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Full Length Research Paper

Biofixation of carbon dioxide from coal station flue gas using *Spirulina* sp. LEB 18 and *Scenedesmus obliquus* LEB 22

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The atmospheric concentration of CO_2, the main greenhouse gas (GHG), has increased rapidly since the beginning of the industrial age. Anthropogenic emissions from coal-fired power plants are responsible for approximately 7% (w/w) of global CO_2 emissions. Microalgae CO_2biofixation is a technology that reduces GHGs based on the use of solar energy through photosynthesis to capture and use the CO_2 that is produced by thermal and other sources. We determined the kinetic characteristics of the cyanophyte*Spirulina* sp. LEB 18 and the chlorophyte*Scenedesmus obliquus* LEB 22 and their capacities for CO_2 fixation from the flue gas of a thermoelectric plant. The kinetic growth parameters of *Spirulina* increased when flue gas was used as the sole carbon source. At the end of cultivation, the biomass production of *Spirulina* sp. LEB 18 was 35% higher in the medium with flue gas compared to the Zarrouk medium, resulting in 5.7% CO_2biofixation and a 24% reduction in flue gas CO_2. Thus, the biofixation of CO_2 from flue gas may be useful in helping to alleviate global warming.

Key words: fossil fuel, microalgae, cyanophyte and chlorophyte.

INTRODUCTION

The burning of fossil fuels, especially coal, in thermo-electric power plants is problematic due to the emission of the greenhouse gas carbon dioxide (CO_2) and the elevated temperature production of environmentally damaging sulfur and nitrogen oxides (SO_x and NO_x). The sustainability of coal use is linked to the reduction of these pollutants. Processes need to be developed for CO_2 capture and sequestration, with one alternative being

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the use of photosynthetic and autotrophic microorganisms that are capable of removing CO_2 from gas streams; cyanophytes and chlorophytes (microalgae) can remove up to 50% of CO_2 and survive in the presence of toxic compounds, such as SO_2 and NO (Radmann et al., 2011).

Microalgae use CO_2 to multiply and produce bio-compounds of interest, such as proteins, fatty acids and pigments. These microorganisms present antioxidant properties and are sources of carbohydrates, lipids, vitamins, essential amino acids and polyunsaturated fatty acids. The microalgae CO_2biofixation mechanism is based on the ability of these microorganisms to perform photosynthesis but with higher fixation rates than those of higher plants. Another important feature is that gases that are emitted may be injected directly into microalgal cultivation through photobioreactor-coupled systems, while higher plants capture gases from the environment. Injection culture tanks may be used without the need to cool these gases because many microalgae have extremophile characteristics that allow them to withstand high temperatures (Pandey et al., 2014).

The 446-MWh President Medici Thermal Power Plant (UTPM) Candiota II is operated by the Eletrobras Thermal Generation Electrical Energy Company (Companhia de Geração Térmica de Energia Elétrica - Eletrobrás CGTEE) in the southern Brazilian state of Rio Grande do Sul. A methodology approved by the Intergovernmental Panel on Climate Change (IPCC) was used to estimate that the CO_2 emitted by Candiota II between 1998 and 2002 was as high as an average of 2.0265 Mt per year, equivalent to a specific CO_2 emission of 1.41 t MW^{-1} h^{-1}. The flue gas emitted by Candiota II contained approximately 120 g L^{-1} CO_2, 2 to 2.5 g L^{-1} SO_X and 0.3 to 0.5 g L^{-1} NO_X (Migliavacca, 2005; De Morais and Costa, 2008).

The objective of this study was to use Candiota II flue gas (hereafter simply called 'flue gas') for the cultivation of *Spirulina* sp. LEB 18 and *Scenedesmus obliquus* LEB 22 and to measure their kinetic characteristics and capacities for CO_2 fixation.

MATERIALS AND METHODS

Culture media and growth conditions

The cyanophyte *Spirulina* sp. LEB 18, isolated from the Mangueira Lagoon in southern Brazil (S33°30'13'', W53°08'59'') (De Morais et al., 2008), was maintained in Zarrouk medium containing the following (g L^{-1}): $NaHCO_3$, 18; $NaNO_3$, 2.5; K_2HPO_4, 0.5; K_2SO_4, 1.0; NaCl, 1.0; $CaCl_2$, 0.04; Na_2EDTA, 0.08; $MgSO_4{\cdot}7H_2O$, 0.2; $FeSO_4{\cdot}7H_2O$, 0.01; and 10^{-3} cm^3 of trace element solution containing (g L^{-1}): H_3BO_3, 2.86; $(NH_4)6Mo_7O_{24}$, 0.02; $MnCl_2{\cdot}4H_2O$, 1.8; Cu_2SO_4 0.08; and $ZnSO_4{\cdot}7H_2O$, 0.22 (Zarrouk, 1966). The chlorophyte *Scenedesmus obliquus* LEB 22, which was isolated from the stabilization pond at Candiota II (S 24°36'13'', W52°32'43'') (De Morais and Costa, 2007), was maintained in MC medium containing the following (g L^{-1}): KNO_3, 1.25; $MgSO_4.7H_2O$, 1.25; KH_2PO_4, 1.25; $FeSO_4.7H_2O$, 0.02; and 10^{-3} cm^3 of trace element solution containing the following (g L^{-1}): H_3BO_3, 2.86; $(NH_4)6Mo_7O_{24}$, 0.02; $MnCl_2{\cdot}4H_2O$, 1.8; Cu_2SO_4, 0.08; and $ZnSO_4{\cdot}7H_2O$, 0.22 (Watanabe, 1960).

For the *Spirulina sp*.LEB 18 experiments, the Zarrouk medium carbon source ($NaHCO_3$) was omitted and replaced with flue gas containing a mean CO_2 concentration of 102 g L^{-1}. For the *Scenedesmus obliquus* LEB 22 trials, we used MC medium with the carbon source ($NaHCO_3$) replaced with the same concentration of CO_2 as in the*Spirulina*trials. In both cases, the control experiments were performed using unmodified Zarrouk or MC medium as the controls.

We grew the organisms separately in acrylic raceway-type photobioreactors (length = 0.68 m, width = 0.18 m, and height = 0.07 m) with a working volume of 5 L, agitated by blades rotating at 18 rotations min^{-1}. The experimental conditions were maintained between 14 and 28°C for 960 h (40 days) at a light intensity of 32.5 µmol m^{-2} s^{-1} provided by 40 W daylight-type fluorescent lamps (white) and a 12 h photoperiod. The initial biomass concentration of the cultures was 0.15 g L^{-1} (Radmann and Costa, 2008). To maintain the original volume, evaporation was compensated for by adding sufficient distilled water each day.

The flue gas, which came from burning coal under normal operating conditions at Candiota II, contained 102 g L^{-1} CO_2, 0.654 g L^{-1} carbon monoxide (CO) and 0.087 g L^{-1} NO_X. The flue gas was collected daily during each experimental run and compressed and stored in industrial cylinders for aspiration into the cultures. Before the flue gas was aspirated into the photobioreactors, the gas stream passed through a column of 100 g L^{-1} hydrogen peroxide (H_2O_2) to remove sulfur dioxide (SO_2) (Colle et al., 2005). The flue gas was aspirated into the culture media using sprinklers, which were uniformly distributed over the base of the photobioreactors, and aspiration was carried out for 15 min every 2 h during the 12 h photo period.

The biomass concentration (X, g L^{-1}) was measured every 24 h by reading the optical density (O.D.) at 670 nm using a 700 Plus spectrophotometer (Femto) and a previously established biomass versus optical density calibration curve. The maximum and minimum temperatures and pH were measured at the same time as the O.D. values, and we determined the alkalinity of the culture medium every three days (American Public Health Association, 1998). These temperature and pH values were used to calculate the dissolved CO_2, HCO_3^- and CO_3^{2-} concentrations (Carmouze, 1994). All of the samples were collected in triplicate.

