

Growth heterogeneity of *Streptococcus suis* in host-mimicking conditions is independent of clinical origin

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Abstract

Background

Streptococcus suis is an important swine and zoonotic pathogen whose virulence relies on complex, niche-specific metabolic adaptation within the host. However, the role of non-virulent *S. suis* strains in the host remains poorly understood. In this study, using host-mimicking media and biological fluids, we compared the growth of *S. suis* strains from different anatomical origins, including clinical isolates from diseased piglets and upper respiratory tract and vaginal isolates from healthy animals.

Results

Strains were evaluated in Todd-Hewitt Broth supplemented with yeast extract (THY), Human Plasma-Like Medium (HPLM), Synthetic Nasal Medium (SNM), and SNM supplemented with mucin (SNM + M). Most isolates exhibited robust growth in THY, whereas proliferation was reduced in HPLM and SNM. Notably, five isolates (two systemic, one nasal and two vaginal) displayed comparatively enhanced growth in SNM compared to the rest of the strains, although this phenotype was not associated with the anatomical origin of the strains. Mucin supplementation consistently increased growth across all tested isolates, suggesting that mucin availability may support *S. suis* proliferation under nutrient-limiting conditions. Experiments using biological fluids revealed that while all strains grew consistently in saliva, vaginal isolates showed comparatively reduced growth in vaginal fluid, indicating limited adaptation to this environment. No evidence of growth inhibition of virulent strains was observed during co-cultivation, while interactions were predominantly positive and influenced by nutrient availability.

Conclusion

In summary, *S. suis* displays strain-specific variability in growth under different in vivo-like conditions independently of clinical origin. These findings suggest a role of metabolic adaptation in shaping bacterial fitness and highlight the importance of using host-mimicking environments to uncover phenotypic differences and interactions masked under standard laboratory conditions.

Background

Streptococcus suis is a Gram-positive facultative-anaerobic bacterium that naturally colonizes the upper respiratory, genital and gastrointestinal tracts of pigs [1]. Although often a commensal organism, *S. suis* acts as a pathogen and certain strains are capable of breaching host barriers and causing systemic disease [2]. *S. suis* is also an important zoonotic pathogen, as human infections linked to contact with pigs or pork meat can result in meningitis, septicemia, arthritis, or a streptococcal toxic shock-like syndrome [3]. In addition to its public health impact, this microorganism is responsible for significant economic losses in pig production and is one of the main reasons for antimicrobial treatments on farms [4]. The restriction of prophylactic and metaphylactic antimicrobial use has complicated disease control, particularly because no broadly protective vaccines are currently available. Current guidelines emphasize

the need for integrated approaches that address pathogenesis, epidemiology, antimicrobial resistance, diagnostics, and prevention strategies, including the use of inactivated autogenous vaccines [4].

Virulence in *S. suis* is a multifactorial and context-dependent process, and the presence of some putative reported virulence factors does not necessarily confer pathogenic potential [5]. Recent evidence suggests that bacterial metabolism plays a central role in the adaptation of pathogens to the host, directly influencing virulence and the immune response during infection [6]. In line with this concept, metabolic adaptation is a key component of the pathogenesis of *S. suis*. Throughout infection, this pathogen encounters host niches that differ markedly in nutrient availability, immune pressure, and physicochemical conditions, requiring continuous metabolic adjustment to sustain survival and dissemination [7]. In this context, metabolic regulators and pathways involved in carbohydrate and amino acid utilization as well as stress responses, have been described as closely linked to bacterial fitness and the expression of virulence-associated traits [7]. In agreement with the central role of metabolic adaptation proposed by [7], *in vivo* transcriptomic analysis showed that *S. suis* extensively reprograms its gene expression during infection in a niche-specific manner [8]. In this study [8] revealed that the bacterium displays distinct transcriptional profiles in blood and infected organs, with major changes in genes involved in metabolism, nutrient acquisition, stress responses, and virulence, supporting the idea that successful host colonization and dissemination depend on the ability of *S. suis* to adjust its physiology to different host environments. Further supporting this connection, carbohydrate availability has been shown to directly regulate virulence gene expression, suggesting that metabolic cues such as carbon source availability coordinate niche adaptation with pathogenicity [9].

On the other hand, *S. suis* comprises genetically and phenotypically heterogeneous populations that differ in virulence and invasive capacity [10]. This variability has been further supported by comparative genomics showing that disease-associated isolates differ from carriage isolates in their genomic composition [11–13]. However, most phenotypic studies have been performed using only virulent and, in many cases, reference strains. Consequently, the role of non-virulent *S. suis* strains in the host remains incompletely understood, and their putative role in protection deserves further analysis.

Due to the considerable genetic and virulence diversity of *S. suis* strains, and given the role of metabolism in pathogenesis, differences in survival and host adaptation among strains with distinct pathogenicity may underline their competitive fitness across host niches and highlight physiological advantages during systemic infection. Therefore, this study compares the growth dynamics of *S. suis* strains from different clinical origins (including systemic strains and commensal isolates from the upper respiratory tract and vagina) under controlled conditions that mimic biologically relevant metabolic environments.

Material and Methods

Bacterial strains

A total of 39 *Streptococcus suis* strains isolated from pig systemic lesions (n = 12), lower respiratory tract (n = 2), nasal cavity (n = 13), tonsil (n = 1) and vagina (n = 11) from our *S. suis* collection were included in this study (Table 1). Strains included 4 isolates from wild boar origin, WB2-3, WB4-2, SS15144-T1 and SS15055-N2. In addition, reference strains P1/7, kindly provided by Dr. Marcelo Gottschalk from the Université de Montreal (Canada) [11] and T15, kindly provided by Dr. Norbert Stockhofe from Wageningen Bioveterinary Research (The Netherlands) [14] were used as controls.

Table 1
Streptococcus suis strain used in this study.

Strain ID	Isolation site
P1/7	Meninges
895-LCR	Cerebrospinal fluid
N218/17	Meninges
18/17	Meninges
32/17	Joint
ID116-A	Abdominal cavity
ID1-2	Bronchoalveolar lavage fluid
BR1-1LJ	Joint
506-1	Joint
AD 2-LP	Pericardial fluid
47/03	Meninges
171/05	Meninges
234/04	Meninges
N233-16	Brain
RC4-1L	Lung
T15	Palatine tonsil
AR-1	Nasal cavity
CR-1	Nasal cavity
CR-8	Nasal cavity
CR-9	Nasal cavity
GM2-2	Nasal cavity
GM8-2	Nasal cavity
VL1-2	Nasal cavity
VL3-1	Nasal cavity
VL5-4	Nasal cavity
VL8-7	Nasal cavity
WB2-3	Nasal cavity

Strain ID	Isolation site
WB4-2	Nasal cavity
SS15144-T1	Palatine tonsil
SS15055-N2	Nasal cavity
R 1V-4S	Vagina
R 3V-3S	Vagina
R 5V-3S	Vagina
A 2V-2S	Vagina
A 3V-1S	Vagina
A 4V-2S	Vagina
CP 9V-1S	Vagina
CP 11V-2S	Vagina
C 2V-2S	Vagina
C 3V-1S	Vagina
C 4V-3S	Vagina

Staphylococcus aureus EJ41-2 [15] and *Escherichia coli* NCM3722 [16] were also included as controls in some experiments.

