

1 **Influence of nutrient availability on *in vitro* growth of major bovine mastitis pathogens**

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**Supplementary File**

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18 **Material & Methods**

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20 *Preparation of Bacterial Suspensions*

21 Bacteria were stored at -20°C on bacterial preservation beads until use (Microbank™ – Mixed,  
22 cat. no. PL.170C/B, Pro-Lab Diagnostics, Richmond Hill, Canada). With an inoculation loop  
23 (cat. no. 251586, Thermo Fisher Scientific, Waltham, MA, USA) bacteria were streaked onto  
24 Brain Heart Infusion (BHI) Agar plates (Brain Heart Infusion Agar, cat. no. 70138, Sigma-  
25 Aldrich, St. Louis, MO, USA; prepared according to the manufacturer's protocol; Petri Dish 92  
26 X 16mm, cat. no 82.1473, Sarstedt AG & Co. KG, Nuembrecht, Germany). Plates were cultured  
27 aerobically for 16-20 h at 37°C. Then, a single colony from the plate was used to inoculate a  
28 sterile tube (Fisherbrand Disposable Centrifuge Tubes, cat. no. 05-539-4, Thermo Fisher  
29 Scientific, Waltham, MA, USA) containing 10mL of TB (Thioglycollate Broth U.S.P.  
30 Alternative, cat. no. CM0391, Oxoid Ltd, Basingstoke, England; prepared according to the  
31 manufacturer's protocol). The bacterial suspension was cultured aerobically during 16-20 h at  
32 37°C under gentle shaking (180rpm).

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**Supplementary Table S1.** Compositions (g/L) of the different types of cow milk.

<b>Medium</b>	<b>Fat</b>	<b>Protein</b>	<b>Carbohydrates</b>
Skim milk (Léger Milk UHT, Migros, Zurich, Switzerland)	1	35	50
Fat-reduced milk (1.5%; Valflora Drink 1.5% UHT, Migros, Zurich, Switzerland)	16	35	50
Fat-reduced milk (2.5%; Valflora Drink 2.5% UHT, Migros, Zurich, Switzerland)	27	35	50
Whole milk (Valflora Milch 3.5% UHT, Migros, Zurich, Switzerland)	37	35	50
Cream <sup>1</sup> (Valflora Halbrahm UHT, Migros, Zurich, Switzerland)	25	25	40
High protein milk <sup>2</sup> (Oh! High Protein Milk UHT, Migros, Zurich, Switzerland)	1	70	45
Lactose-free milk (Vollmilch laktosefrei aha!, Migros, Zurich, Switzerland)	36	35	50

<sup>1</sup>added by thickening agent (E 407)

<sup>2</sup>skimmed and lactose-free

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**Supplementary Table S2.** Nutrients added to TB and whole milk.

<b>Nutrient</b>	<b>Name</b>	<b>Source</b>
Lactose	Lactose-Monohydrate, cat. no. G7656	Dr. Grogg Chemie AG, Stettlen-Deisswil, Switzerland
Glucose	D-(+)-Glucose, cat. no. G8270	Sigma-Aldrich, St. Louis, MO, USA
Urea	Urea, cat. no. 51456	Sigma-Aldrich, St. Louis, MO, USA
Ammonium Sulphate	Ammonium sulphate, cat. no. 101217	Merck KGaA, Darmstadt, Germany
L-glutamine	L-glutamine, cat. no. G-5763	Sigma-Aldrich, St. Louis, MO, USA
Amino acid mixture [used in trials with milk]	MEM Non-essential Amino Acid Solution (100x), cat. no. M7145	Sigma-Aldrich, St. Louis, MO, USA
Amino acid mixture [used in trials with thioglycolate broth]	Amino acid standard, cat. no. AAS18	Sigma-Aldrich, St. Louis, MO, USA
Trace elements	Murashige and Skoog Basal Salt Macronutrient Solution, cat. no. M0654	, Sigma-Aldrich, St. Louis, MO, USA
Bulk elements	Murashige and Skoog Basal Micronutrient Solution, cat. no. M0529	Sigma-Aldrich, St. Louis, MO, USA
Vitamin mixture	Vitamins Kit, cat. no. V1	Sigma-Aldrich, St. Louis, MO, USA

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**Supplementary Table S3.** Compositions of the two amino acid solutions.

<b>Components (mg/L)</b>	<b>MEM Amino Acid Solution</b>	<b>Amino Acid Standard Solution</b>
L-Alanine	890	223
Ammonium Chloride		134
L-Arginine		436
L-Asparagine x H <sub>2</sub> O	1500	
L-Aspartic Acid	1330	333
L-Cystine		300
L-Glutamic Acid	1470	368
Glycine	750	188
L-Histidine		388
L-Isoleucine		328
L-Leucine		328
L-Lysine		366
L-Methionine		373
L-Phenylalanine		413
L-Proline	1150	288
L-Serine	1050	263
L-Threonine		298
L-Tyrosine		453
L-Valine		293

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**Supplementary Table S4.** Compositions of the bulk and trace element solutions.

<b>Components (mg/L)</b>	<b>Bulk Elements Solution</b>	<b>Trace Elements Solution</b>
Ammonium nitrate	1650.0	
Boric acid		6.2
Calcium chloride anhydrous	332.2	
Cobalt chloride x 6H <sub>2</sub> O		0.025
Cupric sulfate x 5H <sub>2</sub> O		0.025
Na <sub>2</sub> -EDTA		37.3
Ferrous sulfate x 7H <sub>2</sub> O		27.8
Magnesium sulfate	180.7	
Manganese sulfate x H <sub>2</sub> O		16.9
Molybdic acid (sodium salt) x 2H <sub>2</sub> O		0.25
Potassium iodide		0.83
Potassium nitrite	1900.0	
Potassium phosphate monobasic	170.0	
Zinc sulfate x 7H <sub>2</sub> O		8.6

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**Supplementary Table S5.** Composition of the vitamin b mixture

<b>Components</b>
P-aminobenzoic acid
D-biotin
Folic acid
Niacinamide
D-pantothenic acid
Pyridoxal hydrochloride
Pyridoxamine dihydrochloride
Pyridoxine hydrochloride
Riboflavin
Thiamine hydrochloride
DL-6,8-thioctic acid

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