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ISOLATION AND CHARACTERIZATION OF *STREPTOMYCES RISHIRIENSIS* (VY31) WITH ANTIBIOTIC ACTIVITY AGAINST VARIOUS PATHOGENIC MICROORGANISMS

Ivana Charousová[1], Soňa Javoreková[1], Joachim Wink[2]*

Address(es): Dipl.-Ing. Ivana Charousová,
[1] Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Microbiology, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, +421 376414433.
[2] Helmholtz Centrum for Infection Research, Microbial Strain Collection Group, Inhoffenstraβe 7, 38124 Braunschweig, Germany.

*Corresponding author: ivanacharousova@gmail.com

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ABSTRACT

Actinomycete strain VY31 was isolated from agriculture soil of region Východná, Slovakia. Morphological, physiological and biochemical studies indicated that this isolate belongs to the genus *Streptomyces.* The 16S rRNA sequence data supported the assignment of the isolate to the genus *Streptomyces rishiriensis* (sequence similarity 97%). Tested isolate was able to produce melanin dark pigment and exopigments on ISP6, ISP7 and SSM+T cultivating media. The optimal pH range was from 6-8 and optimal temperature at 30 °C. The strain exhibited salt tolerance up to 5 % and utilized the carbon sources such as glucose, arabinose, xylose, inositol, mannose, fructose, rhamnose and rafinose. Using ApiZym® stripes, the highest production of enzymes was determined for phosphatase alkaline, leucinearylamidase, valinearylamidase, phosphatase acid, naphtol-AS-BI-phosphohydrolase, galactosidase and glucosidase (>40 nmol). According to ApiCoryne® results, positive reaction was confirmed in case of esculin, alkaline phosphatase, and this strain was also able to hydrolyze gelatine. Minimum Inhibitory Concentration (MIC) of the purified extract of isolate was evaluated against Gram-positive bacteria *Staphylococcus aureus* and *Enterococcus faecium*, Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* and against yeast *Candida albicans*. On the basis of MIC results, strain VY31 had noticeable antibacterial activity against *Staphylococcus aures* N315 (MRSA) from collection database of University Hospital in Hamburg, Germany. This isolate could be used in the development of new antibiotics for pharmaceutical purposes.

Keywords: Soil, *Streptomyces* sp., minimal inhibitory concentration, pathogenic microorganisms

INTRODUCTION

Microorganisms are the main colonizers of the earth, bestowed with inherent physiological and functional diversity and have found applications in agriculture, medicine, industry and environment. Among the various industrially important microorganisms, actinomycetes are of prime importance and are primarily recognized as organisms of academic curiosity **(Rana *et* Salam, 2014)** because of its wide antimicrobial activity **(Thakur *et al.*, 2007)**. Actinomycetes are gram-positive, filamentous **(Holt *et al.*, 2000; Varghese *et al.*, 2012)**, free-living, saprophytic bacteria **(Rahman *et al.*, 2011)** with high G+C contents in DNA inhabiting mainly the soils **(Deepika *et* Kannabiran, 2009),** where decompose organic matter, especially biopolymers such as lignocellulose, starch, and chitin **(Crawford *et al.*, 1993)**. From the 22,500 biologically active compounds that have obtained from microbes, 45% are produced by actinomycetes, 38% by fungi, and 17% by unicellular bacteria **(Berdy, 2005)**. The species belong to the genus *Streptomyces* constitute 50% of the total population of soil actinomycetes and are well known for producing a variety of bioactive secondary metabolites including antibiotics, immunomodulators, anticancer drugs, antiviral drugs, herbicides, and insecticides **(Sanglier *et al.*, 1993)**. Screening of microorganisms for the production of novel secondary metabolites has been intensively pursued for many years by scientists **(Oskay *et al.*, 2004)**, due to intensely increasing resistance of clinically important bacterial strains **(Sahin, 2003; Patel *et al.*, 2014).** Actinomycetes and their bioactive compounds show antibacterial and antifungal activity against various pathogens and multi drug resistant pathogens e.g. methicillin-resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa* **(Singh *et al.*, 2012)**, *Escherichia coli*, *Enterococcus faecium* **(Sharma *et al.*, 2011)** and also against yeasts e.g. *Candida* genera **(Spadari *et al.*, 2013)**. Thus, it is necessary to continue antimicrobial screening and evaluate and identify the potential of microbial taxa especially against drug-resistant bacteria **(Kurtboke, 2005).**
The present investigation describes the isolation, morphological, physiological, biochemical and genotypic characterization of *Streptomyces rishiriensis* actinomycete strain with antibiotic activity against various pathogenic microorganisms.

