International Journal of Biology Research
ISSN: 2455-6548, Impact Factor: (RJIF 5.22)
www.biotechjournals.com
Volume 1; Issue 1; March 2016; Page No. 36-39

Optimization of growth parameters on multi drug resistant *Streptococcus mutans*

S Karikalan, A Mohankumar

Division of Microbial Technology, PG and Research Dept. of Zoology, Chikkanna Govt. Arts College, Tamil Nadu, India.

Abstract
Streptococcus mutans is an etiological agent for worldwide distribution of Tooth decay and it is a costly disease compare than other diseases. Evidences on *S.mutans* as a Multidrug resistant species arose in each country by vast research investigation reports on tooth decay disease. This present investigation was conducted for determining growth optimization parameters of *Streptococcus mutans.* Fifty decayed samples were collected in dental clinics and dental hospitals around Tirupur, Tamilnadu. Only five isolates were selected for this growth optimization assay. Growth parameters were effects of *p*H, Temperature, NaCl and Nitrogen sources on *S.mutans*. In conclusion, growth of the *S.mutans* isolates exhibited the tolerance at different concentrations of all parameters.

Keywords: Streptococcus mutans, MDR, pH, Temperature, NaCl, Nitrogen sources

1. Introduction

Recent research shows that *S.mutans, S. sanguis, Actinomyces viscosus, Prevotella intermedia, Lactobacillus acidophilus,* and *Candida albicans* are common cariogenic microorganism (Takahashi, 2008) [1] and the *S.mutans* colonizes the oral cavity by metabolizing the dietary carbohydrates ingested by its host to produce glucan and to anchor itself to the tooth surface, forming densely populated microbial communities known as biofilms, commonly referred to as dental plaque, that include hundreds of other species of oral bacteria (Tanzer *et al.*,2001) [2].

Conditions in the oral cavity are diverse and complex, frequently changing from one extreme to another. Thus, to survive in the oral cavity, *S.mutans* must tolerate rapidly harsh environmental fluctuations and exposure to various antimicrobial agents in order to survive (Biswas and Biswas, 2011) [3].

Increasing hospital and community-acquired infections due to bacterial multidrug-resistant (MDR) pathogens for which current antibiotic therapies are not effective represent a growing problem. Antimicrobial resistance is, thus, one of the major threats to human health (Walker *et al.,* 2009) [4] since it determines an increase of morbidity and mortality as a consequence of the most common bacterial diseases (Klevens *et al.,* 2007) [5]. Today the oral bacteria are resistant to tetracyclines, aminopenicillins and cephalosporins have been reported (Sweeney *et al.,* 2004) [6]. The emergence of resistance against newly developed antibiotics (Long and Vester, 2012) [7] further supports the need for innovation, monitoring of antibiotic consumption, prevention, diagnosis and rapid reduction in the misuse of these drugs.

S.mutans can thrive; tolerate all condition of oral cavity like *p*H, Temperature, NaCl and Dietary consumption of sugar and Nitrogen products by human beings, also these conditions created by the resistance in *S.mutans*. This resistance characters were investigated in this study to access the growth tolerance against *S.mutans* in different parameters and different characters with different concentrations.

2. Materials and methods

2.1. Sample collection of tooth decay sample

Totally fifty caries samples were collected at various dental clinics and dental hospitals in and around Tirupur district of Tamilnadu. Samples were collected from the male and female caries patients.

2.2. Isolation and Identification of *S.mutans*

Mitis-Salivarius agar was used for isolation of *S.mutans.* All the plates were incubated aerobically at 37 °C for 24 h (Hardie *et al.,* 1986) [8]. Identification was performed by phenotypic and Biochemical reactions (Collee *et al.,* 1996 and Holt *et al.,* (1994) [9, 10].

2.3. Optimization assay of Growth parameters on *S.mutans*

(Towhid, 2014) [11]

Only five selected isolates were performed on each various parameters of optimization assay.

2.4. Effect of *p*H on the growth of *S.mutans*

- Prepared the nutrient broth medium.
- Selected the *p*H ranges from 5.5, 6.5, 7.5, 8.5 and 9.5
- Tested organism was inoculated at aseptic condition in the broth.
- Incubated at 37 ºC for 24 h.
- OD values were measured at 600 nm in spectrophotometer.

2.5. Effect of Temperature on the growth of *S.mutans*

- Test organism was inoculated into sterile nutrient broth tubes
- Tubes were incubated at various temperatures (30 ºC, 35 ºC, 37 ºC, 40 ºC and 45 ºC) 24 h
- Observed growth and measured the optical density at 600 nm using spectrophotometer.

