

# **Impact of spontaneous fermentation and inoculum with natural whey starter on peptidomic profile and biological activities of cheese whey: a comparative study**

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## **Supplementary Materials**

Supplementary Table S1. Phenotypic and molecular characterization of LAB isolates from NWS used as inoculum in this study.

Strains	Isolation	Cell morphology	Gram staining	Catalase reaction	16S-ARDRA with <i>Hha</i> I <sup>1</sup>	Species attribution
DSM20072 <sup>T</sup>	DSMZ <sup>2</sup>	rod	+	+	880-210-150-130-100	<i>L. delbrueckii</i> subsp. <i>lactis</i>
RO4F06	MRS pH 6.5, 42°C, anaerobiosis	rod	+	+	880-210-150-130-100	<i>L. delbrueckii</i> subsp. <i>lactis</i>
RO4F16	MRS pH 6.5, 42°C, anaerobiosis	rod	+	+	880-210-150-130-100	<i>L. delbrueckii</i> subsp. <i>lactis</i>
DSM20617 <sup>T</sup>	DSMZ	cocci	+	+	600-220-190-180-130-80	<i>S. thermophilus</i>
RO4F01	M17-SSW, 42°, aerobiosis	cocci	+	+	600-220-190-180-130-80	<i>S. thermophilus</i>
RO4F03	M17-SSW, 42°, aerobiosis	cocci	+	+	600-220-190-180-130-80	<i>S. thermophilus</i>
RO4F04	M17-SSW, 42°, aerobiosis	cocci	+	+	600-220-190-180-130-80	<i>S. thermophilus</i>
RO4F05	M17-SSW, 42°, aerobiosis	cocci	+	+	600-220-190-180-130-80	<i>S. thermophilus</i>
RO4F09	M17-SSW, 42°, aerobiosis	cocci	+	+	600-220-190-180-130-80	<i>S. thermophilus</i>
RO4F12	M17-SSW, 42°, aerobiosis	cocci	+	+	600-220-190-180-130-80	<i>S. thermophilus</i>
RO4F13	M17-SSW, 42°, aerobiosis	cocci	+	+	600-220-190-180-130-80	<i>S. thermophilus</i>
RO4F15	M17-SSW, 42°, aerobiosis	cocci	+	+	600-220-190-180-130-80	<i>S. thermophilus</i>
RO4F08	M17-SSW, 42°, aerobiosis	cocci	+	+	600-220-190-180-130-80	<i>S. thermophilus</i>
DSM 20075 <sup>T</sup>	DSMZ	rod	+	+	640-400-260-140-90	<i>L. helveticus</i>
RO4F02	MRS pH 6.5, 42°C, anaerobiosis	rod	+	+	640-400-260-140-90	<i>L. helveticus</i>
RO4F07	MRS pH 6.5, 42°C, anaerobiosis	rod	+	+	640-400-260-140-90	<i>L. helveticus</i>

	MRS pH 6.5, 42°C, anaerobiosis	rod					
RO4F10			+	+	640-400-260-140-90		<i>L. helveticus</i>
RO4F14	MRS pH 6.5, 42°C, anaerobiosis	rod	+	+	640-400-260-140-90		<i>L. helveticus</i>
RO4F11	MRS pH 6.5, 42°C, anaerobiosis	rod	+	+	640-400-260-140-90		<i>L. helveticus</i>

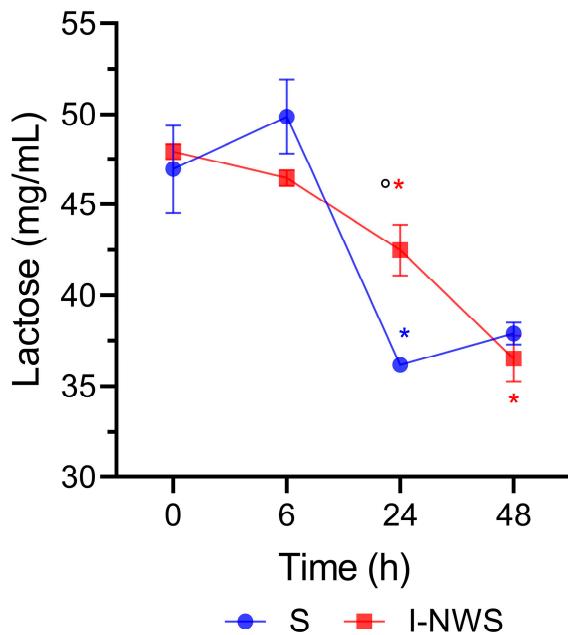
<sup>1</sup> Fragment size is in bp. <sup>2</sup> DSMZ, Deutsche Sammlung von Mikroorganismen und Zellkulturen.

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**Supplementary Table S2.** Phenotypic and molecular characterization of yeast isolates from NWS used as inoculum in this study.

Strains	Isolation	Cell morphology	PCR amplicon	RFLP with <i>Hae</i> III <sup>1</sup>	Species attribution (Yeast-ID score)
RO6107	YPDA, 27°C	Cellipsoidal	880	325-230-170-125	<i>S. cerevisiae</i> (100%)
RO6108	YPDA, 27°C	Cellipsoidal	880	325-230-170-125	<i>S. cerevisiae</i> (100%)
RO7107	YPDA, 27°C	Cellipsoidal	740	655-80	<i>K. marxianus</i> (100%)
RO7108	YPDA, 27°C	Cellipsoidal	740	655-80	<i>K. marxianus</i> (100%)
RO6109	YPLA, 42°C	ellipsoidal	740	655-80	<i>K. marxianus</i> (100%)
RO6110	YPLA, 42°C	ellipsoidal	740	655-80	<i>K. marxianus</i> (100%)
RO7109	YPLA, 42°C	ellipsoidal	740	655-80	<i>K. marxianus</i> (100%)
RO7110	YPLA, 42°C	ellipsoidal	740	655-80	<i>K. marxianus</i> (100%)

<sup>1</sup> Fragment size is in bp.



**Supplementary Figure S1.** Trends of lactose concentration (mg/mL) during S (blue) and I-NWS (red) whey fermentation. Values are expressed as means of at least three replicates. Asterisks (blue for S and red for I-NWS) indicate significant differences over time in the same fermentation batch, while dots significant differences among samples at the same fermentation time ( $p < 0.05$ ).