Growth kinetics and statistical analysis

The kinetic characteristics of both organisms were evaluated by measuring the maximum specific growth rate (μ_{max}, d^{-1}) as calculated from the exponential regression of the logarithmic phase of the cell growth curve (Bailey, 1986). The generation time (tg, d) was calculated by $tg = \ln 2/\mu_{max}$ and the productivity (g $.L^{-1}.$ d^{-1}) according to the methods of Borzani et al. (2008).P_{max}represents the maximum productivity obtained.

The efficiency of carbon biofixation (F) was calculated according to Equation 1:

$$F = \frac{P * X_{C/X} * V_{FBR} * \left(\frac{M_{CO2}}{M_C}\right)}{m_{CO_2}} * 100 \quad (1)$$

Where F = biofixation efficiency (%, v/v); P = productivity (g L^{-1} d^{-1}); X_C/X = mass fraction of carbon in the cell (0.402 g_C/g_X); V_{BR} = volume of the culture medium in the bioreactor (L); m_{CO2} = mass of carbon (as CO_2) supplied in each experiment (g d^{-1}); and M_{CO2} and M_C = molar masses of CO_2 and C (M_{CO2} = 44 g, M_C = 12 g), respectively. The concentration of carbon in the final biomass (g_C)

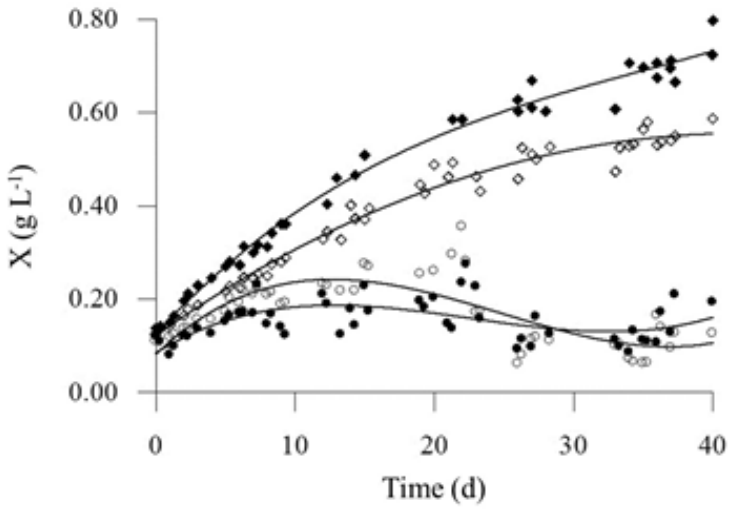

Figure 1. The biomass concentration for the microalgae *Spirulina* sp. LEB 18 with flue gas (♦) and Zarrouk medium (◊) and *S. obliquus* LEB 22 with flue gas (●) and MC medium (○) during cultivation.

was determined using the elemental analyzer CHNS / O (Perkin-Elmer 2400, USA), and standard certificate acetanilide was used to calibrate the equipment (Perkin Elmer, USA) (Baumgarten, Wallner-Kersanach; Niencheski, 2010).

The fraction of dissolved carbon (C, % v/v) in the culture medium derived from the carbon supplied by the gas in each cycle was calculated by the ratio between the mass of inorganic carbon (CO_2, HCO_3^- and CO_3^{2-}) and the flue gas in the culture medium using Equation 2:

$$R(\%) = \frac{(C_{t=15} - C_{t=0}) * V_{FBR}}{0.18} * 100 \quad (2)$$

Where $C_{t=15}$ = carbon concentration from dissolved CO_2, HCO_3^- or CO_3^{2-} at the end of the flue gas injection (g L^{-1}); $C_{t=0}$ = carbon concentration from dissolved CO_2, HCO_3^- or CO_3^{2-} at the start of the flue gas injection (g L^{-1}); V_{FBR} = volume of the culture medium in the bioreactor (L); and 0.18 = carbon mass supplied in each injection cycle (g).

The kinetic data for both organisms were evaluated using Analysis of Variance (ANOVA) at p=0.05.

RESULTS AND DISCUSSION

Biomass (X_{max}), specific growth rate (μ_{max})and productivity (P_{max})

Several compounds that are present in flue gas cannot only affect the efficiency of carbon biofixation, but are also potentially toxic to the cultivation of *Spirulina* LEB 18 and *Scenedesmus obliquus* LEB 22. The CO_2 concentration in the flue gas ranged from 0.507 to 0.703 g L^{-1}, while that of NO_X was 0.087 g L^{-1} and that of NO was 0.084 g L^{-1}.

The presence of flue gas in the culture medium increased the biomass concentration of LEB 18 by 35.7% to a maximum of 0.78 g L^{-1}, which was significant at p > 0.05 (Figure 1). The production of up to 1.50 g L^{-1} of a *Spirulina* sp. has been reported, but this occurred when pure CO_2, free from the other toxic components of flue gas, was used (Lodi et al., 2003). In our experiments, the μ_{max} in the presence of flue gas was 0.026 d^{-1} for LEB 18 and 0.017 d^{-1} for the control; thus, the addition of flue gas increased the growth rate of LEB 18 by 34.6%. The μ_{max} from our previous LEB 18 experiments, in which *Spirulina* was cultivated by injecting 120 g L^{-1} CO_2, was 0.028 d^{-1} (De Morais and Costa, 2007). Other studies have shown that *Spirulina* exhibits high μ_{max} values between 10 and 40°C (Richmond, 1990; Vonshak, 1997), as confirmed in our study, which showed high μ_{max} values between 14 and 28°C. Although lower cultivation temperatures may decrease photosynthetic activity and thus impair CO_2 fixation (Torzillo and Vonshak, 1994), there have also been reports of higher concentrations of phenolic compounds and unsaturated fatty acids, such as ω-3 and ω-6, in *S. platensis* grown at temperatures below 35°C (Colla et al., 2007). An increased concentration of CO_2 leads to an increase in the specific growth rate, yield and biomass concentration (Maeda et al., 1995), and an increase in the maximum kinetic values was observed in the LEB 18 experiments with the addition of flue gas.

The *S. obliquus* LEB 22 experiments showed no increase in the biomass concentration when flue gas was added to the medium (Figure 1). This result contrasts with a previous study by our team, which evaluated CO_2 fixation byLEB 22 in the presence of NO and SO_2 and showed that this organism produced a maximum biomass concentration of 0.81 g L^{-1} at 35°C and grew attemperatures between 20 and 38°C (Radmann and Costa, 2008). The relatively low cultivation temperatures of 14°C and 28°C, typical spring temperatures in southern