2.6. Effect of NaCl on the growth of *S.mutans*

- BHI broth medium was prepared

- Add the different concentrations of NaCl into the broth medium
- Inoculated the tested organism and incubated at 37 ºC for 24 h
- Measure the growth of the organism at 600 nm in spectrophotometer.

2.7. Effect of Nitrogen sources on the growth of *S.mutans*

- Prepared the peptone, beef extract and yeast extract medium as for nitrogen sources
- Sterilized and inoculated the tested organism
- Incubated at 37 ºC for 24 h to several days
- Observed and measured the results at 600 nm in spectrophotometer.

3. Results

Hundred samples were collected from dental caries patients at the Dental Hospitals in and around Tirupur District, Tamilnadu. The samples were collected from different age both male and female patients. *S.mutans* was isolated by using selective media based on Mitis Salivarius Agar (MS).

The growth medium of *S.mutans* was adjusted at different *p*H values: *p*H 5.5, *p*H 6.5, *p*H 7.5, *p*H 8.5 and *p*H 9.5. The results indicated that the moderate growth was obtained from *p*H 6.5 to *p*H 7.5 compared than *p*H 7, and declined in *p*H 4. Maximum growth was recorded in *p*H 7 followed by *p*H 6 and *p*H 5 it indicates the optimum *p*H for the growth of *S.mutans* was recorded at *p*H 7 (Table 1 and Fig 1).

The growth medium of *S.mutans* was adjusted at different temperature: 30 ˚C, 35 ˚C, 40 ˚C and 45 ˚C. The results indicated that the rapid growth was obtained in temperature from 30 ˚C, 35 ˚C and 40 ˚C, declined in 45 ˚C. Maximum growth was recorded in 35 ˚C, it indicates the optimum temperature for the growth of *S.mutans* was recorded at 35 ˚C (Table 2 and Fig 2).

The growth medium of *S.mutans* was supplemented with different concentrations of salt ranging from 0%, 2%, 4%, 6% and 8%. The results specified that the predominant growth obtained from 2% to 8% and declined in 0%. Maximum growth was recorded in 2%-4% concentration of NaCl (Table 3). The optimum growth of *S.mutans* was survived in 4% concentration of NaCl. This shows that as the percentage NaCl was increased from 4 to 6% and the growth rate was improved at 8%. It was slower over the experimental period. Among the five isolates only four isolates were thrived at 8% of NaCl and least level OD value obtained at 0.2. The strain KK3 was inhibited and growth completely stopped (Fig 3).

The growth medium was supplemented with different nitrogen sources namely peptone, beef extract and yeast extract (each at a concentration of 1%). The effect of different incubation hours of optimized nitrogen source was studied to enhance active *S.mutans* growth. It was resulted that nitrogen sources could increase the growth of *S.mutans* Different incubation hours 24, 48, 72 and 96 were taken to observe the growth of *S.mutans* by utilized the nitrogen sources and the nitrogen sources were found to have significant effect on growth by *S.mutans*. Among the nitrogen sources tested, beef extract showed 0.44 OD value at 96th hour and it was found to be the best for growth followed by peptone 0.40 OD value it is followed by yeast extract was showed 0.38 OD value (Table 4). The utilization of nitrogen sources for the growth was reported to isolates. There was a significant and quick growth was achieved by *S.mutans* that using the beef extracts (Fig 4).

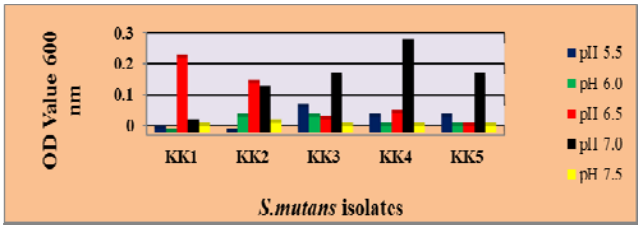

Fig 1: Effect of *p*H on *S.mutans*

Optimization of growth parameters on *S.mutans*

Table 1: Effect of *p*H on *S.mutans*

Strain No	*p*H 5.5	*p*H 6.0	*p*H 6.5	*p*H 7.0	*p*H 7.5
KKI	0.01	0	0.24	0.03	0.02
KK2	0	0.05	0.16	0.14	0.03
KK3	0.08	0.05	0.04	0.18	0.02
KK4	0.05	0.02	0.06	0.29	0.02
KK5	0.05	0.02	0.02	0.18	0.02

