Protein Purification and Characterization Examples

Bio-Technical Resources (BTR)

www.biotechresources.com

1035 S 7th Street, Manitowoc, WI 54220

Phone: (920) 684-5518

Fax: (920) 684-5519

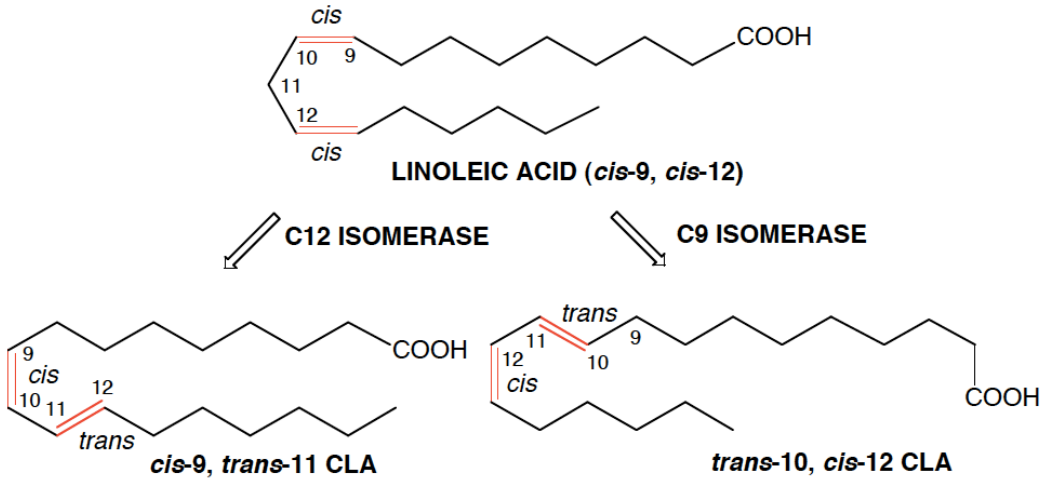
Email: info@biotechresources.com

Protein Purification and Characterization

- BTR scientists have purified soluble and membrane proteins from native and recombinant hosts. These proteins are enzymes for bioconversion or for food, nutraceutical and pharmaceutical applications. Highlighted below are purification of linoleic acid isomerases that catalyze the formation of single isomers of conjugated linoleic acid (CLA), intended for health and nutraceutical applications.

- BTR has the capacity and experiences in preparation of gram level purified protein for clients, involving fermentation, harvesting, cell lysis or removal, ultrafiltraion, and purification to required purity.

Example: Purification and Characterization of Linoleic Acid Isomerases from Different Bacteria



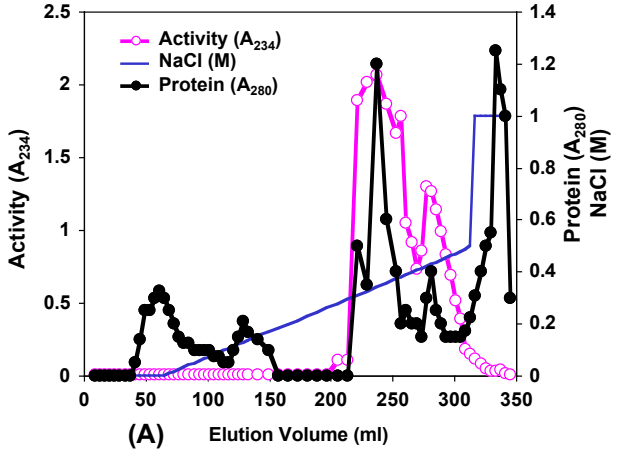
- Enzymatic conversion of linoleic acid to single isomers of conjugated linoleic acid
- The soluble C9 isomerase was purifed from *P. acnes*.
- The membrane bound C9 isomerase was purified from *L. reuteri* and *C. sporogenes*

