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BIOCHEMICAL CHARACTERISTICS OF *STREPTOCOCCUS SUIS* STRAINS ISOLATED FROM HEALTHY AND DECEASED PIGS

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Abstract: The aim of this study was to determine the biochemical properties of *Streptococcus suis* strains isolated from healthy and deceased pigs. For this research we tested 34 *S. suis* strains isolated from deceased pigs that had clinical signs of septicemia and meningitis, as well as from clinically healthy pigs. The strains that have been already confirmed with specific antisera were tested using commercial battery of biochemical tests (API 20 Strep and ID 32 Strep) to determine the dominant biochemical characteristics that can be used in diagnosis of bacterial infection if specific *S. suis* antisera are not available. The main results showed that all *S. suis* strains were postive in esculine, trehalose, glycogen, lactose, sacharose, starch, leucine aminopeptidase, alanine-phenyl-alanine-proline arylamidase tests, while negative in Voges-Proskauer, hipurate, ribose, arabinose and sorbitol tests. *S. suis* strains were in high percentage positive in arginine dihydrolase, β-glucoronidase, α-galactosidase, β-galactosidase, methyl-β-d-glucopyranoside, glycyl-tryptophan arylamidase and inulin tests. Athough *S. suis* is in highly positive in some tests, it can be concluded that Voges-Proskauer, hipurate, trehalose, esculine tests, along with β-glucoronidase (βGUR) and α-galactosidase (αGAL), were significant in differentiation of this bacteria from other similar streptococci, along with some other crucial features (α hemolysis on blood sheep agar, absence of growth in 6,5% NaCl broth).

Key words: *Streptococcus suis*, biochemical characteristics, pigs

Introduction

Streptococcus suis is a facultative anaerobic, coccoid, gram-positive bacterium with the ability to synthesize capsule and secrete hemolysin. It has components of the cell wall antigens similar to those displayed by group D

streptococci. However, *S. suis* is not genetically associated with group D streptococci.

S. suis is a very heterogeneous species. So far, 35 of *S. suis* serotypes have been described on the basis of the composition of the capsular polysaccharide (1-34 and ½). During the last 20 years, *S. suis* has been considered to be one of the main pathogen that causes severe economic losses in countries with developed pig industries. *S. suis* is a normal inhabitant of the pigs respiratory system, mostly of the tonsils and nasal cavities, and can often be isolated from the genital and gastrointestinal systems in healthy animals *(Higgins and Gottschalk, 2005)*. Since it is a very good colonizer of the mucosal surfaces, clinically healthy pigs are the main reservoir of infection, and the most important link in the epidemiology of human infections caused by *S. suis (Gottschalk et al., 2010)*. *S. suis* can be also easily isolated from noses and tonsils of live pigs, as well as from pig carcasses and butchers' knives (*Stanojkovic et al., 2012*).

All age categories, including suckling piglets, older piglets and pigs are prone to disease caused by *S. suis*. Animals at different production stages harbored isolates with similar phenotypic and genetic profiles, highlighting the importance of healthy animals in the maintenance of strains responsible for outbreaks of clinical disease (*Luque et al., 2010*). Even if pigs are infected with *S. suis,* the emergence of a clinically apparent disease varies periodically and is generally below 5% (*Sihvonen et al., 1988*).

The most prominent feature of *S. suis* infection in pigs and humans is meningitis. Infections caused by this species may also manifest as arthritis, endocarditis, pneumonia, rhinitis, vaginitis, and abortion (*Sanford and Tilker, 1982; Sihvonen et al., 1988*) . Human infections caused by *S. suis* are considered to be sporadic, mostly in people who come in contact with pigs and their products (*Arends and Zanen, 1988*). However, in China in 2005, the outbreak caused by *S. suis* affected more than 200 people, with almost 20% mortality rate. This epidemic has completely changed the perception of the danger which this pathogen presents to human health.

A preliminary diagnosis of *S. suis* infection in pigs is usually made on the basis of clinical signs and macroscopic lesions. However, the diagnosis is confirmed by bacteria isolation and detection of microscopic lesions in tissues. It is demonstrated that *S. suis* accumulates in the kidney during *S. suis* infection. These findings might be useful for diagnosis of streptococcal infection (*Nakayama et al., 2011*).