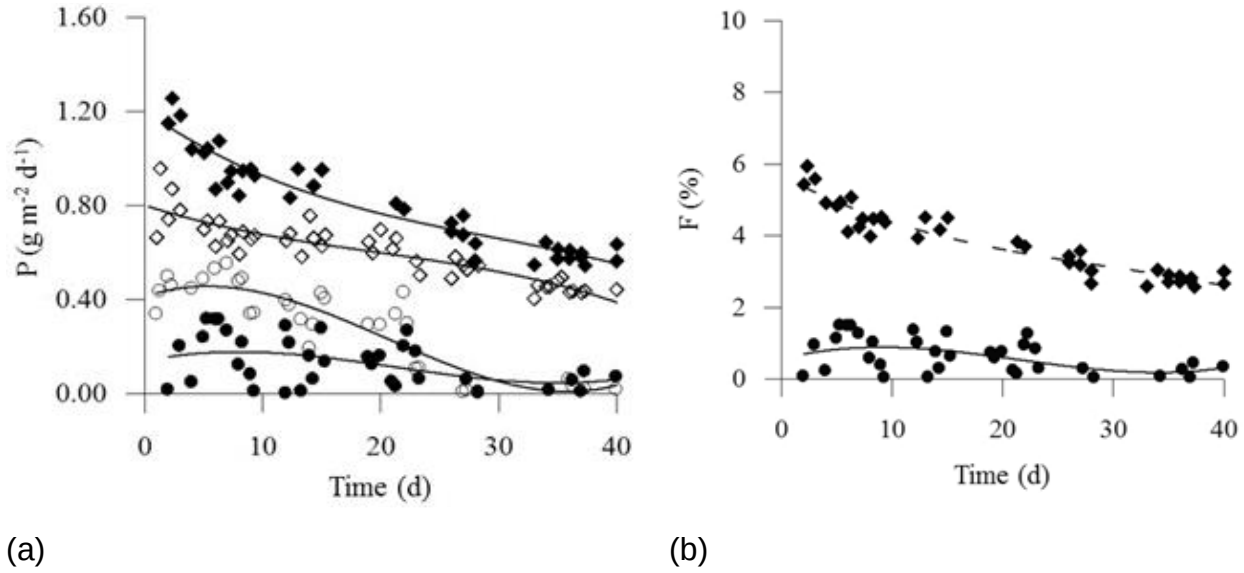

Figure 2.(a) Productivity (P) and (b) fixation of CO_2(F) for the microalgae *Spirulina sp*. LEB 18 with flue gas (♦) andZarrouk medium (◊) and *S.obliquus*LEB 22 with flue gas (●) and MC medium (○) during cultivation.

Brazil, may have affected growth in the presence of flue gas. Several studies by other groups have indicated that *S. obliquus* grows in the presence of combustion gases, containing up to 500 g L^{-1} CO_2 (Hanagata et al., 1992) and 0.300 g L^{-1} NO (Jin et al., 2008). Previous studies by our team have shown that the growth of LEB 22 was not impaired by the addition of flue gases and removed 27% of NO from a culture containing 60 g L^{-1} CO_2 (De Morais and Costa, 2008). The LEB 22 experiments had μ_{max} values of 0.013 d^{-1} in the presence of flue gas and 0.016 d^{-1} for the control experiments, while previous experiments by our group had μ_{max} values between 0.04 d^{-1} and 0.18 d^{-1} in culture medium that was supplemented with pure CO_2, NO and SO_2 (Radmann and Costa, 2008).

The productivity of LEB 18 and LEB 22 (Figure 2a) showed that the media that were supplemented with flue gas LEB 18 and gave the highest yields, with P_{max}= 1.1 g m^{-2} d^{-1} ± 0.1 g m^{-2} d^{-1} versus a control value in unsupplemented medium of P_{max} = 0.8 g m^{-2} d^{-1} ± 0.1 g m^{-2} d^{-1}. For LEB 22, the values were much lower at P_{max} = 0.2 g m^{-2} d^{-1} in flue gas-supplemented medium and 0.5 g m^{-2} d^{-1} for the unsupplemented control. The fact that LEB 22 productivity was lower in the supplemented medium may have been because the flue gas contained toxic components that inhibit growth. The final biomass of the LEB 18 grown with flue gas contained 46.8% protein and 4.8% lipid, while the LEB 22 biomass comprised 40.6% protein and 6.2% lipid. We have reported similar lipid values in two previous studies, one using media supplemented with 120 g L^{-1} CO_2, which produced a final biomass with a lipid concentration of 5.2% for LEB 18, 3.3% for LEB 22 and 4.6% for the *Chlorella vulgaris* strain LEB 106 (De Morais and Costa, 2007), and another employing media supplemented with 120 g L^{-1} CO_2, 60 µL L^{-1} SO_2 and 100 µL L^{-1} NO, which resulted in lipid values of 5.97% for LEB 18, 6.18% for LEB 22, 5.21% for LEB 106 and 5% for *Synechococcus nidulans* LEB 25 (Radmann and Costa, 2008).

Other researchers have reported 57.61% protein and 8.16% lipid in *Spirulina platensis* biomass when grown in Zarrouk medium without the addition of flue gas (Colla et al., 2007), and the protein content of *Spirulina* has been reported to vary from 64 to 74% and the lipid content from 6 to 13 % (Vonshak, 1997). All the values are w/w on a dry-mass basis. Our experiments have shown that the cultivation of LEB 18 and LEB 22 in media that were supplemented with Candiota II flue gas produced a biomass that was rich in protein and lipid and has potential for use in, among other things, the production of fertilizers, biofuels and biopolymers.

Biofixation efficiency

The maximum biofixation efficiency (F_{max}) of CO_2 was 5.66 ± 0.88% for LEB 18 and 0.86 ± 0.56 % for LEB 22 (Figure 2b). The differences that were observed between the experimental and control trials appear to be related to the concentration of free CO_2 in the culture medium, especially for LEB 18, in which the CO_2 concentration in the medium was 25 mg L^{-1} higher than in the control experiments (Figure 3). For LEB 22, we found a significant difference (p=0.086) in the amount of CO_2absorbed into the culture medium up to 360 h (day 15 d) of cultivation.

The addition of 150 g L-1 of CO2 to C. vulgaris cultures

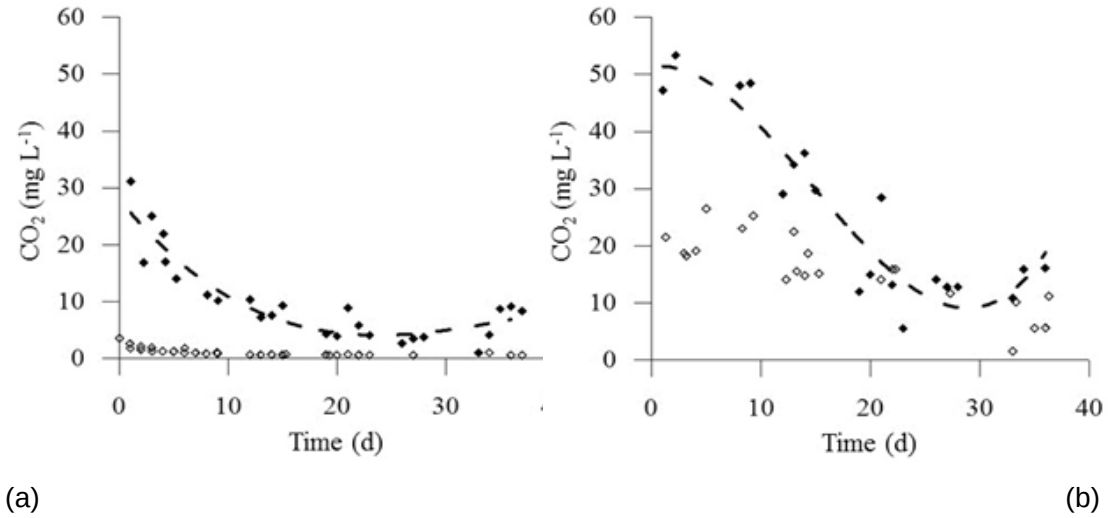

Figure 3.CO_2 concentration in the cultures of *Spirulina* sp. LEB 18 (a) and *Scenedesmus obliquus* LEB 22 (b) with flue gas (♦) and control experiments (◊) during the cultivation period.