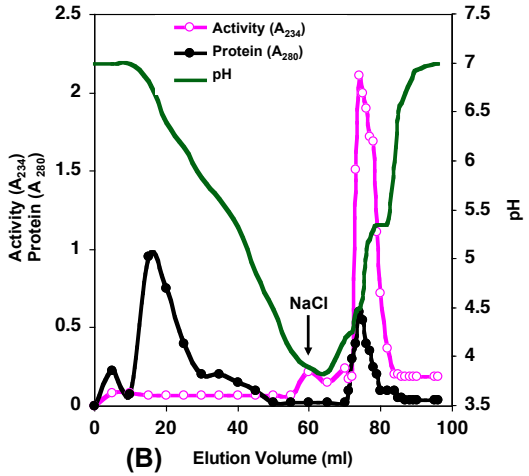
Organism	CLA isomer	Optimal pH	Km (µM)	Vmax (nmol/min/mg)	Enzyme solubility	Substrate inhibition
Propionibacterium acnes ATCC6919	t10,c12	7.3	17.2	478	Soluble	No
Lactobacillus reuteri LA8	c9,t11	7.5	8.1	880	Membrane bound	Yes
Clostridium sporogenes ATCC25762	c9,t11	7.5	11.9	548	Membrane bound	Yes

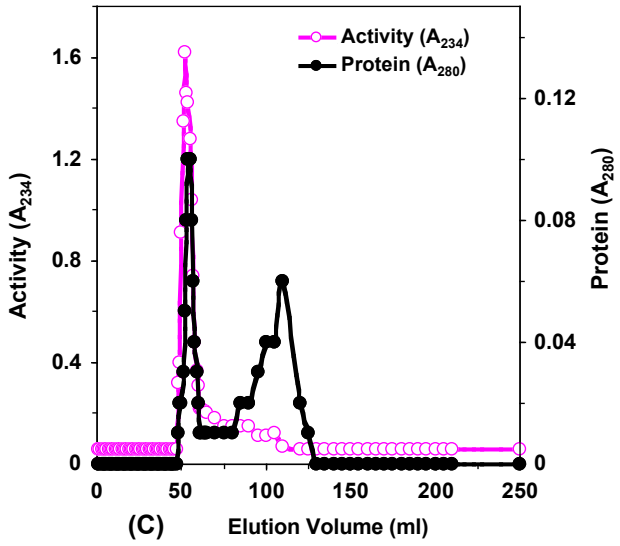
Purification of a Membrane-Bound Linoleic Acid Isomerase from *Clostridium sporogenes*

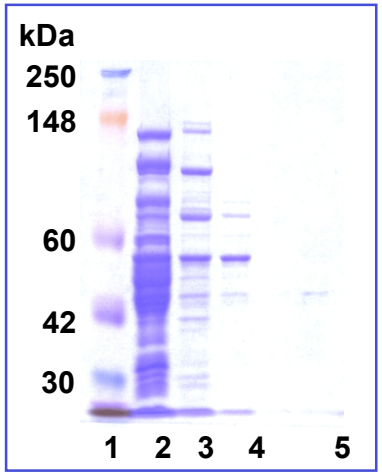
- *C. sporogenes* ATCC 25762 is capable of isomerizing linoleic acid to *cis*9, *trans*11 conjugated linoleic acid (CLA, 18:2)
- The isomerase is membrane-associated and was very unstable, especially after being solubilized by detergents
- Isomerase extraction, solubilization and stability were greatly improved by optimizing buffer compositions and pH, and by minimizing detergent and protein precipitation
- The isomerase was purified by DEAE, chromatofocusing and size exclusion column chromatography, achieving an a 350-fold purification and a specific activity of 400 nmol min^{-1} mg^{-1} protein

Purification of the Linoleic Acid Isomerase from *C. sporogenes*









(D)

A. DEAE
B. Chromatofocusing
C. Size exclusion
D. SDS-PAGE

Summary of Purification of *C. sporoges* Isomerase

Step	Protein (mg)	Total activity (nmol/min)	Specific activity (nmol/min/mg)	Yield
Crude extract	570	653	1.1	100.0
OG extract	157	470	3.0	72.0
DEAE-5PW	12	132	11.0	20.2
Chromatofocusing	0.677	64	94.6	9.8
Gel-Filtration	0.030	10.5	350.0	1.6

Contract Research Service at Bio-Technical Resources

Bio-Technical Resources (BTR) has the capacity and proven experiences in protein science, including protein expression, directed evolution, protein purification and enzyme kinetics. Please contact us for further discussion for your needs and we look forward to working with you.

References

Deng MD et al, Linoleic acid isomerase from *Propionibacterium acnes*: purification, characterization, molecular coning, and heterologous expression. Applied Biochem and Biotechnol 2007**, 143**:199-211

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