Synthetic culture media

Different culture media were used to characterize the growth of *Streptococcus suis* strains. To capture some of the diversity of host-associated environments encountered by *S. suis*, two complementary host-mimicking media were employed in this study. Human Plasma-Like Medium (HPLM) was selected as a physiologically relevant in vitro environment because its metabolite and salt composition closely resembles human plasma [17, 18]. Human Plasma-Like Medium (HPLM; Thermo Fisher Scientific, USA) contains over 60 polar metabolites, including amino acids, nucleotides, sugars, and small organic acids. The second host-mimicking medium was a synthetic nasal medium (SNM), which mimics the nutrient composition of human nasal secretions, based on reported average concentrations. This chemically defined medium, adapted from [19] and originally designed to simulate *Staphylococcus aureus* growth in the nasal environment, was used throughout the study [19]. This medium was prepared using M9 base salts (Supplementary Table 1) supplemented with the components detailed in Supplementary Table 2. In addition, SNM supplemented with mucin (SNM + M) was used to better mimic glycan-rich mucosal environments. The mucin stock solution was prepared following a previously described protocol [20].

SNM + M consisted of 3V SNM and 1V of 2% porcine gastric mucin (Sigma-Aldrich, Merck, Germany), resulting in a final mucin concentration of 0.5%. This concentration represents a non-limiting condition commonly used to assess bacterial metabolic adaptation to mucosal carbohydrates [20]. SNM + M was used as a reference condition to evaluate the impact of mucin on *S. suis* growth under nasal-like conditions. Finally, the rich medium Todd–Hewitt Broth (Sigma-Aldrich) supplemented with 2% (w/v) yeast extract (THY) was used as control [21].

Porcine biological fluids used as culture media

Porcine saliva and vaginal fluid were used as culture media for the growth of *S. suis* strains. The sampled pigs were housed on a commercial farm under standard husbandry conditions, and the owner provided consent for sampling as part of this study. Saliva samples were collected from healthy pigs using salivettes (Sarstedt, Spain) and pooled before centrifugation at 3220 x g for 30 min at 4 °C. Supernatants were filtered through 0.45 µm pore-size filters (Sigma-Aldrich) before storage at -20 °C. Vaginal fluids were collected from sows that had recently given birth using salivettes. Pooled samples were centrifuged at 3,220 x g for 30 min at 4 °C. Due to the high viscosity of the resulting supernatant, samples were sequentially filtered using a 70 µm pore-size filter (Sigma-Aldrich) prior to storage at -20 °C. Sterility of saliva and vaginal fluid preparations was confirmed by incubating aliquots under standard culture conditions to verify the absence of bacterial contamination.

Bacterial growth

For all cultivation experiments, strains were first plated on chocolate agar from -80 °C stocks and incubated overnight at 37 °C in a 5% CO₂ enriched atmosphere. To characterize bacterial growth across diverse metabolic environments, cell suspensions were freshly prepared from agar plates and adjusted to an OD₆₀₀ of 0.5 in sterile PBS. These suspensions were then diluted 1:20 in 100 µL of different growth media in a 96-well plate format (Thermo Fisher Scientific). Subsequently, 100 µL of mineral oil (M3516, Sigma-Aldrich) was added to each well to prevent evaporation while maintaining adequate aeration. Bacterial growth (OD₆₀₀) was monitored every 30 min for 24 h using an automated plate reader (Tecan, Switzerland) set to 37 °C with a non-shake protocol, except for a 10 s linear shaking step (2 mm amplitude) immediately before each measurement in line with previously described cultivation conditions for *S. suis* [22]. Raw OD₆₀₀ values for each well were blanked. Growth curves obtained in the 96-well format were plotted using GraphPad Prism Vs 8.3 (La Jolla, USA). For each growth curve, the area under the curve (AUC) was calculated and the maximum growth was determined.

After the incubation period, the culture from each well was carefully collected, avoiding contact with the mineral oil. Three technical replicates for each condition were pooled into a 1.5 mL microcentrifuge tube and centrifuged at 16,000 x g for 5 min. The resulting supernatant was transferred to a clean tube and stored at -20 °C for subsequent metabolite quantification analyses.

Metabolite quantification

Supernatants obtained after each growth experiment in the different media were subjected to D-glucose, L-lactate, acetic acid, and pyruvic acid quantification using the corresponding enzymatic assay kit (KGLUC, KDLATE, KACETRM, and KPYRUV Megazyme, Ireland), following manufacturer's instructions. Results were plotted using GraphPad Prism. In addition, relative metabolite abundances in the supernatants (normalized to fresh medium) of HPLM and SNM for 6 strains were determined by gas chromatography-mass spectrometry (GC-MS) as described previously [23]. Briefly, samples were extracted and derivatized following established protocols [23] and analyzed using an Agilent 7890A GC system coupled to a 5975C mass detector. Metabolites were manually annotated using TagFinder software and reference mass spectra and retention indices from the Golm Metabolome Database [24]. Data were normalized to the signal intensity of each metabolite in fresh medium and log₂-transformed prior to analysis.

Co-cultivation assays

To assess possible competition between strains, co-cultures of P1/7 (serotype 2) or 895-LCR (serotype 9) with four additional isolates representing different anatomical origins: one systemic (171/05), two nasal (CR-8 and GM2-2), and one vaginal strain (A 4V-2S) were performed in the different culture media. After preparing PBS suspensions at OD₆₀₀ = 0.5 for each strain, both were inoculated at a 1:20 ratio into the corresponding medium. Mineral oil (100 µL) was added to each well, and bacterial growth was monitored as described above. DNA was extracted from bacterial pellets, and PCR assays targeting serotypes 2 and 9 were performed following previously described protocols [25].

Results

Growth of *Streptococcus suis* in host-like synthetic media

As the starting point of this study, we examined the ability of reference *S. suis* strains to grow in different host-mimicking environments. Specifically, we focused on two environments, one that mimics the human nasal environment (SNM), and one that mimics human plasma (HPLM) as examples of habitats found during colonization and systemic infection, respectively. Growth of the *S. suis* reference strains P1/7 (virulent) and T15 (non-virulent) was assessed in THY, HPLM, and SNM with and without mucin supplementation (Fig. 1). As expected, both *S. suis* strains exhibited robust growth in THY, but growth in HPLM and SNM was markedly reduced. To confirm that these media were able to support bacterial proliferation in principle, we used a prototrophic *E. coli* strain (NCM3722), as well as a porcine *S. aureus* isolate (EJ41-2), both of which showed clear growth under these restrictive conditions (Fig. 1). Supplementation of SNM with mucin (SNM + M) resulted in a modest but reproducible increase in growth for both *S. suis* strains (and *S. aureus*, also an upper respiratory tract colonizer) when compared to non-supplemented SNM.

To evaluate whether the growth patterns observed for the reference strains were conserved across a broader range of *S. suis* isolates, we extended the analysis to a larger collection of strains from different clinical origins. *S. suis* isolates from our collection (Table 1), including strains obtained from porcine systemic and mucosal sites, as well as isolates of wild boar origin, were analyzed in THY, HPLM, SNM, and SNM + M. In rich medium (THY), the majority of isolates displayed robust growth; nevertheless, a small number of strains, including one systemic isolate (N218/17) and one wild boar isolate (WB4-2), consistently failed to grow under these conditions (Figs. 2 and S1). In contrast, in HPLM, all strains exhibited reduced growth compared with the THY control (Figs. 2 and S1). Growth in SNM was also reduced for most isolates, except for three strains (895-LCR, 171/05 and CR-8), which exhibited comparatively higher growth in SNM than the remaining isolates (Figs. 2 and S1). Supplementation with mucin (SNM + M) resulted in increased growth across all strains, including the isolate (N218/17) that was unable to grow in THY (Figs. 2, S1 and S2).