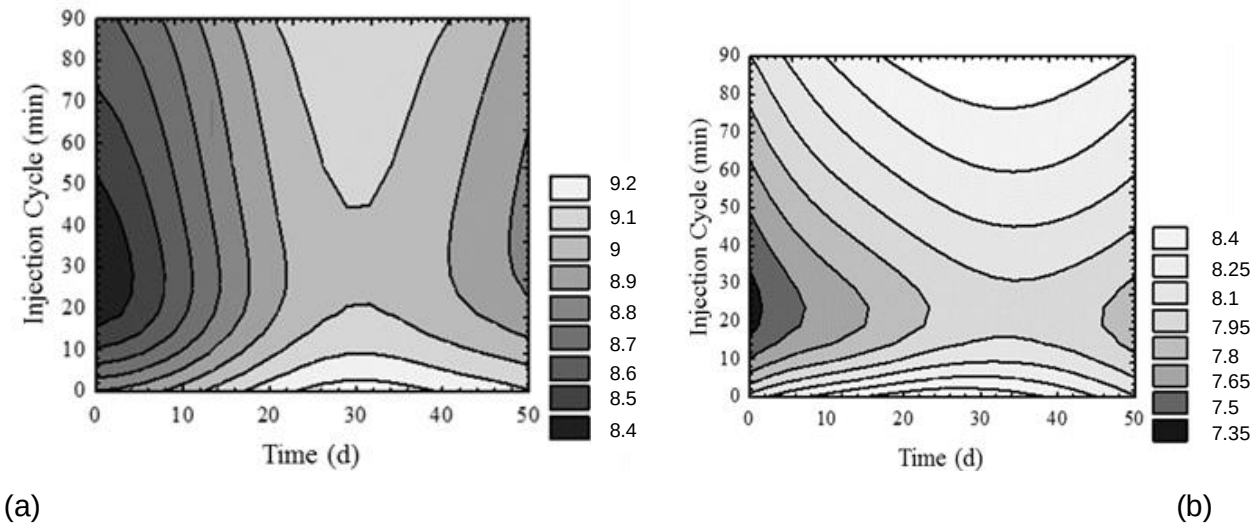

Figure 4. Changes in pH during the injection cycle and time of operation of reactors for *Spirulina sp*. LEB 18 (a) and *Scenedesmus obliquus*LEB 22 (b) when grown with flue gas.

resulted in a CO_2 fixation rate of 0.624 g L^{-1} d^{-1} (Yun et al., 1997). Membrane technology has been used to aid in CO_2 dispersion in experiments designed to remove this gas from the air. The resultant 10 g L^{-1} of CO_2 that is dispersed in the culture medium using filter modules increases pH of CO_2biofixation efficiency from 2 to 20 % (Cheng et al., 2006). Other studies have reported that 4.4 kg of CO_2 from burning natural gas is required to produce 1 kg of *Chlorella* biomass.

We also discovered that during flue gas injection, the dissolution of CO_2 reduced the pH by altering the equilibria between HCO_3^-, CO_3^{2-} and CO_2. In the last 75 min of the experiment, the pH value increased as a result of the CO_2 utilization by the microorganism in the media and the formation of bicarbonate and carbonate (Figure 4). The injection of flue gas can be carried out over a wide pH range without significantly affecting photosynthetic activity (Olaizola, 2003). We found that when flue gas was injected, the largest pH variations occurred at the beginning of cultivation (t < 10 d) and that LEB 18 showed less severe changes in pH than LEB 22.

The differences in pH were associated with the alkalinity of the culture media, which averaged 53.1 ± 14.9 g L^{-1} $CaCO_3$ for LEB 18 and 16.3 g L^{-1} ± 6.4 g L^{-1}

Table 1. The fraction (%) of inorganic carbon from the flue gas dissolved in the culture medium in the form of CO_2, $HCO_3^-$and CO_3^{2-}, fixed by microalgae (F) and the total CO2 removed.

Factors	*S. obliquus*LEB 22	*Spirulina* sp. LEB 18
CO_2 (%)	7.41 ± 4.58	2.08 ± 3.39
HCO_3^- (%)	3.34 ± 2.47	14.15 ± 2.92
CO_3^{2-} (%)	1.57 ± 1.28	3.93 ± 1.35
F (%)	0.86 ± 0.56	4.04 ± 1.02
Total (%)	13.2	24.2

$CaCO_3$ for LEB 22. The higher the alkalinity, the greater the pH stability during cultivation (Vonshak,1997). Because the rate of flue gas injection is associated with pH buffering capacity, drastic changes in pH to non-optimal values must be avoided. An alkaline medium favors the formation of highly soluble chemicals, such as HCO_3^- and CO_3^{2-}, with some of the CO_2 in the injected flue gas converted to these chemical species and thus removed from the effluent gas. The concentrations of HCO_3^-, CO_3^{2-} and CO_2 derived from the flue gas dissolved in the culture medium are given in Table 1. In the LEB 18 culture, most of the CO_2 supplied by the flue gas remained dissolved in the form of HCO_3^-, which helped to maintain the pH, whereas in the LEB 22 culture, the largest carbon supply remained in the form of CO_2. The percentage of CO_2 removed from the flue gas was 24.2 % for LEB 18 and 13.2 % for LEB 22.

Conclusions

At the end of the cultivation period, we found that Candiota II flue gas increased *Spirulina* sp. LEB 18 biomass production by 35%, with a 24% reduction in CO_2 from the flue gas and a 5.66% biofixation of CO_2. When supplemented with flue gas, the final biomass of LEB 18 contained 46.8% protein and 4.8% lipid, while LEB 22 contained 40.6% protein and 6.2% lipid. Our results indicate that these microorganisms have the potential to be grown in power plants to biofix CO_2 from the flue gas that is produced from coal and can thus contribute to reducing global warming.

Conflict of interests

The authors have not declare any conflict of interest.

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Phenotypic and genotypic characterization of *Staphylococcus aureus* isolates recovered from bovine milk in central highlands of Ethiopia

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Antimicrobial resistance is becoming an extremely serious global problem. The goal of this study was to determine the prevalence and phenotypic and genotypic characteristics of *Staphylococcus aureus* isolated from milk and milk product samples in Ethiopia and also to determine the presence of methicillin resistant *Staphylococcus aureus* (MRSA). A total of 577 milk and milk product samples were collected from central Ethiopia and *Staphylococcus* spp. were isolated using the method described in FDA Bacteriological Analytical Manual (BAM). Resistance of *S. aureus* isolates to 12 antimicrobials was determined by using the Kirby-Bauer disk diffusion method. PCR detection of *mecA* and nuc gene was also conducted. To determine the clonal relatedness of *S. aureus* isolates, DNA fingerprinting of selected isolates was performed by PFGE. Of the 577 milk and milk product samples investigated, *S. aureus* isolates were recovered from 120 (21%) of the sample. In addition, coagulase negative *Staphylococcus* species were also isolated from 361/577 (63%) of the samples. The highest frequency of resistance was observed for penicillin (83%) and the lowest was noted for amoxicillin/clavulanic acid (3%) and gentamicin (3%). Fourteen (14) isolates (13%) recovered from raw milk were found to be susceptible to all the tested antimicrobials while 57% of the isolates were resistant to more than one of the antimicrobials. All the isolates were susceptible to vancomycin and none were found to be methicillin resistant *S. aureus* based on *mec*A gene carriage. PFGE analysis of 39 *S. aureus* isolates identified three separate clonal clusters and also several sporadic isolates. *S. aureus* isolates in this study were found to be resistant to multiple antimicrobials. This warrants a larger representative study to fully understand the extent of the problem and design better strategies for regulation of antimicrobial use in both the medical and veterinary sectors in central Ethiopia.

Key words: Bovine milk, Ethiopia, genotypic resistance, *Staphylococcus aureus.*

INTRODUCTION

Different studies conducted in Ethiopia have shown that *Staphylococcus aureus* is implicated in nearly 40% of mastitic cows (Workineh et al., 2002; Dego and Tarke, 2003; Getahun et al., 2008; Abera et al., 2010). Another study in and around Sebeta, Ethiopia, that looked into the prevalence of mastitis in 180 local and crossbreed dairy cows showed that mastitis (majority of which is sub-clinical in form) can reach a prevalence rate of 52.78% (Hundurra et al., 2005). The prevalence of *S. aureus* as a cause of mastitis was 44% and that of *Staphylococcus epidermis* was 14.93% in the study conducted by Hundurra et al. (2005). With such high prevalence of sub-clinical mastitis in the Ethiopian context, the public health importance of zoonotic pathogens would be of great concern as there is a trend of using raw milk for direct consumption from apparently healthy cows. In addition, most rural Ethiopians have a tradition of consuming milk and milk product as a ready to eat food items in unpasteurized form such as raw milk, Ethiopian cottage cheese (Ayib) and yoghurt (CSA, 2001).