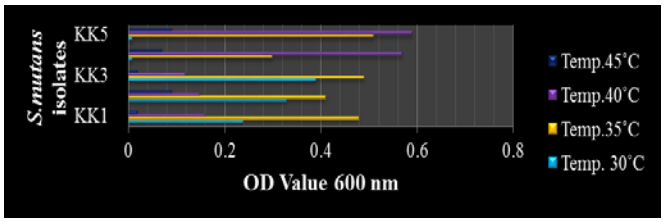

Fig 2: Effect of Temperature on *S.mutans*

Table 3: Effect of NaCl on *S.mutans*

Strain No	NaCl 0%	NaCl 2%	NaCl 4%	NaCl 6%	NaCl 8%
KKI	0.68	0.45	0.42	0.5	0.28
KK2	0.68	0.41	0.55	0.34	0.09
KK3	0.60	0.57	0.48	0.42	0
KK4	0.57	0.46	0.57	0.46	0.24
KK5	0.59	0.51	0.49	0.4	0.28

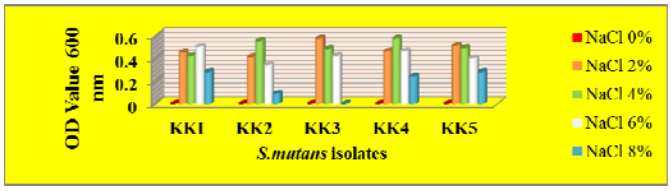

Fig 3: Effect of NaCl on *S.mutans*

Table 4: Effect of Nitrogen sources on *S.mutans*

S. No	Nitrogen sources	OD Value (680nm)			
		24h	48h	72h	96h
1.	Peptone	0.19	0.21	0.30	0.40
2.	Yeast	0.25	0.27	0.32	0.38
3.	Beef	0.08	0.24	0.41	0.44

KK – KariKalan, 1 to 5- *S.mutans* isolates

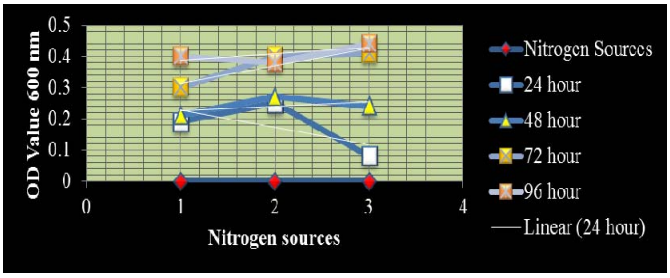

Fig 4: Effect of Nitrogen sources on *S.mutans*

4. Discussion

The growth of *S.mutans* was documented on *p*H 5.0 that similarly observed at a low *p*H range of Chen *et al.,* (2012) [12]. Acidophilic bacteria can grow at lower *p*H levels (Whiley and Beighton, 1998) [13]. However, it has been suggested that *S.mutans* may be better adapted to lower *p*H levels than some other oral bacteria (Van Ruyven, 2000) [14].

The effect of different temperature on *S.mutans* in this study that similarly growth was observed at temperature ranges from 30 to 47ºC, and the optimal growth around 37ºC (Ma and Marquis, 1997) [15]. *S.mutans* was thrived in the temperature ranging from 18-40ºC (European Bioinformatics Institute, 2011) [16].

NaCl tolerance is the most important character and most of the studies were showed that NaCl tolerance at 4%, but in this study *S.mutans* was tolerated at 6% of NaCl. This result was totally different from other studies because of the tolerant ability of *S.mutans*. The 4% NaCl tolerance of *mutans streptococci* was proved and was considered criteria for distinguished species of the *mutans streptococci* (Holt *et al.,* 1994) [10]. It was proved by one more evidence that salt concentrations are one of the essential physical factors. Twenty one isolates of *S.mutans* with a tolerance 4% NaCl while eight isolates showed no growth (Al-Jumaily, 2014) [17].

The nitrogen sources have an undefined composition with small amounts of carbohydrates, lipids and minerals. Sometimes the natural sources contain stimulators like vitamins and minerals, which influence the enzymes like amylase production. Nitrogen is needed for the synthesis of amino acids, purines, pyrimidines, carbohydrates, lipids, enzyme cofactors and other substances (Suribabu, 2014) [18]. The effect of different nitrogen sources like peptone, beef and yeast stimulates accumulation of *S.mutans* in the culture medium. These sources could strengthen high release of nitrogenous components during the growth in the media. Different nitrogen sources were found to have a significant enhancement of the growth effect on *S.mutans*. Among the nitrogen source beef was chosen as it induces the growth of *S.mutans* and is also, a substrate for the growth curve activity.