To assess if these strains are nevertheless metabolically active, we further quantified one of the key metabolic traits in streptococci, namely the fermentation of glucose to lactate [26]. As expected, growth of *S. suis* strains was linked with glucose consumption and lactate production (Figs. 3 and S3). We also tested two other intermediary metabolites, acetate and pyruvate, but we did not detect significant production of either by none of the strains in the tested conditions (Figure S3).

To investigate growth profiles potentially associated with maternal-to-neonate bacterial transmission during parturition, 11 vaginal *S. suis* isolates were analyzed in the same synthetic media conditions. All vaginal strains were able to grow in the rich medium THY, displaying growth dynamics comparable to the reference strain P1/7 (Figs. 4 and S4). As observed for respiratory and systemic isolates, growth in HPLM and SNM was markedly reduced for most isolates (Figs. 4 and S4). However, two isolates (R 1V-4S and A 4V-2S) exhibited comparatively better growth in SNM, highlighting variability in growth performance among the vaginal strains. In agreement with other *S. suis* strains, growth was associated with glucose consumption and lactate production (Figure S5).

Notably, even strains exhibiting reduced growth in the two synthetic media (HPLM and SNM) still appeared to remain metabolically active (Fig. 5A and 5B). Additional exometabolomics quantification revealed that all tested strains consumed at least some of the metabolites present in the medium, regardless of whether they grew in the respective condition. Most notably, we found that all tested strains depleted L-arginine (but not other amino acids) in SNM, suggesting that this condition triggers the activation of the arginine deiminase pathway.

Growth of *Streptococcus suis* in porcine biological fluids

To further assess the ability of *S. suis* to proliferate in more physiologically relevant environments, we next evaluated the bacterial growth of selected strains in porcine biological fluids. First, we confirmed that reference strains P1/7 and T15 were able to grow in saliva and vaginal fluid (Figure S6). Then, several *S. suis* strains isolated from systemic lesions, nasal cavity, tonsils and the vagina were selected to assess their growth in porcine saliva and vaginal fluid. In saliva, all strains exhibited measurable

growth (Figs. 6 and S7) and lactate production, independently of their clinical origin and in apparent absence of glucose (Figs. 7 and S8). Surprisingly, vaginal isolates showed reduced growth in vaginal fluid compared with strains from other origins, with two vaginal isolates (R 1V-4S and R 5V-3S) showing no apparent growth (Figs. 6 and S7). In general, glucose consumption in vaginal fluid was common to all the tested strains, but lactate production was restricted to few of them (Fig. 7).

Co-culture of *Streptococcus suis* strains

In an attempt to explore potential competitive interactions that could limit the growth of virulent strains, we performed co-cultures of pairs of the reference strain P1/7 (serotype 2) or the systemic strain 895-LCR (serotype 9) with four selected strains: 171/05 (systemic), CR-8 and GM2-2 (nasal), or A 4V-2S (vaginal). To confirm that carryover from the initial inoculum was not interfering with the results, a second passage of the mono and co-cultures was performed, and serotype specific PCRs were performed to detect P1/7 and 895-LCR (strains CR-8, GM2-2, and A 4V-2S were not assigned to either serotype 2 or 9, and strain 171/05 was confirmed as serotype 9). Although all the strains grew and consumed glucose in the rich medium THY, competition with P1/7 or 895-LCR was not observed (Figures S9 and S10). Consistent with the limited growth detected during the first passage in SNM, after the second passage, with some exceptions, no growth was observed in monocultures or co-cultures. In contrast, growth in SNM + M resulted in reproducible growth for both monocultures and co-cultures (Figs. 8 and S11). Using the serotype-specific PCR, we were able to demonstrate that P1/7 (serotype 2) was not inhibited by the presence of another *S. suis* strain under the conditions tested (Figs. 8 and S9). In fact, several conditions suggested potential positive interactions, such as those observed between strains 171/05 and P1/7 in SNM and SNM + M. As a result, P1/7, which did not grow in monoculture in SNM, was able to grow when co-cultured with 171/05, as detected by PCR (Fig. 8). Similarly, strains GM2-2 and CR-8 showed a modest positive effect on P1/7 growth in SNM + M, whereas no detectable effect was observed in those co-cultures involving strain A 4V-2S (Fig. 8). Similar results were obtained in co-cultures including the systemic strain 895-LCR, with no evidence of inhibition when this strain was mixed with the others, as determined by specific-serotype PCR. In the co-culture with strain 171/05, discrimination between strains was not possible using this method due to their shared serotype (Figures S10 and S11).

After the first passage in HPLM, we observed very limited growth both in monocultures and co-cultures, and a second passage was not performed (Figures S9 and S10).

Discussion

In this study, we examined the growth of multiple *Streptococcus suis* strains under *in vivo*-like conditions using synthetic media as well as porcine biofluids. Compared to conventional rich media, host conditions imposed strong limitations on bacterial growth, revealing substantial nutritional constraints faced by *S. suis* within the host. The observed strain-dependent differences further suggest that

adaptation to nutrient-limiting conditions may vary among strains, with potential implications for survival and colonization in the host.

Previous studies have shown that the host environment significantly influences the physiology of *S. suis* and other streptococcal species [27–32]. To distinguish between metabolic responses driven by nutrient availability and those modulated by host immune factors, synthetic media can reproduce the metabolic composition of animal fluids, providing controlled and physiologically relevant conditions for studying bacterial metabolism in host-associated contexts. Two media, HPLM and SNM, were employed in this study to investigate bacterial metabolism under host-like conditions, while excluding other host-derived factors, such as immunomediators and the complexity of the nasal microbial interactions. These media reproduce key metabolic aspects of human plasma and the nasal environment, respectively, and enable the investigation of bacterial adaptation under defined conditions that can reveal biological responses not detected under conventional laboratory conditions [17–19]. Under these *in vivo*-like conditions, *S. suis* strains showed reduced growth in both media compared to rich laboratory conditions, supporting the notion that host niches impose significant nutritional constraints on bacterial proliferation. Previous studies have shown that key bacterial genes are expressed in SNM in patterns comparable to those observed *in vivo* within the nasal niche, supporting its relevance as a surrogate system for the *in vitro* study of nasal colonization processes [19]. We observed that five strains displayed relatively higher growth in SNM compared to the other isolates. Of these, two were derived from clinical cases, one from a nasal isolate, and two from vaginal isolates, indicating that the clinical and anatomical origin of the isolates did not determine their growth capacity. Considering the reported high genetic heterogeneity of *S. suis* [13], we can hypothesize that strains originating from different anatomical niches or clinical contexts may belong to evolutionarily distinct lineages and exhibit phenotypic variability, including differences in growth patterns. Taken together, these findings reinforce the notion that, in *S. suis*, the relationship between isolation source, virulence potential, and phenotypic behavior is complex and cannot be explained by a simple dichotomy between commensal and pathogenic strains. The strain set analyzed here was diverse and included isolates from different clinical origins, but to encompass the full genetic variability of *S. suis*, studies with isolates from different genetic lineages may be informative.