With the ever-increasing situation of foodborne diseases worldwide, the development of antimicrobial resistance in foodborne pathogens has become a very important public health issue. Antimicrobial resistance is becoming an extremely serious global health problem (Carlet et al., 2012). The liberal use of antimicrobials in hospitals and treatment centers as well as the sub-therapeutic use in livestock for growth promotion and prophylaxis has greatly contributed to the emergence and persistence of resistant strains of bacteria (Helmuth, 2000; Ray, 2004).

There is a concern that the extensive use of antimicrobials in animal husbandry potentially leads to maintenance of selective pressure and higher frequency of resistance for wide variety of antimicrobial agents (Kumar et al., 2005; Silbergeld et al., 2008). Furthermore, over the last few decades, community- and healthcare-related infections have become a common phenomenon globally (Carlet et al., 2011; Gagliotti et al., 2011). Specifically, *Staphylococcus aureus* has been involved in infections associated with in patients in hospitals and community settings (Millar et al., 2007). Livestock-associated methicillin resistant *Staphylococcus aureus* (LA-MRSA) multilocus sequence type 398 (ST398) has also emerged in the past 10 years.

Initially, it was described in pigs and pig farmers who were carriers, but soon it was also detected in patients in hospitals. Several lineages of this epidemic strain have also been reported to be commonly present in other livestock species (Armand-Lefevre et al., 2005; Voss et al., 2005). The detection of *mec*A as a confirmatory test for methicillin-resistant *S. aureus* (MRSA) is widely accepted. However, recent reports indicate that there appears a new *mec*A homologue, $mec_{ALGA251}$ that is only 70% nucleotide homology to the conventional *mec*A gene. This makes the confirmatory test of the conventional *mec*A gene detection questionable (Stegger et al., 2012).

Although large-scale studies and documentations regarding the problem of antimicrobial resistance (AMR) in Ethiopia do not exist, the available reports indicate that it is already a growing problem and should be considered a public health concern (Gebre-Sealsssie, 2007; DACA, 2009). In the veterinary area, a number of reports indicate that resistance of *S. aureus* to antimicrobials is a common problem prevailing in different parts of Ethiopia (Legesse et al., 2009; Getahun et al., 2008; Abera et al., 2010; Sori et al., 2011; Daka et al., 2012; Tamiru et al., 2013; Mekuria et al., 2013). A number of studies have been conducted in Ethiopia with a major focus on the prevalence of *S. aureus* in bovine milk (Getahun et al., 2008; Abera et al., 2010; Workineh et al., 2002; Dego and Tarake, 2003). Most of these researches, however, were concentrated on the importance of this pathogen as a cause to clinical and subclinical mastitis and all of these studies conducted in Ethiopia were done using the conventional phenotypic methods and none of them managed to use a modern molecular technique that involves *mec*A gene detection. Despite the expense, application of molecular techniques like PCR and DNA fingerprinting renders many advantages over the conventional phenotypic assay in the study of antimicrobial resistance profile of pathogens (Fluit et al., 2001). The current study aim was to determine the prevalence and antimicrobial resistance of *S. aureus* isolates recovered from raw bovine milk collected in central Ethiopia using both phenotypic and genotypic molecular techniques.

MATERIALS AND METHODS

Sampling areas and sample types

Small-scale bovine milk producing co-operatives were conveniently selected from urban and per-urban regions of central Ethiopia [*Asela, Debre-Zeit, Addis Ababa (Akaki/Kaleti)* and *Selale*, the areas surrounding and supplying milk to the capital city, Addis Ababa, with a population of 2.7 million people (CSA, 2007). A total of 577 samples were conveniently selected from milk collection centers and milk processing plants in the aforementioned regions and were analyzed. The samples included raw on-farm pooled milk (n=433) and combined bulk tank milk (n=44), pasteurized milk (n=65), yogurt (n=20) and cheese (n=15) samples.

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Table 1. Primer-pairs used for the detection of different genes of *S. aureus* using PCR.

Gene	Primer[1]	Sequence (5'-3')	Amplicon size (bp)	Location within gene	Reference
nuc	NUC-F	gcgattgatggtgatacggtt	270	48-70	(Brakstad et al., 1992)
	NUC-R	agccaagccttgacgaactaaagc		303-328	
mecA	MEC-F	tccagattacaacttcaccagg	162	1190-1211	(Oliveira & Lencastre 2002)
	MEC-R	ccacttcatatcttgtaacg		1351-1332	

[1]Primer synthesized by Integrated DNA Technology (IDT, Coralville, IA).

Culture and identification of *S. aureus*

Raw/pasteurized milk samples (25 ml) and 25 g of milk products (cheese and yoghurt) were analyzed following methods as recommended by the U.S. Food and Drug Administration: Bacteriological Analytical Manual (BAM: http://www.cfsan.fda.gov/_ebam/bam-5.html). Briefly, 25 ml of milk were pre-enriched in 225 ml of Mueller-Hinton broth (Difco, Detroit, MI, USA) with 6.5% NaCl. After incubation at 37C for 24 h aerobically, aliquots were plated onto Mannitol Salt agar plates (Difco). Presumptive positive colonies were confirmed biochemically using Gram stain, catalase, and coagulase tests. Confirmation of *S. aureus* isolates was done by *nuc* gene detection using PCR.

Phenotypic antimicrobial susceptibility testing

S. aureus isolates were tested against 12 antimicrobials. Resistance to antibiotics was determined by the Kirby-Bauer disk diffusion test using commercially prepared filter paper disks as determined by the Clinical laboratory Standards Institute (CLSI, 2008). Briefly, after pre-inoculation in Tryptone soy broth and adjustment of turbidity to a 0.5 McFarland turbidity standard, bacterial suspensions were plated as a full lawn onto freshly prepared Mueller-Hinton agar plates using sterile swabs. The antimicrobials (Becton Dickinson and Company Sparks, MD USA) used and their respective disc potencies were as follows: ampicillin (amp) (10 µg), amoxicillin-clavulinic acid (amc) (30 µg), ceftriaxone (cro) (30 µg), ciprofloxacin (cip) (5 µg), erythromycin (ery) (15 µg), gentamicin (gen) (10 µg), penicillin (pen) (10 µg), streptomycin (str) (10 µg), tetracycline (tet) (30 µg), trimethoprim-sulfamethoxazole (sxt) (1.25 + 23.75 µg=25 µg), cefoxitin (fox) (30 µg) and vancomycin (van) (30 µg). The findings were interpreted as resistant, intermediate or susceptible to each antimicrobial. *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, and *Pseudomonas aeruginosa* ATCC 27853) were used for quality control for culture, drug sensitivity testing. The criteria used to select the antimicrobial agents tested were based on local clinical need and global use for treatment of food borne pathogens.

Genomic DNA extraction

DNA was extracted directly from all the 109 *S. aureus* isolates. Genomic DNA extraction was performed using DNeasy blood and tissue kit (QIAGEN, Hilden, Germany). Bacterial DNA was extracted according to the protocol provided by the manufacture.

Species identification

PCR was done for the detection of the *nuc* genes as previously described (Table 1) (Brakstad et al., 1992). This gene is a marker gene used to confirm isolates as *S. aureus*.