5. Conclusion

S.mutans isolates were showed the growth; tolerate ability at each different condition and concentration of every parameter. Thus, concluded (MDR) Multidrug resistant species of *S.mutans* can thrive at any fluctuating conditions and any different concentrations of growth parameters in oral cavity. Avoid the over consumption of any food and other items that affecting the normal conditions of the oral cavity.

6. References

1. Takahashi N, Nyvad B. Caries ecology revisited: microbial dynamics and the caries process. Caries Res. 2008; 42:409-418.
2. Tanzer JM, Livingston J, Thompson AM. The microbiology of primary dental caries in humans. J Dent. Educ. 2001; 65:1028-1037.
3. Biswas S, Biswas I. Role of Vl tAB, an ABC transporter complex, in viologen tolerance in Streptococcus mutans. J Antimicrobial agents and chemotherapy. 2011; 55(4):1460-1469.
4. Walker B, Barrett S, Polasky S, Galaz V, Folke C, Engstrom G, *et al*. Environment looming global-scale failures and missing institutions. Science. 2009; 325:1345-1346.
5. Klevens RM, Morrison MA, Nadle J, Petit S, Gershman K, Ray S, *et al*. Invasive methicillin-resistant Staphylococcus aureus infections in the United States. J.A.M.A. 2007; 298:1763-1771.
6. Sweeney LC, Dave J, Chambers PA, Heritage J. Antibiotic resistance in general dental practice – a cause for concern? J Antimicrob. Chem. 2004; 53:567-576.
7. Long KS, Vester B. Resistance to linezolid caused by modifications at its binding site on the ribosome. Antimicrob. Agents Chemother. 2012; 56:603-612.
8. Hardie JM, Sneath PHA. Genus *Streptococcus.* Bergey's Manual of Systematic Bacteriology, Williams & Wilkins, Baltimore. USA. 1986; 2:1043-1071.
9. Collee JG, Marmion BP, Fraser AG, Simmons A. Mackie and McCartney Practical Medical Microbiology. 14th Edn. Churchill Livingstone, New York. 1996, 263-298.
10. Chen F, Mao T, Cheng X. pH and effects on Streptococcus mutans growth of denture adhesives: An invitro study. Key Lab for Oral Biomedical Engineering of Ministry of Education, School of Stomatology, Wuhan University, Wuhan, China. Gerodontology. 2012; 31(2).
11. Towhid Hossain MD, Kamal Uddin MD. Optimization of culture conditions for a biofilm forming bacterium. Int J Pharm Bio Sci. 2014; 5(1):(B):11-20.
12. Whiley RA, Beighton D. Current classification of the oral streptococci. Oral Microbiol. Immunol. 1998; 13:195-216.
13. Van Ruyven FOJ, Linstrom P, van Houte J, Kent R. Relationship between mutans streptococci, low pH bacteria and iodophilic polysaccharide-producing bacteria in dental plaque and early enamel caries in humans. J Dent. Res. 2000; 79:778-784.
14. Ma Y, Marquis RE. Thermo physiology of Streptococcus mutans and related lactic-acid bacteria. Antonie Van Leeuwenhoek. 1997; 72(2):91-100.
15. European Bioinformatics Institute. http://www.ebi.ac.uk/2can/genomes/bacteria/Streptococcus_mutans.html. 2011.
16. Holt JG, Sneath PHA, Krieg NR, Staley JG, Williams ST. in: Hensyl WR (Ed.). Bergey's Manual of Determinative Bacteriology, Library of Congress Cataloguing-in-Publication Data, United States. 1994, 559-564.
17. Al-Jumaily EF, AL-Seubehawy HMZ, Al-Toraihy FA. Isolation and Identification of Streptococcus mutans (H5) produced glucosyltransferase and cell-associated glucosyltransferase isolated from dental caries. Int. J. Curr.Microbiol.App.Sci. 2014; 3(6):850-864.

18. Suribabu K, Lalitha Govardhan T, Hemalatha KPJ. Optimization of various Nitrogen sources for the production of -Amylase using Brevibacillus borstelensis R1 by Submerged fermentation Int. J Curr. Microbiol. App. Sci. 2014; 3(4):791-800.