, pg 5819-5823 (2001)
5. ISO 8914:1990, Microbiology -- General Guidance for the Detection of Vibrio parahaemolyticus
6. Color Atlas and Textbook of Diagnostic Microbiology, 5th edition, Lippincott Williams &Wilkins (1997)

Using ISOGRO® Supplementation of M9 Minimal Media to Enhance Recombinant Protein Expression

By Dr. Natosha Finley....*Contact: Lisa Roth, lisa.roth@sial.com*

Stable isotope enrichment of recombinant proteins is necessary for most structure-function studies using NMR spectroscopy. The uniform incorporation of stable isotopes such as 13C, 15N and D into proteins is routinely accomplished by using bacterial expression systems such as Escherichia coli (*E. coli*). Protein expression in *E. coli* typically involves the growth of bacteria in defined media where 13C and 15N containing glucose and ammonium salt are the sole sources of carbon and nitrogen. While bacterial protein production systems offer high cell mass and protein yields in rich media, the level of protein expression and cell mass obtainable in defined media is often lower. To compensate for reduced cell mass and recombinant protein yields, the large-scale production of protein in *E. coli* cells grown in M9 minimal or complex media is required which can be labor-intensive and expensive. Economical and efficacious production of adequate quantities of isotopically enriched protein for NMR experimentation is often the rate-limiting step in structural biology. Therefore, considerable interest exists in the development of protocols for the improvement of growth rates and recombinant protein expression levels in *E. coli*.

One strategy for the cost-effective optimization of protein expression in *E. coli* is the supplementation of M9 media with ISOGRO, an algal lysate-derived complex labeling medium. The inclusion of ISOGRO in M9-based growth cultures provides the cells a metabolic boost that often decreases lag time, facilitates the attainment of growth saturation, and promotes recombinant protein production. The presence of algal cell lysate-derived complex growth media in cultures conserves cellular energy by limiting the requirement for de novo synthesis of cellular machinery and metabolic precursors. Minimizing energy expenditure permits more cellular resources to be directed towards recombinant protein expression. This protocol describes the usage of ISOGRO as a supplement in minimal media to augment *E. coli* cell growth and protein production.

Advantages of Supplementation (Proposed side table)

- Cost-effective protein production in *E. coli* with as little as 10% ISOGRO to M9
- Decrease lag time by as much as 60%
- Maximize OD and recombinant protein expression
- High level of recombinant protein expression with ISOGRO supplementation

Method

Competent *E. coli* BL21(DE3) pLysS cells were freshly transformed with the expression plasmid containing the gene encoding for troponin C amino acid residues 1-89 (cTnC1-89) controlled by the T7 promoter, spread aseptically on LB plates containing carbenicillin (Carb) and chloramphenical (Chl), and grown overnight at 37 °C. A single colony was grown at 37 °C in LB with antibiotic selection until slightly turbid with shaking at 250 rpm. From this starter culture, 1 mL was transferred to 50 mL of LB/Carb/Chl, grown at 37 °C with shaking, and the optical density was monitored at 600 nm (OD_{600}). The cells were harvested at OD_{600} ~ 0.9 by centrifugation and resuspended in 50 mL of sterile filtered minimal media (pH 7.0) containing 7 g/L Na_2HPO_4, 3 g/L KH_2PO_4, 2.5 g/L NaCl, 10.5 g/L K_2HPO_4, 0.5 g NaOH, 1 g/L $^{15}NH_4Cl$, 4 mM $MgSO_4$, 10 µM $FeCl_3$, 125 µM $CaCl_2$, 50 µM $ZnSO_4$, 2 g/L D-Glucose-$^{13}C_6$, 107 µg/L $MgCl_2 \cdot 6H_2O$, 20 µg/L $FeCl_2 \cdot 4H_2O$, 0.16 µg/L $MnCl_2 \cdot 4H_2O$, 16 ng/L $CuCl_2 \cdot 2H_2O$, 2.4 ng/L $Na_2MoO_4 \cdot 2H_2O$, 0.26 µg/L H_3BO_3, 1 mg/L choline chloride, 100 µg/L riboflavin, 50 mg/L niacin, 1 mg/L folic acid, 1 mg/L pyridoxal phosphate, 50 mg/L thiamine, 1 mg/L biotin, 1 mg/L D-pantothenate. The minimal media starter culture was equally divided between 500 mL regular minimal medium and 500 mL of minimal media supplemented with 10% ISOGRO 13C,15N powdered growth medium (0.5 g ISOGRO powder/500 mL medium) and grown at 37 °C with shaking. At an OD_{600} ~ 0.9 the protein expression was induced by addition of isopropyl β-D-1-thiogalactopyranoside (IPTG) to a final concentration of 0.1 mM. Protein production was analyzed by SDS-PAGE.