S. suis is α hemolytic on sheep blood agar plates with variable biochemical properties. *Kilper-Balz and Schleifer (1987)* presented and described the following biochemical characteristics of *S. suis*: acid from fermentation of D-glucose, sucrose, lactose, maltose, salicin, trehalose, and inulin, no fermentation of L-arabinose, D-mannitol, D-sorbitol, glycerol, melezitose, or D-ribose, positive hydrolysis of L-arginine, esculin, salicin, starch, and glycogen; no hydrolysis of hippurate; no production of acetoin (Voges-Proskauer - VP negative). According

to these authors *S. suis* is acid phosphatase and alkaline phosphatase negative, L-ornithine decarboxylase, N-cetylglucosaminidase, α-galactosidase, β-glucuronidase, and leucine arylamidase positive; β-alactosidase variable, resistant to optochin and doesn not grow in 6.5% NaCl or 0.04% tellurite.

According to *Tarradas et al. (1994), S. suis* can be confirmed using only a few tests: no growth in broth with 6.5% NaCl, postive esculine and trehalose reactions and negative VP test. *Higgins and Gotsschalk (1990)* and *Gottschalk et al. (1991)* proposed the following indicators as specific for *S. suis*: VP negativity, negativity for growth in the presence of 6,5% NaCl, salicin and trehalose positivity. There is an opinion of many authors *(Facklam et al, 2002; Princivalli et al., 2009*; *Gottschalk et al. 2010)* that it is sometimes very difficult to distinguish *S. suis* from viridans streptococci. Although serotyping and PCR are at the moment the only definitely methods to determine *S. suis* infections, these methods are available only to small number of diagnostic laboratories.

The aim of this study is to determine dominant biochemical characteristics of *S. suis* that can be used by large number of laboratories to be able to diagnose infection caused by this bacterium in high rate of precision.

Materials and methods

The material analysed in this study included 186 tonsil and nose swabs of clinically healthy pigs and 40 meningeal, renal and joint samples (swabs and parts of organs) of deceased pigs that had symptoms resembling those associated with *S. suis* infection. Swabs and samples were transported in trypton soy broth (Oxoid, England) within 2 h of sampling. All samples were inoculated on Columbia agar with added 5% sheep blood (CBA) (bioMérieux, France), and incubated for 24 h aerobically at 37 °C. Parts of diseased organs of pigs were homogenized, inoculated on CBA and incubated aerobically for 24 h at 37 °C. Bacterial strains were selected on the basis of colony morphology, hemolytic characteristics that they produce on blood agar, absence of growth in 6.5% NaCl broth and their microscopic appearance.

In order to definitely determine isolated strains, serological typing with antisera (Statens Serum Institute, Denmark) specific for capsular *S. suis* antigens (Quellung reaction) was used. Strains already confirmed with specific antisera were tested using salicin fermentation test in the tube (peptone water with 1% salicine and indicator added) and commercial battery of biochemical tests (API 20 Strep and ID 32 Strep) to determine dominant biochemical characteristics.

Results and discussion

From the 226 tested samples, 34 strains of *S. suis* were isolated and thus confirmed with specific antisera for bacterial capsular antigens. All strains were

tested with the battery of commercial biochemical tests API 20 Strep and ID 32 Strep.

S. suis in this study showed very variable results in most of the tests and only a few test were indicator of *S. suis* infection. Table 1 shows dominant biochemical characteristics of *S. suis*.

In the present study all of *S. suis* strains were α hemolytic on sheep blood agar plates, showed no growth in broth with 6,5% NaCl, esculine and trehalose positive and VP negative. These results are in agreement with those described by most of the autors *(Kilper-Balz and Schleifer, 1987*; *Tarradas et al., 1994)*. Also, all strains were positive in glycogen (GLYG), lactose (LAC), sacharose (SAC), starch (AMD), leucine aminopeptidase (LAP), alanine-phenyl-alanine-proline arylamidase tests (APPA), and negative in hipurate (HIP), ribose (RIB), arabinose (ARA) and sorbitol (SOR) tests. *S. suis* strains were in high percent positive in arginine dihydrolase (ADH), β-glucoronidase (βGUR), α-galactosidase (αGAL), β-galactosidase (βGAL), methyl-β-d-glucopyranoside, glycyl-tryptophan arylamidase (MβDG) and inulin (INU) tests. These results are similar to those presented by *Kilper-Balz and Schleifer (1987)* and *Facklam et al. (2002)*.