MATERIAL AND METHODS

The tested actinomycete strain was isolated from soil sample collected at region Východná (geographical coordinates: latitude 49°4´N, longitude 19°54´E), Slovakia, using classical plate dilution method on Pochon medium **(Korzeniewska *et al.*, 2009)** complemented with nystatin (50µg/ml). After gently rotating, the plate was incubated at 28°C for 7 days. Strain was transferred from mixed culture of the plate into selective yeast extract-malt extract medium **(Shirling *et* Gottlieb, 1966)** for purification, identified on the basis of various criteria and determined for inhibition activity against selected pathogenic microorganisms.

Morphological characterization

Standard media for colony description were described by **Shirling *et* Gottlieb (1966)** in International Streptomyces project (ISP). The morphological characteristics were observed by culturing isolate on ISP2-ISP7 media. For morphological characteristic four parameters were used - growth, reverse colors, colors of aerial mycelium and colors of soluble pigments. The colors were described by the RAL-code. ISP6 and ISP7 media were also used together with the synthetically Suter medium **(Suter, 1978)** with and without tyrosine for the detection of melanin production. The pigment production of test strain was controlled after 5, 10 and 14 days and recorded by using "+" for a visible formation or "–"for none. For the light microscopic classification of the strain, well grown agar plate with GYM medium **(Větrovský *et al.*, 2014)** was used for observation of spore chain morphology.

Physiological testing

The physiological testing holds four different approaches for the characterization of actinobacteria strains, including optimal pH and temperature for growth, resistance toward sodium chloride and utilization of various carbon sources. pH tolerance was tested in tubes with liquid ISP2 medium at pH levels of 2, 3, 4, 5, 6, 7, 8, 9, and 10. After 5-7 days of incubation was documented visible growth.

For determination of optimal temperature ISP2 agar plates were incubated at 25, 30, 45 and 60 °C. Sodium chloride tolerance was tested on microtiter plates (six-well) using a technique based on the method of to **Kutzner (1981).** The growth of strain was determined on medium (casein peptone – 10.0 g/L, yeast extract - 5.0 g/L, agar – 20.0 g/L, deionized water – 1000 ml) with 0, 2.5, 5, 7.5 and 10 % of sodium chloride. Utilization of different carbon sources was determined on the basis of **Shirling *et* Gottlieb (1966)** methodology using a microplate technique with twelve well plates. Physiological tests included ability to utilize 10 different carbon sources (arabinose, cellulose, fructose, glucose, inositol, mannitol, raffinose, rhamnose, sucrose and xylose). The evaluation was carried out using "-" for lesser growth like in case of negative control (plate without C - source) and "+" if growth was similar to positive control (plate with addition of glucose).

Biochemical characteristic with Api® stripes

For detection of enzymes we used two different Api® systems - ApiCoryne® and ApiZym®. For conducting the Api® tests, culture was grown in shaking flask with GYM medium for one week and after that the strain was inoculated followed by manufacturer´s manual. Stripes were incubated 24 hours (30°C). After incubation we added one drop of ZYM A and one drop of ZYM B reagents to each cupule of ApiZym® stripes. In case of ApiCoryne® we added one drop of NT1+NT2 reagents to the first cupule, PYZ reagent to the second cupule and one drop of ZYM A and ZYM B to the next six cupules. The rest of cupules were not filling with any reagents. We let the colors develop and after five minutes we evaluated stripes according to manual criteria.