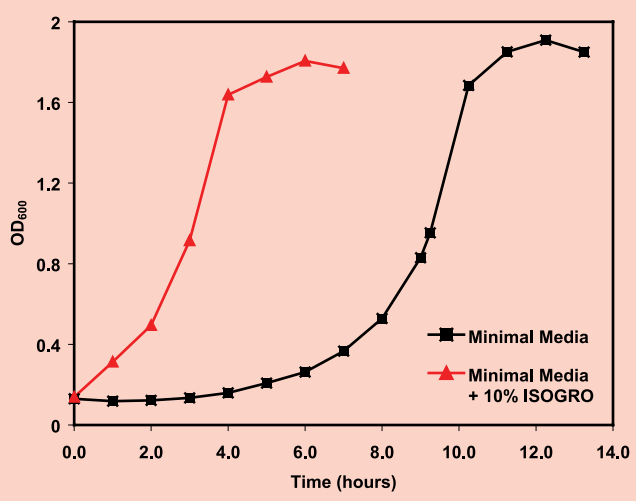


Figure 1: Growth Curve cTnC(1-89) p LysS. OD_{600} versus time (hours). Red curve is ISOGRO supplemented minimal media and the black curve is minimal media alone.

Results

Bacterial Growth Rates

Supplementation of minimal media with 10% ISOGRO substantially reduced the lag time of cells by more than 60% as evidenced by the faster transition of cultures into log phase growth (see Figure 1). Cells grown in ISOGRO supplemented minimal media reached the target induction point of an OD_{600} ~ 0.9 in approximately 3 hours, versus 9 hours in standard M9 media. ISOGRO is composed of an algal lysate containing metabolic precursors, which permits the cultures to initiate growth more rapidly, thus eliminating the need for de novo production of the various components of the metabolic pathways.

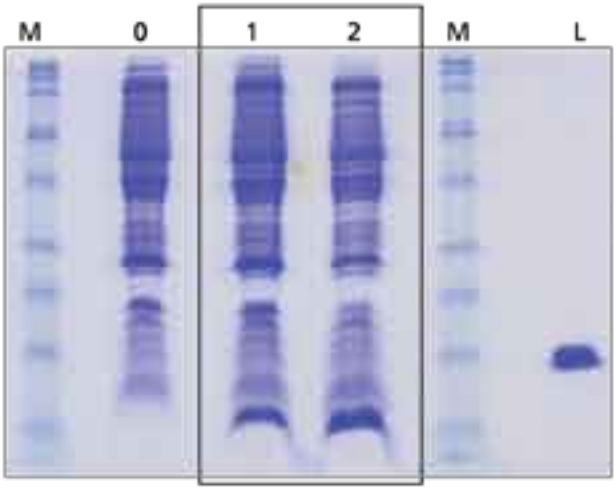


Figure 2: SDS-PAGE cTnC(1-89) cell lysates. M = low molecular weight marker, 0 = uninduced ; 1 = induced minimal media ; 2 = induced minimal media + ISOGRO, L = Lysozyme

Recombinant Protein Expression

The expression levels observed for cTnC(1-89) produced in the ISOGRO enriched minimal media were greater compared to that obtained using unsupplemented minimal media (see Figure 2). In the presence of 10% ISOGRO, there was an increase in the amount of cTnC(1-89) expressed while the contaminating host cellular proteins were produced at a level comparable to that seen in uninduced minimal media and induced minimal media cultures. This observation is important in that subsequent recombinant protein purification is often facilitated when the target protein constitutes a high percentage of the total cellular protein.

