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Protein expression in *Bacillus subtilis*

In 1 collection

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Protocol status: Working
We use this protocol routinely and it works for us

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Abstract

B. subtilis is a gram-positive bacteria used by both academia and industry as a protein production workhorse. This is due to its' their excellent fermentation properties, high production titers, and capacity to secrete proteins into the extracellular medium.

This protocol describes how to express proteins in *B. subtilis.* The protocol is developed using KO7-S, although it might also work for other strains as well. The method is adapted from Rasmussen, M. D.; Bjoernvad, M. E.; Diers, I. Pectate Lyase Fusion for Expression and Secretion of Polypeptides. WO 00/75344, 2000 and Jensen, K.; Østergaard, P. R.; Wilting, R.; Lassen, S. F. Identification and Characterization of a Bacterial Glutamic Peptidase. *BMC Biochem.***2010**, *11* (1), 47. https://doi.org/10.1186/1471-2091-11-47.

Materials

MATERIALS

- Sodium molybdate dihydrate
- manganese sulfate
- Iron(III) chloride hexahydrate **Catalog #**236489
- Zinc sulfate heptahydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**204986
- Magnesium sulfate heptahydrate
- Sodium Phosphate dibasic **Fisher Scientific Catalog #**S373-500
- Copper (II) sulfate pentahydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**209198
- Yeast extract
- Nalgene™ Rapid-Flow™ Sterile Disposable Bottle Top Filters with PES Membrane,150mL, 0.45μm pore, 45mm neck **Thermo Fisher Catalog #**296-4545
- Maltodextrin (DE 13.0-17.0) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**419680
- Pluronic L-61 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**435422

Safety warnings

Be sure to wear protective equipment when adjusting the pH of the media. Follow local safety regulations

Before start

Make sure you have your expression strain freshly streaked on an agar plate.

Cal18-2 media preparations

1 Prepare a stock solution of 2.0g/L Na_2MoO_4. Sterilize by filtration

2 Prepare a trace metal solution consisting of
- 4.48g/L $MnSO_4 \cdot H_2O$
- 3.33g/L $FeCl_3 \cdot 6H_2O$
- 0.625g/L $CuSO_4 \cdot 5H_2O$
- 7.12g/L $ZnSO_4 \cdot 7H_2O$

Sterilize by filtration

3 Fill a blue cap bottle to ~80% of the desired final volume with MQ water.

4 Add a magnetic stirrer to the blue cap bottle and place the bottle on a stirring plate. Turn on the stirring, and make sure it's mixing well.

5 Add the following to the bluecap bottle:
- 40g/L yeast extract
- 1.3 g/L $MgSO_4 \cdot 7H_2O$
- 50 g/L maltodextrin (DE ~ 12)
- 20 g/L $NaH_2PO_4 \cdot 2H_2O$
- 6.7mL/L 2.0g/L Na_2MoO_4 stock solution
- 6.7mL/L Trace metal solution
- 100µL/L Pluronic L-61

6 Make sure that all of the ingredients are dissolved

7 Adjust to pH 6 with 5M NaOH

8 Add MQ water to the desired final volume

9 Sterilize by filtration

Note

The media easily clogs filters, so choose a 0.45µM vacuum bottle top filter for this step and be prepared to use a few filters per liter

10 Store the media at 4 °C until needed

Overnight culture - Day 1

11 Inoculate between 3 mL to 50 mL LB media with a single colony of the expression strain. Depending on the expression volume and overnight OD. The culture can be grown in in a 24-deepwell plate, a falcon tube or a shake flask

12 Grow the strain at 37 °C Overnight

Note

Make sure to not incubate the overnight culture for longer than 16:00:00. Using an overnight culture that has been incubating for longer than this, often results in non-reproducible results

Expression - Day 2 - 4/5

13 Prepare the desired volume of expression media in the desired vessel

Note

Vessel and media volume has a massive influence on the final protein titers. In our experience, 24-deepwell plates in an incubator with a large shaking amplitude and CSTRs work well, while shake flasks give low protein yields. This is likely highly dependant on the equipment (e.g. incubators and the shape and baffle pattern of the shake flasks), and thus should be optimized for the individual labs

14 Inoculate the expression media to an OD_{600} of 0.1

15 Incubate the expression culture at 20 °C with 250 RPM shaking between 48:00:00 and 72:00:00

Note

The expression temperature and duration is dependant on the target protein, although the specified values seem to be a good starting point in our experience

Harvesting - Day 4/5

16 Harvest the culture by centrifuging at 6000 x g, 4°C, 00:05:00

Note

Depending on what the samples are for, the supernatant from the first centrifugation can additionally be centrifuged at 16000 x g, 4°C, 00:30:00 to clear out cell debris and other smaller contaminants

17 Keep the sample On ice when working with it and at -20 °C for storage