Mucin is the main structural glycoprotein of mucus and is abundantly present on the surface of respiratory mucosal membranes, including the porcine nasal mucosa, where it forms a glycan-rich physical barrier that can influence the adhesion and colonization of *S. suis* [33–35]. Although direct evidence demonstrating that *S. suis* uses mucin as a nutrient source is still lacking, previous studies indicate that complex carbohydrate metabolism plays an important role in mucosal colonization and virulence in this species. In particular, carbohydrate availability strongly influenced the expression of virulence-associated genes [9, 36]. The capacity to utilize mucin is not a universal property but rather a trait restricted to specific species and dependent on particular metabolic capabilities [37]. Mucin utilization has been demonstrated more directly in *S. pneumoniae*, where mucin supports bacterial growth in a neuraminidase-dependent manner [38]. Together, these findings support the hypothesis that mucin-derived carbohydrates may also be relevant for *S. suis*. Consistent with this hypothesis, our results show that supplementation of a synthetic nasal medium with mucin significantly enhanced the

growth of *S. suis*, including strains that exhibited limited growth even under nutrient-rich conditions. These findings suggest that the ability to benefit from mucin is common among *S. suis* strains, albeit at different degrees.

Growth assays in porcine saliva and vaginal fluid revealed additional differences in niche permissiveness and strain-specific adaptation. Porcine saliva is a complex biological fluid containing proteins, glycoproteins, metabolites, nucleic acids, and microorganisms [39]. Mucins represent a particularly relevant component of the glycoprotein fraction, contributing to the organization of the mucosal interface and to host-microbiota interactions [40]. Available evidence suggests the idea that *S. suis* is more stably associated with the upper respiratory tract than with the vaginal niche. Microbiota based and targeted studies have consistently identified saliva as a major natural reservoir of *S. suis* [41, 42], whereas its detection in vaginal samples is less consistent and has been mainly linked to maternal-neonatal transmission rather than being the main colonization habitat [43, 44]. In agreement with this ecological framework, all strains analyzed in this study, regardless of anatomical origin, exhibited measurable growth in saliva reinforcing the role of the upper respiratory tract as the primary colonization site for this species. On the other hand, systemic and respiratory *S. suis* strains exhibited significant growth in vaginal fluid, comparable to that observed for *S. aureus*. These observations are consistent with previous reports that describe the sow vaginal microbiota as being predominantly composed of Gram-positive genera such as *Streptococcus*, *Staphylococcus* and *Corynebacterium* [45, 46]. Unexpectedly, vaginal isolates exhibited poor growth in vaginal fluid. The vaginal environment contains host-derived compounds that can support bacterial metabolism, including glycogen-derived products and mucosal components [47, 48]. However, bacterial proliferation within this niche is known to be influenced by contextual factors such as pH, hormonal status, local immunity, and interactions among microorganisms [48] which may have limited the growth of vaginal isolates. This discrepancy likely reflects the inability of these models to capture the full physiological complexity of the *in vivo* environment [49]. Moreover, previous studies in *Streptococcus agalactiae* have demonstrated that vaginal persistence can be a strain-dependent trait, with substantial variability in colonization capacity, epithelial interaction, and host immune activation among isolates [50], which might explain the heterogeneous growth patterns observed here for *S. suis* vaginal strains. It is important to note that biological fluids may exhibit inherent variability depending on the physiological state of the animals. For example, the levels of glucose in saliva are influenced by factors such as feeding status or sampling conditions [51].

Together, these findings indicate that nutrient availability and metabolic flexibility play a role in shaping *S. suis* growth across host-related environments, raising the question of how these factors may influence interactions between coexisting strains. In this context, pathogen exclusion is an appealing concept that motivated us to explore possible competition among *S. suis* strains. However, the co-culture experiments performed did not reveal evidence of competitive inhibition. Instead, interactions appeared to be positive and influenced by nutrient availability. In rich media, co-cultures displayed growth patterns similar to those observed in monocultures, suggesting that competitive effects are largely attenuated when resources are abundant. This supports the notion that non-limiting environments can mask underlying fitness differences between strains [52]. Although competition may still occur in the complex natural

host environment, where *S. suis* coexists with diverse microbial communities, our results point toward a different scenario under nutrient-limiting conditions. Nutrient limitation resulted in positive interactions, potentially driven by metabolic complementation or cross-feeding between strains, pointing to underlying metabolic differences. However, preliminary metabolomic data indicates that strains from different origins do not exhibit marked metabolic differences when grown individually under the conditions tested, although subtle differences may only be detectable using deeper untargeted metabolomic analysis.

This work provides a comprehensive framework for understanding how nutrient availability and host-associated conditions shape strain-specific growth and interactions in *S. suis*, emphasizing the role of ecological context in modulating its colonization and pathogenic potential.

Conclusions

In conclusion, our results demonstrate that *S. suis* exhibits strain-specific differences in growth, interactions, and adaptation under host-associated, nutrient-limiting conditions. While conventional rich media failed to capture these differences, host-mimicking environments, including defined media, mucin supplementation and native host fluids, revealed distinct fitness profiles across strains, independent of their clinical origin. Our results indicate that differences in pathogenic potential are unlikely to be explained by metabolic traits.

Declarations

Ethics approval and consent to participate

Sampling of piglets was done under institutional authorization and followed good veterinary practices. According to European (Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes) and Spanish (Real Decreto 53/2013) normative, this procedure did not require specific approval by an Ethical Committee. Saliva and vaginal fluid samplings were performed only once to each piglet and these procedures are not likely to cause pain, suffering, distress in accordance with good veterinary practice (Chapter I, Article 1, 5 (f) of 2010/63/EU). The animals were commercial pigs housed under conventional husbandry conditions on a commercial farm. Oral consent was obtained from the owner to sample the animals for this study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Author Contribution

Conceptualization and design of the study: VA, FCF and KK. Funding acquisition: VA and FCF. Performed experiments: APF. Performed analyses: APF, IFS, EFP and KK. Supervised the analyses: VA, FCF and KK. Writing of the manuscript: APF. Manuscript reviewing and editing: VA, FCF and KK. All authors read and approved the final manuscript.

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Data Availability

All data generated or analyzed during this study are included in this published article (and its supplementary information files).