16S rRNA analysis

Genomic DNA was isolated according to **Sambrook *et al*. (1989)** methodology**.** The quality of isolated DNA was determined electrophoretic on 2 % agarose gel and visualized by Gel Logic 212 PRO Imaging System (Carestream Health, Inc., USA). The isolated DNA was amplified by PCR reaction using primers according to **Cook *et* Meyers (2003)**. Reaction mixture was made in total volume of 50 µl. Each reaction contained 5 µl of 10 × DreamTaq Green PCR buffer, 5 µl of 2 mmol.dm^{-3}dNTP, 2 µl of each 10 µmol.dm^{-3} primer, 0,3 µl Taq DNA polymerase and 0,5 µl of template DNA (approximately 20 ng). The PCR reaction ran in thermo cycler Biometra T Personal under the following conditions: 95 °C for 3 min, 40 cycles of 95 °C for 30 sec, 56 °C for 30 sec, 72 °C for 90 sec and final extension at 72 °C for 10 min. PCR products were purified using Exonuclease I and Thermosensitive Alkaline Phosphatase according to the manufacturer´s instructions and send for sequencing in MacroGen company, South Korea. The similarity and homology of the 16S rRNA gene sequence was analysed with the similar existing sequences available in the data bank of National Center for Biotechnology Information (NCBI) using BLAST search (http://www.ncbi.nlm.nih.gov/BLAST).

Preparation of extract

For the liquid cultivation a single colony of the strain was transferred in 5254 medium **(Wink *et al.*, 2013)** for metabolite production using a sterile inoculating loop. The culture was incubated at 30°C for 5-10 days at 160-180 rpm on the shaker. 20 ml of 5 days old culture were mixed with 20 ml of ethyl acetate in a 50 ml Falcon tube. After a 12 min shaking step the sample was centrifuged at 9000 rpm for 10 min and the upper phase was transferred into a 50 ml round bottom flask. At about 40°C the ethyl acetate was evaporated in a rotary evaporator (Heidolph). Finally, the extract was solved in 1 ml of ethyl acetate: acetone: methanol (1:1:1) and centrifuged at 14000 rpm for 10 min.

Tested microorganisms and determination of antimicrobial activity

Tested microorganisms were obtained from Microbial Strain Collection Group (MISG) of Helmholtz Centrum for Infection Research (HZI) in Braunschweig, Germany. The selected pathogenic microorganisms used in antimicrobial study were *Escherichia coli* (DSM 116), *Escherichia coli* WT3 (resistance on chinolone), *Staphylococcus aureus* (Newman), *Staphylococcus aureus* N315 (MRSA), *Candida albicans* (DSM 1665), *Pseudomonas aeruginosa* (DSM 19882), *Enterococcus faecium* (DSM 20477) and *Enterococcus faecium* (DSM 17050).

Minimal inhibitory concentration of active ethyl acetate crude extract of isolates was determined by serial dilution method. The whole experiment was conducted in 96-well plates. Initially, the correspondent inoculation volume of the test strains was transferred into 20 ml of the suitable media (Table 1). 150 µl of the test culture medium was transferred into each well and additional 150µl in wells of the first row. Afterwards (A12) was used as negative control by adding 20µl of acetate: acetone: methanol (1:1:1), whereas the other places in the first row were inoculated with 20 µl of the raw extracts. Subsequently the first row was mixed and 150 µl of the mixture was transferred into the new row (B). This procedure was taken out until the last row was reached. Data about optimal concentration of tested microorganisms were obtained from MISG of HZI after long investigations.