Screening for methicillin resistance genes

The primers used in the case of *mec*A gene detection were according to methods described elsewhere (Table 1) (Oliveira and Lencastre, 2002). The thermocycling conditions used for *mec*A gene detection were: Initial denaturing at 94°C for 2 min followed by 30 cycles of 94°C for 2 min, 55°C for 1 min, 72°C for 2 min and a final extension step of 72°C for 2 min. 10 µl of the PCR product of each isolate tested were electrophoresed on 1% agarose gel containing 5 µl of 10 mg/ml ethidium bromide for 1 h at 120 v using 0.5X Tris-borate EDTA (TBE) as running buffer. PCR product size estimation was done using Gene ruler 100-bp DNA ladder (exACTGene 100 bp DNA ladder, Fisher Scientific, Fairlawn, NJ). For PCR amplification of *mec*A gene, the MRSA ATCC 43300 strain was used as a positive control.

PFGE genotyping

Thirty nine (39) *S. aureus* isolates were randomly selected from four geographic locations to determine the clonal relatedness of isolates. DNA fingerprinting of the selected isolates was performed using pulsed-field gel electrophoresis (PFGE). PFGE for *Staphylococcus aureus* was done as previously described (McDougal et al., 2003). Briefly, isolates were grown on Trypticase Soy Agar (TSA) at 37°C for 16-18 h. Cell suspension buffer (10 mM Tris-HCl pH 7.2, 20 mM Nacl, 50 mM EDTA) was used to suspend and adjust the bacterial concentration to an optical density (OD) of 1.35. TE buffer (10 mM Tris-HCl pH 8.0, 0.1 mM EDTA,) was used to prepare agarose embedded cells. After the bacterial cells were lysed (2 µl of 2 mg/ml lysostaphin was added for each sample), intact genomic DNA was digested with 20 µl of *SmaI* restriction enzyme (New England Biolabs, Ipswich, MA, USA) incubated at 25°C for 4 h. The Pulsenet universal strain *Salmonella enterica* serovar Braenderup H9812 was used as a molecular standard marker. The DNA fragments were separated by CHEF-DR® III Pulsed-Field Electrophoresis System (Bio-Rad Laboratories, Hercules, CA, USA). PFGE running conditions were as follows: initial switch 5.2s, final switch 43.9 s, voltage 6 V/cm include angle 120, run time 19 h and pump 50-60. Cluster analysis was performed using the unweighted pair group method with arithmetic averages with 2.0% band position tolerances and 1.5% optimization values using Bionumerics software, version 5.1 (Applied Math inc., Belgium). Similarity coefficients were calculating using Dice Coefficient Similarity Index. PFGE banding patterns with a similarity index >70% were grouped within the same genotypic cluster.

Data analysis

Descriptive statistics were used for the frequency of isolation of *S.*

Table 2. Prevalence of *Staphylococci* spp. in the different sample types.

Staphylococci species	Milk and Milk Products				Total number (%) n=577
	Raw milk number (%) n=477	Pasteurized number (%) n=65	Yoghurt number (%) n=20	cheese Number (%) n=15	
S. aureus	120 (25.2)	0 (0)	0 (0)	0 (0)	120 (20.8)
Coagulase negative Staphylococcus (CoNS)	334 (70.0)	17 (26.2)	2(10)	8 (53.3)	361 (62.6)
Total	454 (95.2)	17 (26.2)	2(10)	8 (53.3)	481 (83.4)

aureus from milk and milk products. The identification of the associations between variables under investigation and the dependent variable (contamination by one or more pathogens) were assessed by non-parametric tests for nominal variables (χ^2). The data was analyzed using SPSS version 20.0. Descriptive statistics were done using frequency and percentages (%).

Presence or absence of bands was determined initially by visual analysis and macro-restriction banding patterns were compared. Clustering of fingerprints was conducted by Dice coefficient similarity index. Isolates were clustered according to UPGMA method using the Bionumerics software, version 5.1 (Applied Math inc., Belgium).

RESULTS

Isolation of *S. aureus* isolates from raw bovine milk and milk products

Overall, 120 *Staphylococcus aureus* isolates were obtained from the milk and milk product samples with a prevalence of 21% (120/577). In addition, Coagulase Negative staphylococcus (CoNS) species were also isolated from 361/577 (63%) milk and milk product samples analyzed. Considering the raw bovine milk samples only, the overall prevalence of *S. aureus* was 24% (103/433) for individual on-farm pooled milk and 39% (17/44) for combined bulk tank milk samples. All of the milk product samples tested were negative for *S. aureus*. However, 17/65 (26%), 2/20 (10%) and 8/15 (53%) of pasteurized milk, yoghurt and cheese samples were contaminated with coagulase negative *Staphylococcus* (CoNS) isolates, respectively (Table 2). Of the 120 *S. aureus* isolates, only 110 were recovered after shipping to USA for molecular analysis. Further analysis of 110 of the *S. aureus* isolates using the *nuc* gene PCR assay revealed that, 109 of the isolates were confirmed to be *S. aureus* that made the overall PCR confirmed prevalence to be 109/577(19%).

Phenotypic and genotypic antibiotic resistance profile of *S. aureus*

Of the total 109 *S. aureus* isolates tested for susceptibility to 12 antimicrobials, all were found to be susceptible to vancomycin. The highest rate of resistance was observed for penicillin (83%) and the lowest rate was noted for amoxicillin/clavulanic acid (amc) (3%) and gentamicin (gen) (3%) (Table 3). Fourteen isolates (13%) recovered from raw milk sample were found to be susceptible to more than one of the antimicrobials tested while 57% of the isolates were multidrug resistant.

Isolates resistant to more than one antimicrobial were detected from all the four geographic locations (Addis Ababa, Debre-Zeit, Selale and Asela) (Table 3). However, the proportions of resistance to each antimicrobial varied by location. For example, penicillin (pen) resistance was higher for isolates from Addis Ababa (92%) as compared to isolates from Selale (88%), Debre-Zeit (75%) and Asela (69%). Resistance to cefoxitin (Fox) was relatively higher in Asela isolates (62%) as compared to 48% for Selale and 58.3% for both Addis Ababa and Debre-Zeit isolates. Resistance to ciprofloxacin (cip) was only found in the isolates from the Selale region (7%). In addition, resistance to erythromycin was only evident in the isolates from the Asela and Selale areas (peri-urban areas) with 8 and 7% respectively. When stratifying the study sites into urban and peri-urban settings, resistance to erythromycin and ciprofloxacin was only evident in the peri-urban areas. Overall, the proportion of *S. aureus* isolates with resistance to more than one antimicrobial from the different regions was as follows: Selale (53%), Asela (69%), Addis Ababa (58%), and Debre-Zeit (58%). These differences were not statistically significant ($p>0.05$). When stratifying the study sites into peri-urban and urban, the resistance proportions were not statistically significant between the two regions.

In addition to the phenotypic characterization, all the 109 *S. aureus* isolates were screened for the presence of the *mec*A gene using PCR. None of the isolates were found to carry the *mec*A gene, indicating the absence of MRSA in the samples. Phenotypically, as shown in Table 3, there were 58 isolates that were resistant to cefoxitin (Fox) and yet were *mec*A negative.

PFGE typing of *S. aureus* isolates

PFGE analysis of 39 *S. aureus* isolates identified three separate clonal clusters and also several sporadic

Table 3. Proportion of antimicrobial resistance among *S. aureus* isolates recovered from different locations.