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Figures

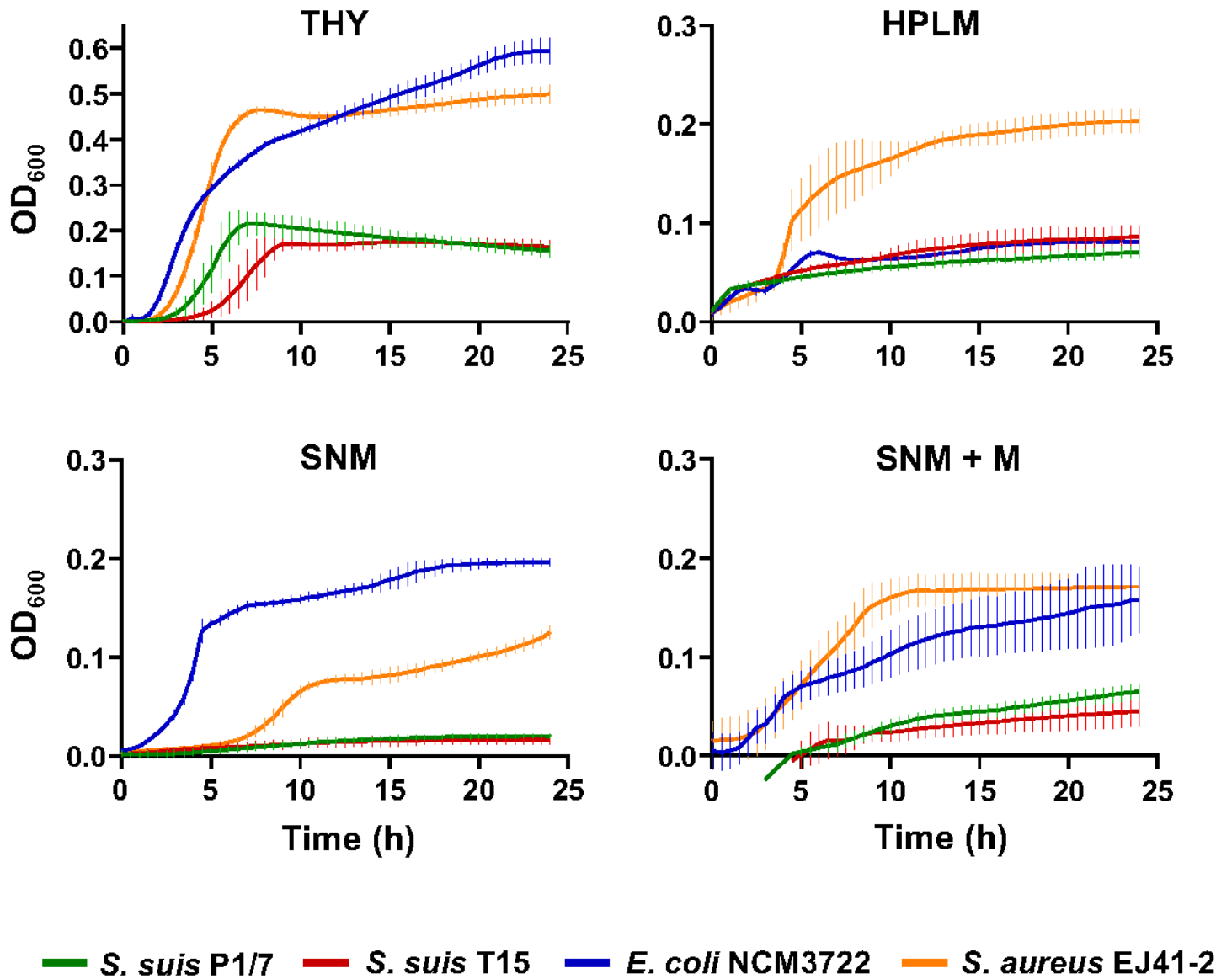


Figure 1

Growth kinetics of *Streptococcus suis* and control strains in different culture media. Growth curves of *S. suis* strains P1/7 and T15, *Escherichia coli* NCM3722, and *Staphylococcus aureus* EJ41-2 were obtained in THY, human plasma-like medium (HPLM), synthetic nasal medium (SNM), and SNM supplemented with 0.5% mucin (SNM+M). Each panel corresponds to a different culture medium, as indicated. Results are presented as the mean $\pm$ standard deviation of optical density measurements at 600 nm (OD₆₀₀) from at least two independent biological replicates, each including three technical replicates.

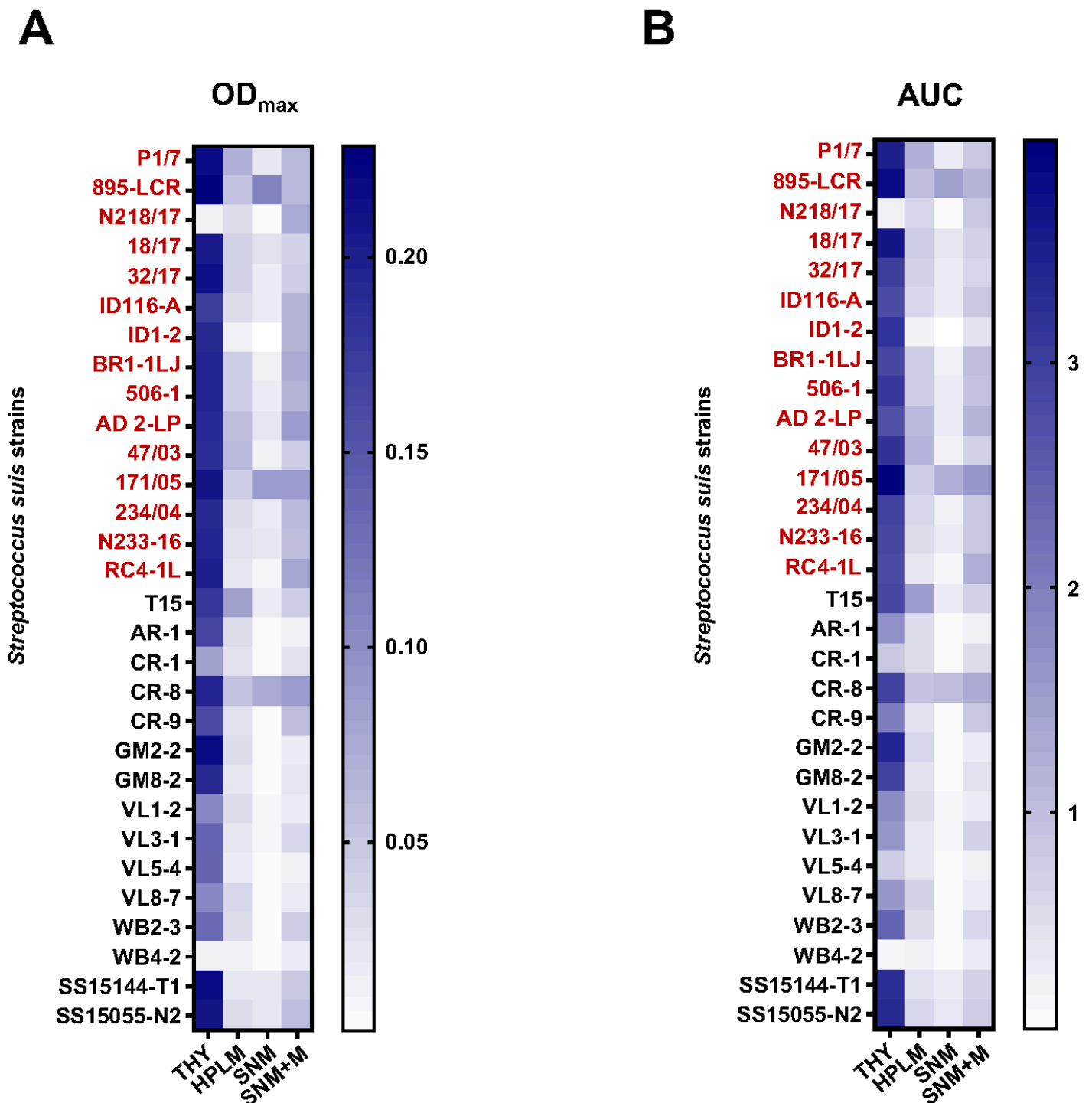


Figure 2

Growth of systemic and upper respiratory tract *Streptococcus suis* strains in host-like media. Growth was quantified as (A), maximal optical density at 600 nm (OD_{max}) and (B), area under the curve (AUC) for *S. suis* strains grown in THY, human plasma-like medium (HPLM), synthetic nasal medium (SNM), and SNM supplemented with 0.5% mucin (SNM+M). Heat maps represent the mean values from two

independent experiments, each performed with three technical replicates per condition. Systemic strains are indicated in red, whereas upper respiratory tract commensal strains are shown in black.