Table 1 Cultural conditions of selected pathogenic microorganisms used for detection of minimal inhibitory concentration

Tested microorganisms	Cultivating medium (20 ml)	Amount (µl)	Temperature of incubation (°C)
***Escherichia coli* (DSM 116)**	MHB	126	37
***Escherichia coli* (WT3)**	MHB	106	37
***Staphylococcus aureus* (Newman)**	MHB	108	37
***Staphylococcus aureus* (N315)**	MHB	104	37
***Candida albicans* (DMS 1665)**	MYC	123	30
***Pseudomonas aeruginosa* (DSM 19882)**	MHB	136	37
***Enterococcus faecium* (DSM 20477)**	TSB	216	37
***Enterococcus faecium* (DSM 17050)**	TSB	218	37

Legend: MHB - Mueller Hinton Broth, MYC- Mycosal medium, TSB - Tryptic Soy Broth

RESULTS AND DISCUSSION

Genus *Streptomyces* is considered as the most secondary metabolite producing genus of actinomycetes. For this reason an attempt to isolate, identify and study antimicrobial activity of *Streptomyces* species from natural soil habitats of microorganisms was screened.

The selected actinomycete strain VY31 exhibited typical morphological characteristics of the genus *Streptomyces* (slow growing, chalky, heaped and folded colony with aerial and substrate mycelium of different colors). In addition, streptomyces strains possessed an earthy odor **(Suneetha, 2011).** Morphological and micro morphological observation of the strain revealed that vegetative and aerial mycelium were abundant, well development and fragmented with rod shaped spores. Sporophore morphology of the strain grown on GYM medium for seven days showed *rectus-flexibilis* pattern. The cultural characteristics of the strain are represented in Table 2. The strain VY31 exhibited good growth on five from eight used media. The colors of substrate and aerial mycelium varied depending on medium (Figure 1). **Sivakumar (2001)** reported that the cultural characteristics could be used as markers by which an individual can be recognized.

Table 2 Morphological characterization of VY31 strain on different cultivating media

Medium	Reverse color	Color of aerial mycelium	Color of diffusible pigments	Speed of growth
Yeast malt agar (ISP2)	Sand yellow	No growth	-	Good
Oatmeal agar (ISP3)	Beige	Light ivory	-	Good
Inorganic salt-starch agar (ISP4)	Ivory	No growth	-	Good
Glycerol asparagine agar (ISP5)	Light ivory	No growth	-	Sparse
Peptone yeast extract iron agar (ISP6)	Terra brown	Agate grey	Pale brown	Sparse
Tyrosine agar (ISP7)	Pale brown	Agate grey	Pale brown	Good
Suter medium with tyrosine (SSM+T)	Signal black	No growth	Terra brown	Good
Suter medium without tyrosine (SSM-T)	Light ivory	No growth	-	Sparse

Baskaran *et al*. (2011) studied a high potential antibacterial actinomycete, which was cultured on six different culture media. This isolate mostly developed dark grey to white colored aerial mycelium and coffee brown colored substrate mycelium. In study of **Naine *et al*. (2012)** the colonies of actinomycetes with powdery appearance had a characteristic feature of concave, convex, flat surface with different colors from grey, white, pink, cream to yellowish. Thus, we can conclude that the nutrient compositions of the medium greatly influenced the growth and morphology of actinomycetes.

Part of the morphological characteristic includes also ability to produce dark-brown substances in the culture media, generally referred to as melanin or melanoid pigments. Melanin is negatively charged composed of multi-functional polymer and polyphenolic compound that is produced by various microorganisms by fermentative oxidation **(Dastager *et al*., 2006)** and have the radioprotective and antioxidant properties that can effectively protect the living organisms from ultraviolet radiation **(Vinarov *et al*., 2002).** *Streptomyces* species also produce brown exopigments on agar media, which mostly but not always correlate with the appearance of melanin pigment with tyrosinase activity **(Claus *et* Decker 2006)**. Strain VY31 produced melanin pigment on SSM+T medium and brown exopigments on ISP6 and ISP7 media (Figure 1).