Table 1. The dominant biochemical characteristics of *S. suis* strains

S. suis	6,5% NaCl	VP	HIP	*GLYG*	ADH	*AMD*	MβDG	βGAL	SAC	*RIB*	*AR*
	0	0	0	100	↑70	100	↑90	↑80	100	0	0
	Hemolysis	ESC	*TRE*	βGUR	αGAL	*INU*	*LAC*	APPA	GT	LAP	SO
	α	100	100	↑80	↑80	↑90	100	100	↑90	100	0

Absence of growth in 6.5% NaCl broth was the test that excluded *Enterococcus* species that have sometimes very similar biochemical patterns as *S. suis*. It was noticed that VP negativity was test that distinguishes *S. suis* from *S. bovis* and *S. salivarius* and that sometimes hipurate negativity and especially esculine and trehalose positive test were critical for distinguishing *S. suis* from some other streptococi (viridans group streptococci).

Only 38.2% of strains fermented salicin which is not in accordance with results presented by *Higgins and Gotsschalk (1990)* and *Gottschalk et al. (1991)* which proposed that positive salicin fermentation test is characteristic for *S. suis*.

Athough *S. suis* dominant features in this study were negativity in sorbitol, ribose and arabinose test, positivity in APPA, LAP, glycogen,sacharose and lactose test, it is noticed that these tests were also in high percentage a feature of other

similar bacteria and thus not critical in diagnosis. On the contrary, tests in which *S. suis* was highly positive, like β-glucoronidase (βGUR) and α-galactosidase (αGAL) were sometimes critical in diagnosis of *S. suis* infection.

Bearing all this in mind, we acknowledge that these results are similar to those of other authors *(Kilper-Balz and Schleifer, 1987*; *Tarradas et al., 1994)*. Despite that, we found that some other biochemical characteristics may be critical in diagnosis of *S. suis* infection, and can be used in all laboratoires that are not specialized in diagnosis of this pathogen.

Conclusion

The results of this study showed that except known growth and biochemical features of *S. suis* (α hemolytic on sheep blood agar, abscence of growth in 6,5% NaCl, Voges-Proskauer and hipurine negativity, esculine and trehalose positivity) some other features may be important and critical for *S. suis* diagnosis if specific antisera or PCR are not available.

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Biohemijske karakteristike sojeva *Streptococcus suis* izolovanih iz zdravih i obolelih svinja

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Rezime

Cilj ovog istraživanja je bio da se utvrde biohemijske osobine sojeva S. suis. U ovom istraživanju biohemijski smo testirali 34 soja *S. suis* koji su izolovani kod uginulih svinja koje su prethodno pokazivale kliničke znake septikemije i meningitisa kao i od klinički zdravih svinja. Sojevi koji su potvrđeni specifičnim antiserumima su testirani komercijalnim nizom testova (API 20 Strep and ID 32 Strep) da bi se definisale njihove biohemijske osobine koje se mogu koristiti pri dijagnozi ukoliko specifični antiserumi nisu na raspolaganju. Svi sojevi *S. suis* u ovom istraživanju su bili pozitivni u testovima razlaganja eskulina, trehaloze, glikogena, laktoze, saharoze, skroba, u leucin aminopeptidaza, alanin-fenil-alanin prolin arilamidaza testovima, i negativni u Voges-Proskauer, hipurat, riboza,

arabinoza i sorbitol testovima. Takođe, *S. suis* je u visokom procentu pozitivan u arginin dihidrolaza, β-glukoronidaza, α-galaktozidaza, β-galaktozidaza, methil-β-d-glukopiranozid, glicil-triptofan arilamidaza i inulin testovima. Iako je *S. suis* često pozitivan u nekim testovima može se zaključiti da su osim već poznatih karakteristika ove bakterije (α hemoliza na krvnom agaru sa ovčijom krvi, odsustvo rasta u bujonu sa 6,5% NaCl) Voges-Proskauer, hipurat , trehaloza i eskulin testovi zajedno sa β-glukoronidaza i α-galaktozidaza testovima najznačajniji u diferencijaciji ove od drugih sličnih streptokoka.

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