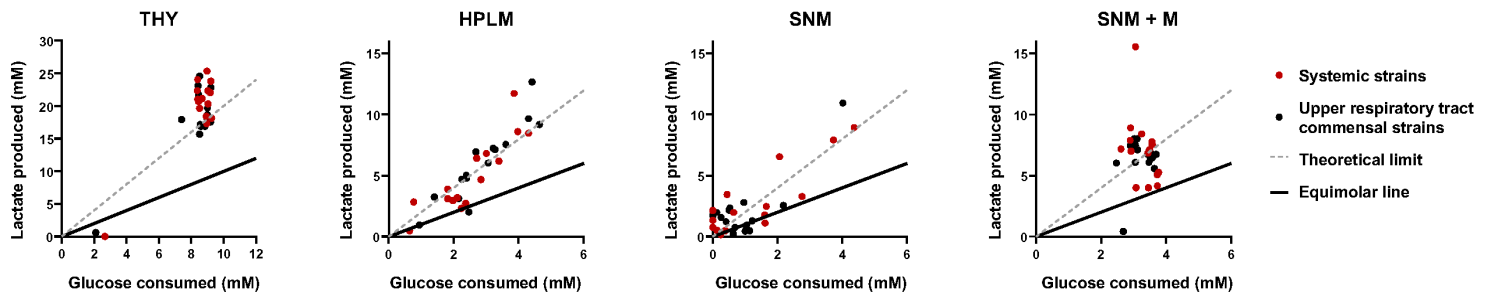
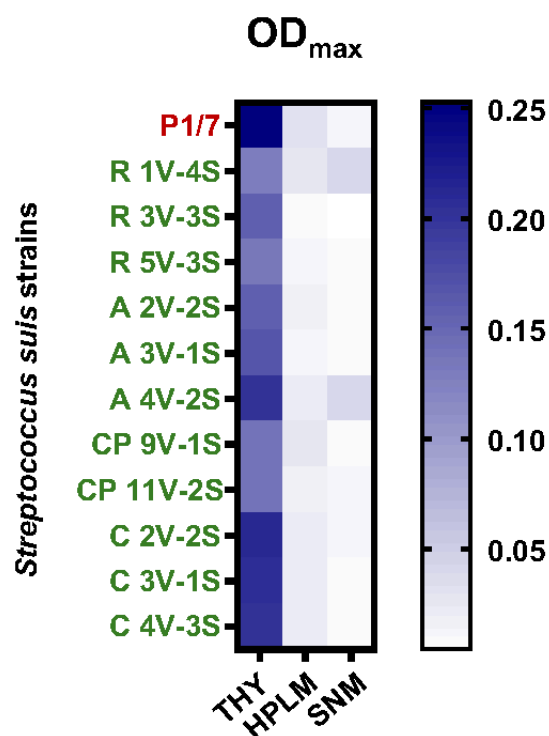


Figure 3

Glucose and lactate in host-like cultures of systemic and upper respiratory tract *Streptococcus suis* strains. Glucose depletion and lactate production (mM) were quantified for *S. suis* strains grown in THY, human plasma-like medium (HPLM), synthetic nasal medium (SNM), and SNM supplemented with 0.5% mucin (SNM+M). Results are shown as the mean of two independent experiments, each performed with three technical replicates per condition. Systemic strains are indicated in red, whereas upper respiratory tract commensal strains are shown in black. Continuous lines denote an equimolar relationship of glucose to lactate, and the dashed lines denote the theoretical limit (i.e. 2 mol lactate per mol glucose) if glucose is the sole carbon source.

A



B

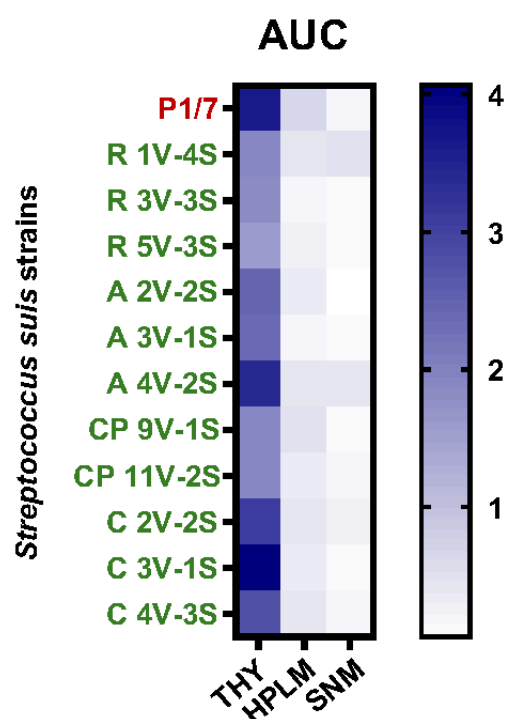


Figure 4

Growth of vaginal *Streptococcus suis* isolates in host-like media. Growth was quantified as (A), maximal optical density at 600 nm (OD_{max}) and (B), area under the curve (AUC) for 11 vaginal *S. suis* strains grown in THY, human plasma-like medium (HPLM) and synthetic nasal medium (SNM). Heat maps represent mean values from two independent experiments, each performed with three technical replicates per condition. The systemic strain P1/7 was included as references and it is indicated in red, whereas vaginal strains are labelled in green.