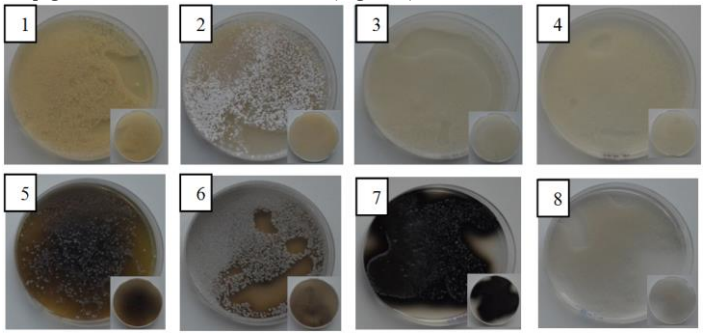

Figure 1 Morphological characteristic of VY31 strain on 1-ISP2, 2-ISP3, 3-ISP4, 4-ISP5, 5-ISP6, 6-ISP7, 7-SSM+T, 8-SSM-T media

Antonova-Nikolova *et al.* (2006-2007) found out, that *Streptomyces* sp. strain 34-1 synthesizes melanin pigment only on the ISP6 medium. Some authors also reported that melanin pigments produced by *Streptomyces* species have antimicrobial activity, for example against *Aspergillus niger* (**Ali *et al.* 2011)**.
The physiological tests are indispensable tools for classification and identification of actinomycetes and influencing the growth rate of actinomycetes **(Shimizu *et al*., 2000).** *Streptomycetes* are known to prefer neutral to alkaline environment pH, optimal growth pH range being 6.5-8 **(Locci, 1989).** Growth of the strain VY31 occurred in the pH range of 6-8 with optimum growth at pH 7.The optimal temperature being 30 °C. For most actinomycetes, the optimum growth temperature is 23-37 °C, whereas **Goodfellow *et* Williams (1983)** reported that most of the actinomycetes behave as mesophiles with an optimum growth 30 °C. The strain exhibited salt tolerance up to 5 % (Table 3), but results of **Cai (2009)** indicated, that actinomycetes are also able to grow on media with 7.0, 10 and also 15% of NaCl. NaCl tolerance of actinomycetes studied by **Naine *et al.* (2012)** was only 1% of NaCl. Utilization of carbon sources by the strain also could be used as an aid for species determination **(Pridham *et* Gottlieb, 1948).** The strain efficiently utilized the carbon sources such as glucose, arabinose, xylose, inositol, mannose, fructose, rhamnose and rafinose, but not utilized sucrose and cellulose (Table 4). The similar results found out **Baskaran *et al.* (2011); Naine *et al.* (2012)** and **Pandey *et al*. (2005).**

Table 3 Physiological testing of strain VY31

	Observed parameters		
	Growth	**Formation of aerial mycelium**	**Diffusible pigment**
0% NaCl	+	+	+
2,5% NaCl	+	+	+
5% NaCl	+	-	-
7,5% NaCl	-	-	-
10% NaCl	-	-	-
Glucose	+	+	-
Arabinose	+	+	+
Sucrose	-	-	-
Xylose	+	+	-
Inositol	+	+	-
Mannose	+	+	-
Fructose	+	+	+
Rhamnose	+	+	+
Rafinose	+	+	-
Cellulose	-	-	-