Location of isolates	Fox	Pn	S	E	CRO	CIP	AmC	Am	G	Te	SXT	>1R*
Selale (n= 60)	29 (48.3%)	53 (88.3%)	4 (6.7%)	4 (6.7%)	4 (6.7%)	4 (6.7%)	2 (3.3%)	19 (31.7%)	2 (3.3%)	15 (25%)	3 (5%)	34 (56.7%)
Asela (n=13)	8 (61.5%)	9 (69.2%)	1 (7.7%)	1 (7.7%)	0 (0%)	0 (0%)	0 (0%)	3 (23.1%)	0 (0%)	3 (23.1%)	0 (0%)	7 (53.8%)
Addis Ababa (n= 12)	7 (58.3%)	11 (91.7%)	2 (16.7%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (16.7%)	1 (8.3%)	3 (25%)	0 (0%)	6 (50%)
Debre-Zeit (n=24)	14 (58.3%)	18 (75%)	2 (8.3%)	0 (0%)	1 (4.2%)	0 (0%)	1 (4.2%)	10 (41.7%)	0 (0%)	8 (33.3%)	2 (8.3%)	15 (62.5%)
[1]Urban (n=36)	21 (58.3%)	29 (80.6%)	4 (11.1%)	0 (0%)	1 (2.8%)	0 (0%)	1 (2.8%)	12 (33.3%)	1 (2.8%)	11 (30.6%)	2 (5.6%)	21 (58.3%)
[2]Peri-urban (n=73)	37 (50.7%)	62 (84.9%)	5 (6.8%)	5 (6.8%)	4 (5.5%)	4 (5.5%)	2 (2.7%)	22 (30.1%)	2 (2.7%)	18 (24.7%)	3 (4.1%)	41 (56.2%)
Total (n=109)	58 (53.2%)	91 (83.5%)	9 (8.3%)	5 (4.6%)	5 (4.6%)	4 (3.7%)	3 (2.8%)	34 (31.2%)	3 (2.8%)	29 (26.6%)	5 (4.6%)	62 (56.9%)

[1]Addis Ababa and Debre-Zeit. [2]Asela and Selale. *Resistance to more than one antimicrobial. Fox, Cefoxitin; Pn, Penicillin; S, Streptomycin; E, Erythromycin; CRO, Ceftriaxone; CIP, Ciprofloxacin, AmC, Amoxicillin-clavulanic acid; Am, Ampicillin G, Gentamicin; Te, Tetracycline; SXT, Trimethoprim Sulphamethoxazole; VA, Vancomycin. >1R*=resistance to more than one antimicrobial.

isolates (Figure 1). Cluster-A contains eight isolates that are 74% clonally related based on banding pattern identity. A total of 7/8 isolates in this cluster share the penicillin resistance phenotype and one isolate was susceptible to all the antimicrobials tested. This cluster also has two sub-clusters; sub-cluster-IA and IIA.

Isolates in sub-cluster-IA share banding similarity at 79% and contains 5 isolates: 3 isolates from Selale (collection centre 6-farmer 1 and 8; collection centre 5-farmer 2); 1 isolate from Asela (collection centre 3-farmer 17) and 1 isolate from Debre-Zeit (collection centre 4-farmer 5). Isolates from Selale region under sub-cluster-IA (collection centre 5-farmer 2 and collection centre 6-farmer 1) had shown a 93% identity based on PFGE banding pattern and phenotypically they share resistance to penicillin. In addition, the third isolate recovered from Asela (collection centre 3-farmer 17) also share banding pattern identity at 92% with the above isolates within the same sub-cluster-IA. This isolate also shares phenotypically Pen-Fox resistance pattern with isolate from Selale collection centre 5-farmer 2 and from Debre-Zeit (collection centre 4-farmer 5).

A 94% banding pattern identity was observed for isolates in sub-cluster-II A. This sub-cluster contains three isolates from three different geographical locations, Selale, Addis Ababa and Debre-Zeit. An isolate from Debre-Zeit collection centre 2-bulk tank 1 and an isolate from Selale collection centre 9-farmer 15 showed a 96.3% banding pattern identity within the sub-cluster. The isolates from Debre-Zeit and Addis Ababa shared the same penicillin resistance profile while the isolate from Selale was susceptible to all the antimicrobials tested.

Cluster-B isolates are 74% clonally related based on banding pattern identity and contains two isolates- one from Asela (collection center 3 farmer-12) and one isolate from Addis Ababa (collection centre 2-farmer 15). These clonal isolates share phenotypically Pen-Fox resistance pattern.

Cluster C contains 2 isolates that were found to be 87% clonally. These isolates were from two geographically distinct regions. One was from Debre-Zeit (collection centre 3-farmer 17) and the other was from Addis Ababa (collection centre 1-farmer 3). These isolate had different resistance patterns.

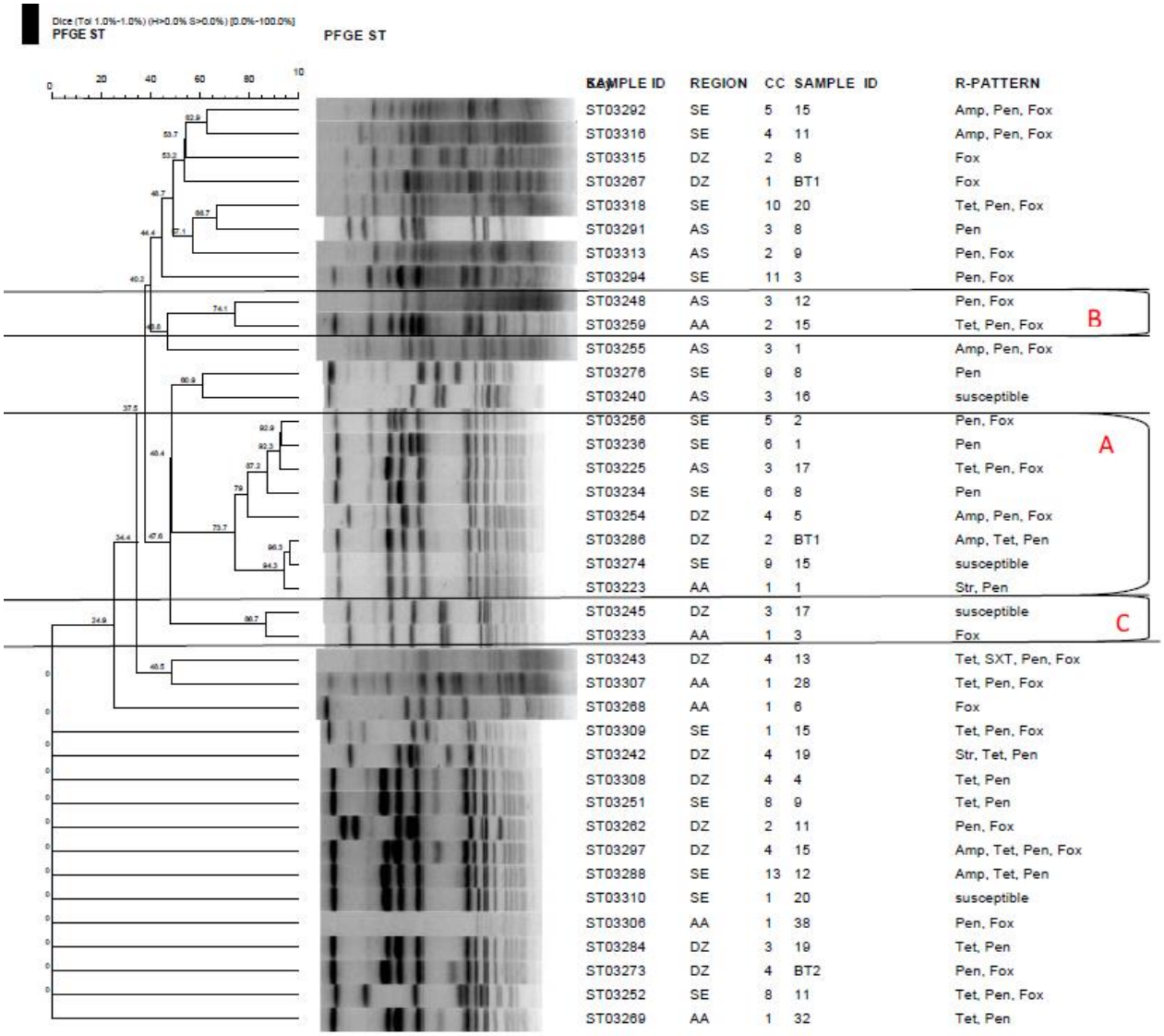

Figure 1. Dendrogram clusters of pulsed field electropheris of *S. aureus* isolates (banding patterns with a similarity index>70% were grouped within the same genotypic cluster). CC, milk collection center; AS, Asela; SE, Seleae; DZ, Debre-Zeit; AA, Addis Ababa; Fox, Cefoxitin; Pn, Penicillin; S, Streptomycin; Am, Ampicillin G; Te, Tetracycline; SXT, Trimethoprim Sulphamethoxazole; Fox, Cefoxitin; susceptible to all tested antimicrobials.