Discussion

Modern NMR techniques require the routine production of milligram quantities of uniformly enriched recombinant protein. It is desirable to develop protocols for the economic and time-efficient isotopic enrichment of target proteins. The methodology presented here offers researchers the potential to reduce the labor hours and isotope expense by utilizing ISOGRO supplementation of minimal media for recombinant protein expression. The inclusion of 10% ISOGRO (0.5g/500 mL of minimal media) in minimal medial reduces the lag time of cultures and promotes the enhancement of target protein levels. It should be noted that further optimization of this protocol is possible by altering variables such as IPTG concentration, induction optical density, and percentage of ISOGRO supplementation.

For more information on ISOGRO or other stable isotope labeled products, please visit sigma-aldrich.com/bionmr or contact Stable Isotopes Technical Service at *isosales@sial.com*

Acknowledgements: Data generously provided by Dr. Paul R. Rosevear. The Department of Molecular Genetics, Biochemistry and Microbiology, University of Cincinnati Medical Center, Cincinnati, OH.

Cat. No.	Description	Isotopic Purity
606863	ISOGRO-13C Powder Growth Medium	99 atom% 13C
616729	ISOGRO-D Powder Growth Medium	97 atom% D
606871	ISOGRO-15N Powder Growth Medium	98 atom% 15N
606839	ISOGRO-13C,15N Powder Growth Medium	99 atom% 13C, 98 atom% 15N
608300	ISOGRO-15N,D Powder Growth Medium	98 atom% 15N, 97 atom% D
608297	ISOGRO-13C,15N,D Powder Growth Medium	99 atom% 13C, 98 atom% 15N, 97 atom% D
299251	Ammonium-15N chloride	98 atom% 15N
299286	Ammonium-15N2 sulfate	98 atom% 15N
617385	Deuterium oxide	99.8 atom% D
552003	D-Glucose-1,2,3,4,5,6,6-d7	97 atom% D
389374	D-Glucose-13C6	99 atom% 13C
552151	D-Glucose-13C6,1,2,3,4,5,6,6-d7	99 atom% 13C, 97 atom% D

Table 1: Products of Interest

Argentina
SIGMA-ALDRICH DE ARGENTINA S.A.
Free Tel: 0810 888 7446
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Fax: (+54) 11 4552 1698

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SIGMA-ALDRICH PTY LTD.
Free Tel: 1800 800 097
Free Fax: 1800 800 096
Tel: (+61) 2 9841 0555
Fax: (+61) 2 9841 0500

Austria
SIGMA-ALDRICH HANDELS GmbH
Tel: (+43) 1 605 81 10
Fax: (+43) 1 605 81 20

Belgium
SIGMA-ALDRICH NV/S.A.
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Free Fax: 0800 14745
Tel: (+32) 3 899 13 01
Fax: (+32) 3 899 13 11

Brazil
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Fax: (+55) 11 5522 9895

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Free Fax: 1800 265 3858
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Fax: (+30) 210 994 3831

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New Delhi: (+91) 11 4358 8000
Mumbai: (+91) 22 2570 2364
Hyderabad: (+91) 40 4015 5488
Kolkata: (+91) 33 4013 8003
Fax
Bangalore: (+91) 80 6621 9650
New Delhi: (+91) 11 4358 8001
Mumbai: (+91) 22 2579 7589
Hyderabad: (+91) 40 4015 5466
Kolkata: (+91) 33 4013 8016

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Free Fax: 1800 600 222
Tel: (+353) 402 20370
Fax: (+ 353) 402 20375

Israel
SIGMA-ALDRICH ISRAEL LTD.
Free Tel: 1 800 70 2222
Tel: (+972) 8 948 4100
Fax: (+972) 8 948 4200

Italy
SIGMA-ALDRICH S.r.l.
Numero Verde: 800 827018
Tel: (+39) 02 3341 7310
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