A huge enzymatic variability was discovered within the range of isolated streptomycetes. We found out, that our isolate produce phosphatase alcaline, leucinearylamidase, valinarylamidase, phosphatase acid, naftol-AS-BI-phosphohydrolase, galactosidase and glucosidase in important values (>40 nmol) (Table 4). Negative activity was observed only for lipase, chymotrypsin, galactosidase, glucuronidase, N-acetyl-glucoseamidase, mannosidase and fucosidase. On the basis of ApiCoryne® results we found out, that our isolate produce only phospahatase alcaline, esculin and also hydrolysed gelatin (Table 5). In study of **Khan *et al.* (2010**) strain *Streptomyces tateyamensis* demonstarted positive activity for *N*-acetyl-*β*-glucosaminidase, acid phosphatase, alkaline phosphatase, *α*-chymotrypsin, gelatin hydrolysis, *α*-glucosidase, *β*-glucosidase, leucine arylamidase, *α*-mannosidase, naphthol-AS-BI-phosphohydrolase, pyrazinamidase, pyrolidonyl arylamidase, trypsin and urease. Negative activity was observed for esterase, esterase lipase, *α*-fucosidase, *α*-galactosidase, *β*-galactosidase, *β*-glucuronidase and lipase. **Nagai *et al.* (2011)** reported positive enzymatic activity of *Streptomyces aomiensis* for acid phosphatase, catalase, β-glucosidase, leucine arylamidase, *N*-acetyl-β-glucosaminidase, naphthol-AS-BI-phosphohydrolase, pyrazinamidase and nitrate reduction and weakly positive for cystine arylamidase, pyrrolidonyl arylamidase and valine arylamidase, but negative for alkaline phosphatase, esterase, esterase lipase, α-chymotrypsin, α-fucosidase, α- and β-galactosidase, β-glucuronidase, α-glucosidase, α-mannosidase, lipase, trypsin, urease and gelatin hydrolysis.
Using ApiZym® and ApiCoryne® stripes is providing the advantage of easy and fast determination between two isolates showing significant appearance by means of the differences in their enzyme profiles. On the other hand, there are doubts about usability of this method as a taxonomic tool **(Vítězová, 2013).** Reliable identification of isolated actinomycetes from soils should involve using a polyphasic taxonomic approach and employing a wide variety of phenotypic and molecular techniques.

Table 4 Enzymes produced by strain VY31 detected by ApiZym® system

	ApiZym® system		
No.	**Enzyme**	**Substrate**	**Amount of enzyme (nmol)**
2	Phosphatase alkaline	2-naphtyl phosphate	>40
3	Esterase (C4)	2-naphtyl butyrate	10
4	Esterase lipase (C8)	2-naphtyl caprylate	10
5	Lipase (C14)	2-naphtyl myristate	0
6	Leucinearylamidase	L-leucyl-2-naphthylamide	>40
7	Valinearylamidase	L-valyl-2-naphthylamide	>40
8	Cystinearylamidase	L-cystyl-2-naphthylamide	20
9	Trypsin	N-benzoyl-DL-arginine-2-naphtylamide	5
10	Chymotrypsin	N-glytaryl-phenylalanine-2-naphtylamide	0
11	Phosphatase acid	2-naphtyl phosphate	>40
12	Naphtol-AS-BI-phosphohydrolase	Naphtol- AS-BI-phosphate	>40
13	Galactosidase	6-Br-2-naphtyl-D-galactopyranoside	0
14	Galactosidase	2-naphtyl-D-galactopyranoside	>40
15	Glucuronidase	Naphtol-AS-BI-D-glucuronide	0
16	Glucosidase	2-naphtyl-D-glucopyranoside	>40
17	Glucosidase	6-Br-2-naphtyl-D-glucopyranose	>40
18	N-acetyl-glucoseamidase	1-naphtyô-N-acetyl-D-glucoseaminide	0
19	Mannosidase	6-Br-2-naphtyl-D-mannopyranoside	0
20	fucosidase	2-naphtyô-L-fucopyranoside	0

Table 5 Enzymatic activities tested by ApiCoryne® system

Test	Reaction	Result	Test	Reaction	Result
Nit	Nitrate reduction	-	Pyz	Pyrazinamidase	-
PyrA	PyrrolidonylArylamidase	-	Pal	Alkaline phosphatase	+
Gur	β-glucuronidase	-	Gal	β-galactosidase	-
Glu	α-Glucosidase	-	Nag	N-acetyl-β-glucosamidase	-
Esc	Esculin (β - Glucosidase)	+	Ure	Urease	-
Gel	Gelatine (hydrolysis)	+	Glu	Glucose fermantation	-
Rib	Ribose fermentation	-	Xyl	Xylose fermentation	-
Man	Mannitol fermentation	-	Lac	Lactose fermentation	-
Sac	Sucrose fermentation	-	Glyg	Glycogen fermentation	-

Figure 2 Visible enzymatic reactions of 1-ApiZym® and 2-ApiCoryne® stripes

For genotypic identification, specific primers - F1 (5´-AGAGTTTGATCITGGCTCAG-3´; I=inosine) and R5 (5´-ACGGITACCTTGTTACGACTT-3´) were used for amplification and sequencing the gene 16S rDNA of the isolate VY31. The partial sequence was aligned and compared with all the 16S rRNA gene sequence available in the GenBank database by using the multisequence advanced BLAST comparison tool that is available in the website of National Centre for Biotechnology Information (Figure 3).