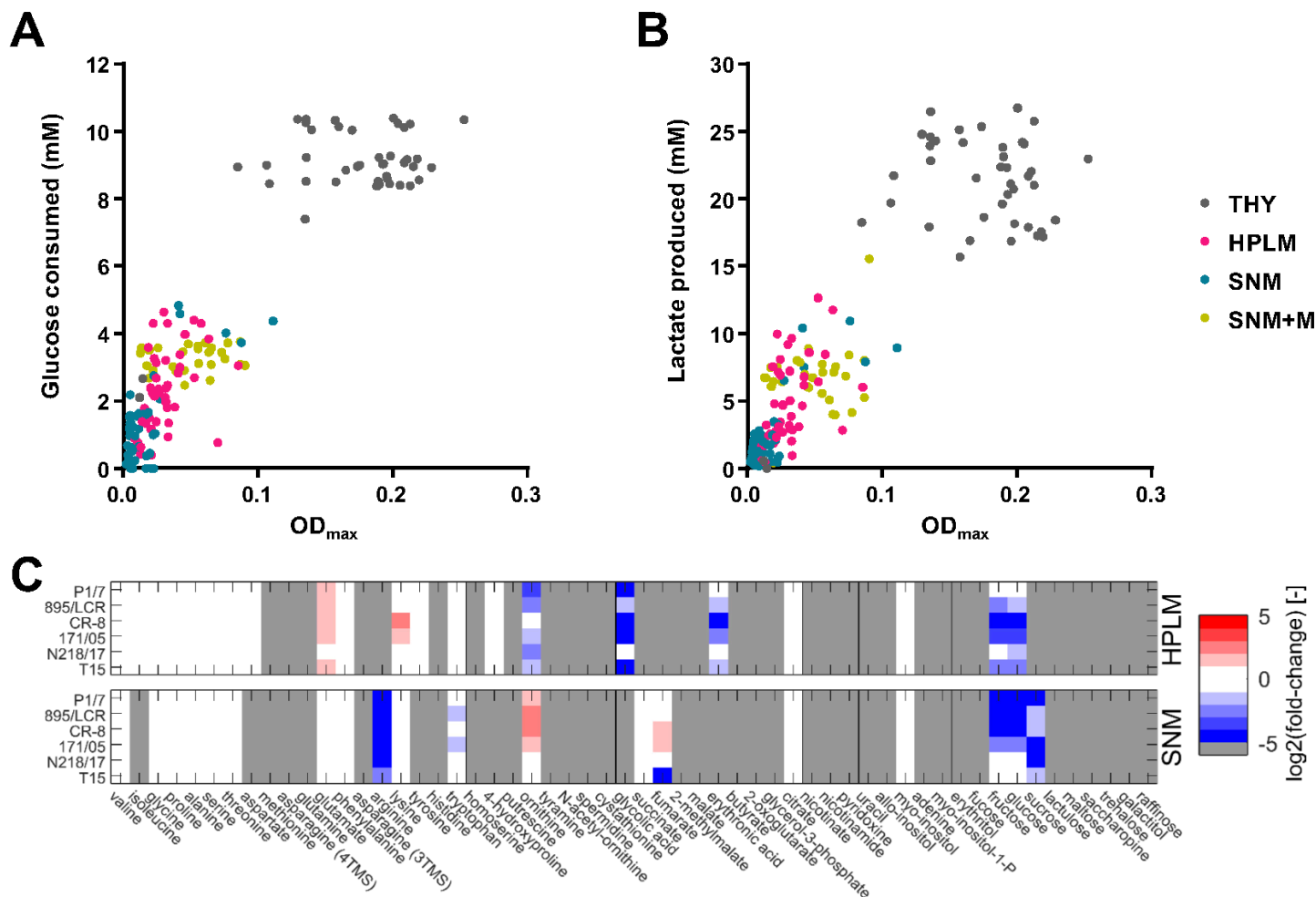
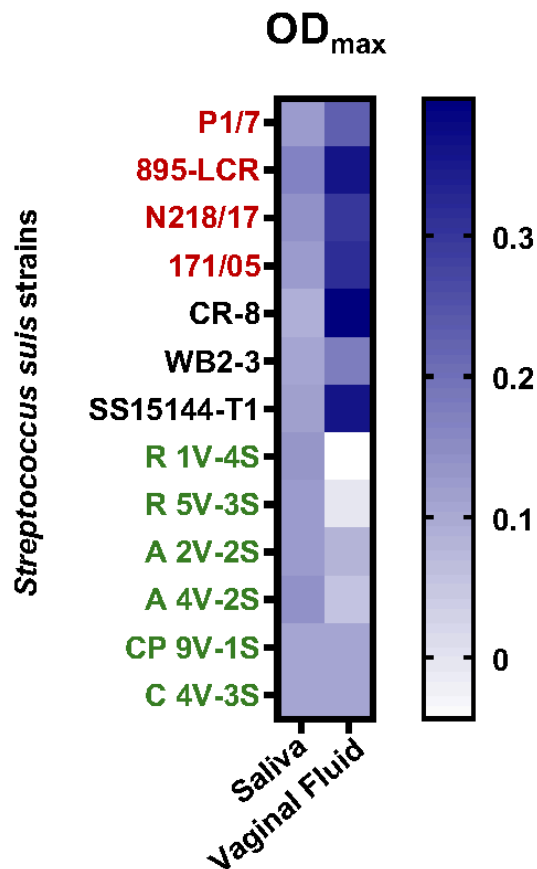
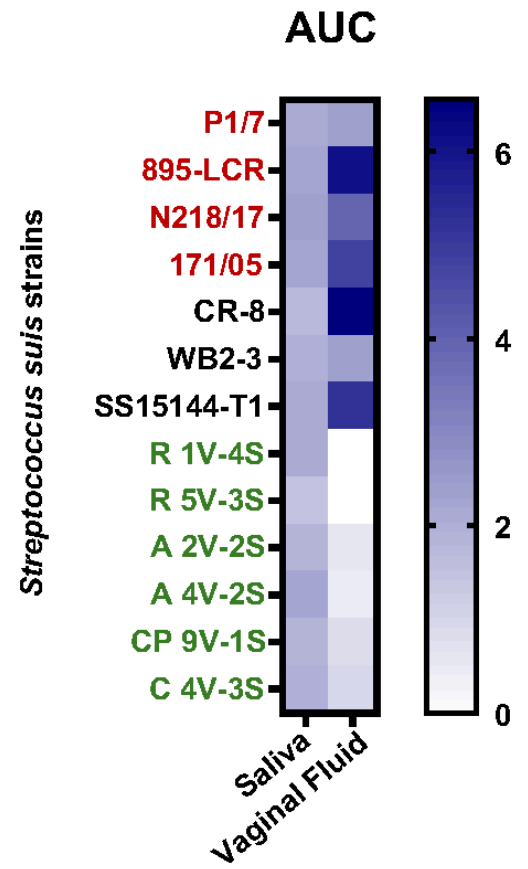


Figure 5

Growth and metabolic insights of *Streptococcus suis* strains grown in host-like media. *S. suis* strains isolated from systemic lesions, upper respiratory tract and vagina were cultured in THY (gray), human plasma-like medium (HPLM; pink), synthetic nasal medium (SNM; blue), and SNM supplemented with 0.5% mucin (SNM+M; dark yellow). Glucose consumption (A) and lactate production (B) together with strain growth in the different media. C) Exometabolomics quantification of consumed/produced metabolites by four systemic isolates (P1/7, 895 LCR, 171/05 and N218/17) and two commensal isolates (CR 8 and T15) in human plasma-like medium (HPLM, top row), and synthetic nasal medium (SNM, bottom row). Data shown are mean metabolite concentrations (n = 2-3) relative to fresh medium. Gray: metabolite included in the quantification, but below the detection limit.

A**B****Figure 6**

Growth of selected *Streptococcus suis* isolates in porcine saliva and vaginal fluid. *S. suis* strains isolated from systemic lesions (n=3), the nasal cavity (n=2), tonsils (n=1), and vaginal samples (n=6) were cultured in porcine saliva and vaginal fluid. Growth was quantified as (A), the maximal optical density at 600 nm (OD_{max}) and (B), the area under the curve (AUC). Heat maps represent mean values from two independent biological experiments, each performed with three technical replicates per condition. Strains are color-coded according to their origin: systemic isolates in red, nasal and tonsillar isolates in black, and vaginal isolates in green.