There are also several sporadic isolates representing the different geographical areas that include: four isolates from Addis Ababa, ten isolates from Selale, four isolates from Asela and nine isolates from Debre-Zeit.

DISCUSSION

A number of studies have been conducted in Ethiopia on the prevalence of *S. aureus* in bovine milk (Workineh et al., 2002; Dego and Tarake, 2003; Getahun et al., 2008; Abera et al., 2010). Most of these researches; however, were concentrated on the importance of this pathogen as a cause to clinical and subclinical mastitis and yet its public health importance has not been well addressed. The prevalence of *S. aureus* in the current study is lower than what was reported previously. A study in Ethiopia (Abera et al., 2010), for example, reported the prevalence of *S. aureus* in mastitic cows to be 42%. The prevalence of *S. aureus* as a cause of mastitis was also reported to be 44% by a different study in Ethiopia (Hundurra et al., 2005). Another study done in Ethiopia also showed a higher prevalence of *S. aureus* (52%) in bovine milk in Jimma (Sori et al., 2011). The discrepancy in prevalence of *S. aureus* among the present study and the above studies can partially be explained by the very type of milk samples analyzed in the respective studies. In the previous studies, mastitic bovine milk was the focus and

cultured for *S. aureus*, while milk samples for the current study was picked regardless of their mastitic status. In addition, the fact that the milk specimen for the current study has been obtained from on-farm pooled and bulk tank, rather than the cow specifically, would also explain the discrepancy in the prevalence rate.

Prevalence of *S. aureus* is reported to vary greatly among different countries and production systems. The prevalence of *S. aureus* reported here is lower than what has been reported elsewhere. Some workers from Brazil reported a 70% prevalence of *S. aureus* from unpasteurized bovine milk samples (Rall et al., 2008) and a study in Zimbabwe also reported a prevalence of 58% for *S. aureus* recovered from raw bovine milk (Gran et al., 2003). Despite a lower prevalence of *S. aureus* in the current study, 21% prevalence is of paramount risk for the consumer as there is a lack of refrigeration facilities and the consumption of raw milk is a common feeding tradition in Ethiopia.

Regarding the antimicrobial resistance profile of *S. aureus*, all the isolates were susceptible to vancomycin. This is in contrast with some reports in Ethiopia which showed 3 to 38% vancomycin resistance rate in *S. aureus* isolates from different parts of Ethiopia (Sori et al., 2011; Daka et al., 2012; Mekuria et al., 2013). The highest rate of resistance was observed for penicillin (83%) and the lowest rate was noted for amoxicillin/ clavulanic acid (3%) and genamicin (3%). The penicillin resistance proportion observed in the present study is lower than what has been reported from mastitis cow in Ethiopia. A study conducted in Adama town in Ethiopia that looked at the antibiotic resistance profile of *S. aureus* from mastitic cows reported a 94% resistance to penicillin (Abera et al., 2010). These workers also reported that resistance to trimethoprim-sulfamethoxazole (SXT), gentamicin (gen) and streptomycin (str) was 58, 0, and 6% respectively. The current study, however, showed a much lower resistance proportion for SXT (5%) and a higher rate of resistance for str (8%). The discrepancy observed here might be due to methods of antimicrobial resistance determination variability based on location, or type of sample collected. The current study revealed that 14 isolate (13%) recovered from raw milk sample were found to be susceptible to the tested antimicrobials while 57% of the isolates were resistant to more than one of the antimicrobials tested. Multidrug resistance reported here is higher than what was reported elsewhere (Adwan, 2006).

A recent study in Ethiopia (Mekuria et al., 2013) reported a 45% prevalence of multidrug resistant *S. aureus* isolates most of which were originating from milk. A study conducted in Palestine looked into the anti-microbial resistance profiles of *S. aureus* isolated from sub-clinical mastitis cases and revealed that 53% of the isolates were resistant to at least three antimicrobials (Adwan, 2006). Higher multidrug resistance (79%) rate were also reported for *S. aureus* isolates recovered from human clinical samples in Gondar University Hospital, located in Northwest Ethiopia (Belay et al., 2013). Further studies focusing on the possible epidemiological link between the human multiple drug resistant *S. aureus* isolates and those of animal/food origin would help in understanding the dynamics of transmission of resistance traits and the public health risk.

Genotypic investigation of all the PCR confirmed *S. aureus* (n=109) isolates showed that none of the isolates were *mec*A positive. However, phenotypically, there were 58 (53%) isolates that were resistance to cefoxitin. In previous studies, isolates that were phenotypically resistant to β-lactams and yet genotypically lacked the *mec*A gene were identified (Stegger et al., 2012; García-Álvarez et al., 2011). A possible explanation for the finding in the present study could be- resistant to cefoxitin, without the *mec*A gene, may be due to either the over-production or over-expression of penicillinase or alteration of other penicillin-binding proteins (Caierao et al., 2004).

PFGE analysis of 39 *S. aureus* isolates identified three separate clonal clusters and also several sporadic isolates. This is the first study of its kind in Ethiopia. The result from molecular characterization (PFGE) indicated that the isolates contained in each cluster and sub-clusters represent two up to four different geographical areas. For example, cluster-A contained isolates from Addis Ababa, Asela, Selale and Debre-Zeit that are hundreds of kilometers apart. The presence of limited diversity among *S. aureus* isolates of bovine origin with a broad geographic distribution is possibly related to the large number of mastitis cases in this region (Fitzgerald et al., 1997; Sabour et al., 2004). However, further studies using genotypic characterization of *S. aureus* isolates collected at the level of the cow are warranted, as other factors may be involved in the origin of isolates in this study since samples were collected from pooled milk.

Most of the isolates in each cluster and sub-clusters phenotypically share antimicrobial resistance pattern indicating the presence of a common resistance gene pool within a broad geographic area. Based on the result, it is also possible that cross contamination between different milk collection centers within the same geographical area may occur. For example, in Selale, a number of milk collection centers that are several kilometers apart share the same bulk tank containers and milk field technician who tests the standard of milk at milk collection centers and this may also be possible source of cross contamination. Study that involves tracing down this line of contamination by milk container, however, is needed to verify this notion.

Conclusion

The result from this study reveals that milk in the central

high lands of Ethiopia was contaminated with *S. aureus* which may imply public health significance. The overall prevalence of *S. aureus* was found to be 21%. PFGE analysis of 39 *S. aureus* isolates identified 3 separate clusters and also several sporadic isolates. Most of *S. aureus* isolates in this study showed resistance to a range of antimicrobials tested. Overall, 13% of all the isolates were found to be susceptible to all the antimicrobials while 57% of them were resistant to more than one of the antimicrobials tested. In addition, *S. aureus* strains isolated from infections in humans or from human nasal colonization should be investigated and compared to those of animal origin in this geographic location to generate a comprehensive picture of the current situation regarding antimicrobial resistance and the public health concern with regards to *S. aureus* in Ethiopia. In summary, *S. aureus* isolates recovered from raw bovine milk in this study were found to be resistant to multiply antimicrobials. This warrants further investigation of antimicrobial usage in both the human and veterinary medical sectors.

Conflict of interests

The authors did not declare any conflict of interest.

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