Figure 3 Sequence producing significant alignment for isolate VY31

The 16S rRNA sequence data supported the assignment of this isolate VY31 to the genus *Streptomyces* and species *rishiriensis* (*Streptomyces rishiriensis* strain Ca407).

Antimicrobial activity of *Streptomyces rishiriensis*

In the course of screening for novel antimicrobial substances (antibiotics) from soil samples, antibiotic-producing actinomycete cultures were recorded from soil samples taken in Východná, Slovakia. During our continuous search for novel antimicrobial metabolites of this region led to the isolation of actinomycetes isolate VY31 on Pochon medium by soil dilution plate technique. The MIC evaluation of the actinomycete isolate was performed against all the tested microorganisms by the dilution method (Table 6). The highest inhibition activity was observed against *Staphylococcus aureus* N315 (MRSA). Identification of novel antagonistic molecules is needed for MRSA due to emerging resistance, because multidrug resistant *Staphylococcus aureus* serves as a hospital borne pathogen and plays a dominant role in many clinical problems globally **(Sharma *et al.*, 2011).** The choice of drugs against MRSA is very less due to their genetic alternation, enzyme variations and permeability changes. Due to harmful effects, there is a need to find out new drug molecules against MRSA **(Parasa *et al.*, 2011)**. Inhibitory activity of soil actinomycetes against MRSA recorded also **Sharma *et al.* (2011)** and **Amirmozaffari *et al.* (2014)** who found out, that three isolates (from 96 strains) with inhibition zones 28, 17 and 15 mm in diameter inhibited this pathogen.

Table 6 Antimicrobial activity of selected strain against selected pathogenic bacteria and yeast

Tested microorganisms	Maximum letter of inhibition on MIC plates
***Escherichia coli* (DSM 116)**	A*
***Escherichia coli* (WT3)**	-
***Staphylococcus aureus* (Newman)**	C
***Staphylococcus aureus* (N315)**	F
***Pseudomonas aeruginosa* (DSM 19882)**	-
***Candida albicans* (DSM 1665)**	D
***Enterococcus faecium* (DSM 20477)**	A
***Enterococcus faecium* (DSM 17050)**	A

*Letter of lowest concentration of an antimicrobial that inhibited the visible growth of a tested microorganisms on MIC plate

Moderate inhibition activity was determined against *Staphylococcus aureus* (Newman) and *Candida albicans* (DSM 1665). Inhibition of *Candida* species growth was observed by many scientists (**Bharti *et al.* (2010); Das *et al.* (2014); Anansiriwattana *et al.*, (2006)).** In contrast, strain VY31 showed weak inhibition activity against *Escherichia coli* (DSM 116) and *Enterococcus faecium* (DSM 20477, DSM 17050) and no inhibition activity against *Escherichia coli* WT3 and *Pseudomonas aeruginosa* (DSM 19882). Results indicated, that tested strain was more active against Gram-positive bacteria in comparison with Gram-negative. Similar results found out also **Silambarasan *et al.* (2012)** in their study.

CONCLUSION

Selected actinomycete isolate (*Streptomyces rishiriensis*) showed significant antibiotic activity against the tested microorganisms, mostly against methicilin-resistant *Staphylococcus aures* N315. Therefore, this isolate proves to be a promising isolate which can be further studied for its applications in producing important pharmaceutical compounds. The present study is mainly involved in the identification of this strain based on the cultural, morphological, physiological, biochemical and molecular methods. Further studies for characterization of secondary metabolites are currently in progress. It is expected that the current attempt for the isolation and characterization of actinobacteria from region Východná will be useful for the identification of novel antibiotics effective against various pathogens.

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