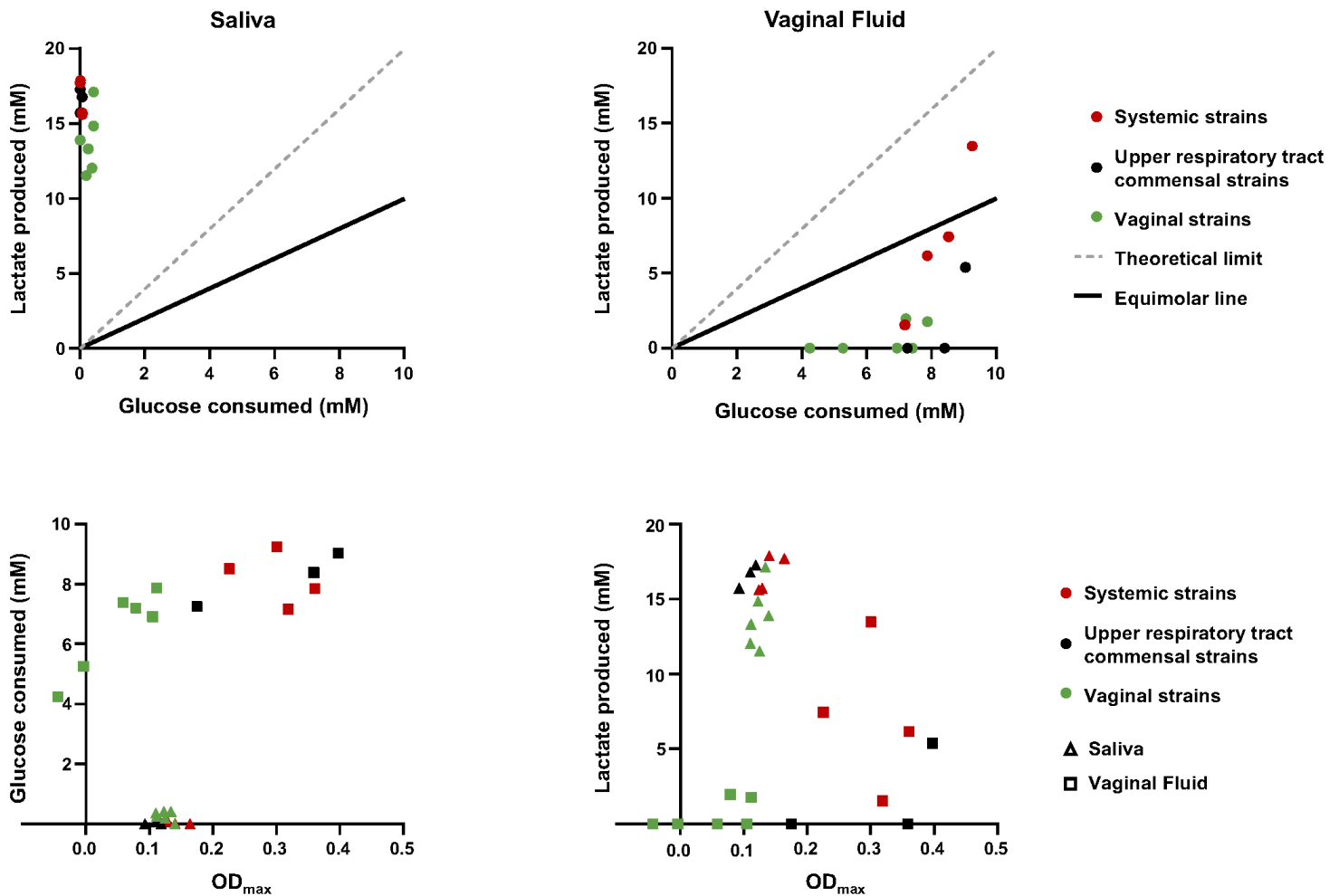


Figure 7

Growth, glucose and lactate in saliva and vaginal fluid of *Streptococcus suis* strains. *S. suis* strains isolated from systemic lesions (n=3), the nasal cavity (n=2), tonsils (n=1), and vaginal samples (n=6) were cultured in porcine saliva and vaginal fluid. Glucose depletion and lactate production (mM) were quantified for *S. suis* strains after growth in saliva (upper left) and vaginal fluid (upper right). Glucose consumption and lactate production are also shown relative to growth in the different media (lower panels). Results are shown as the mean of two independent experiments, each performed with three technical replicates per condition. Systemic strains are indicated in red, upper respiratory tract commensal strains are shown in black and vaginal strains in green. Continuous lines denote an equimolar relationship of glucose to lactate, and the dashed lines denote the theoretical limit (i.e. 2 mol lactate per mol glucose) if glucose is the sole carbon source.

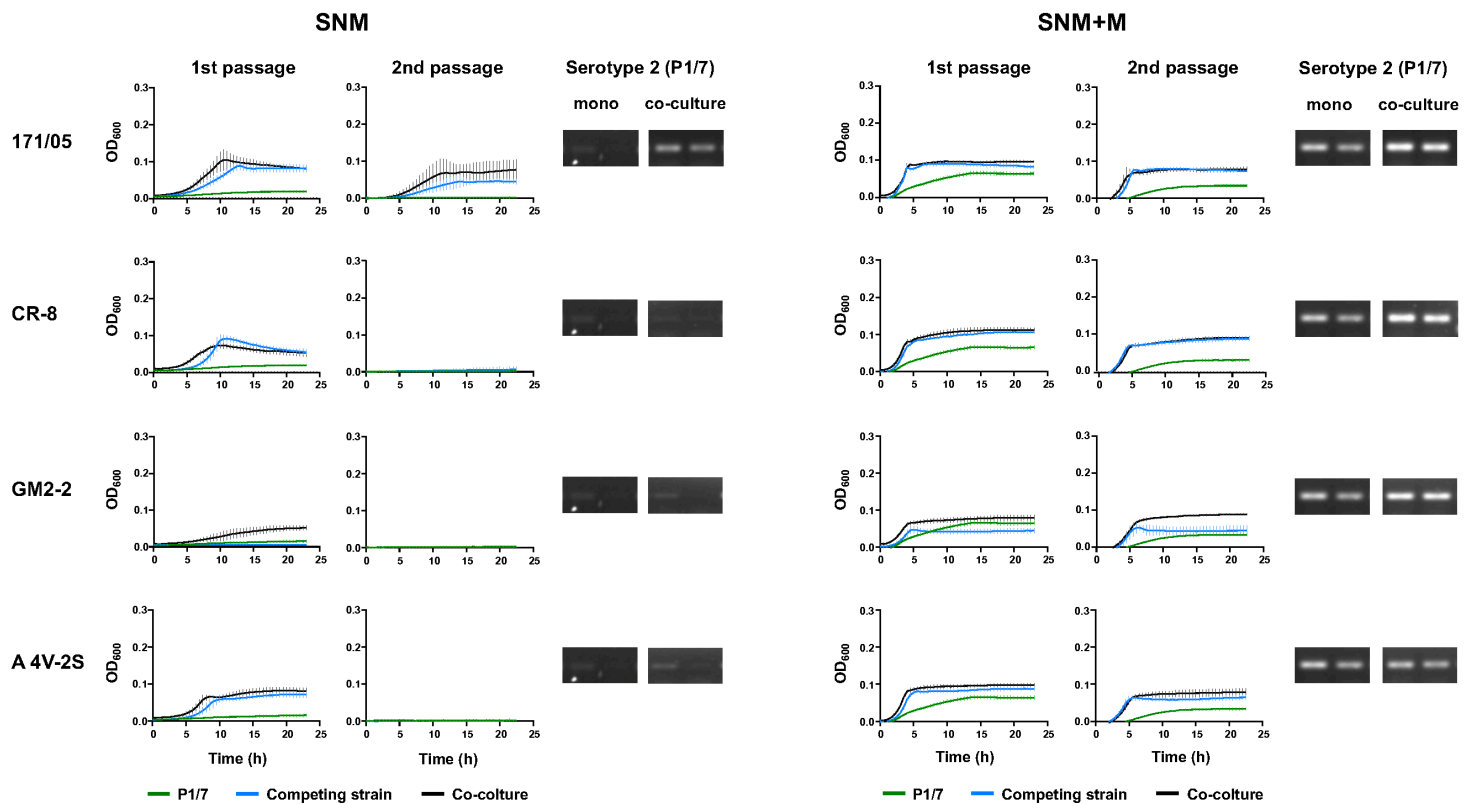


Figure 8

Growth dynamics of *S. suis* P1/7 co-cultures across sequential passages in synthetic nasal media. Co-cultures of *S. suis* P1/7 (serotype 2) with four additional isolates representing different clinical origins: one systemic (171/05), two from nasal (CR-8 and GM2-2) and one from vaginal (A4V-2S) origins, were performed in synthetic nasal medium (SNM) and SNM supplemented with 0.5% mucin (SNM+M). Growth curves correspond to the initial culture (first passage) and the subsequent subculture (second passage). Growth was monitored by measuring optical density at 600 nm (OD₆₀₀). Data represent the mean $\pm$ standard deviation of at least two independent biological replicates, each including three technical replicates. Detection of P1/7 in mono and co-cultures was achieved using a serotype 2-specific PCR. In the growth curves, P1/7 is shown in green, competing strains are shown in light blue, and co-cultures are represented in black.

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